FORENSIC ENTOMOLOGY

The Utility of Arthropods in Legal Investigations

Edited by

Jason H. Byrd

James L. Castner

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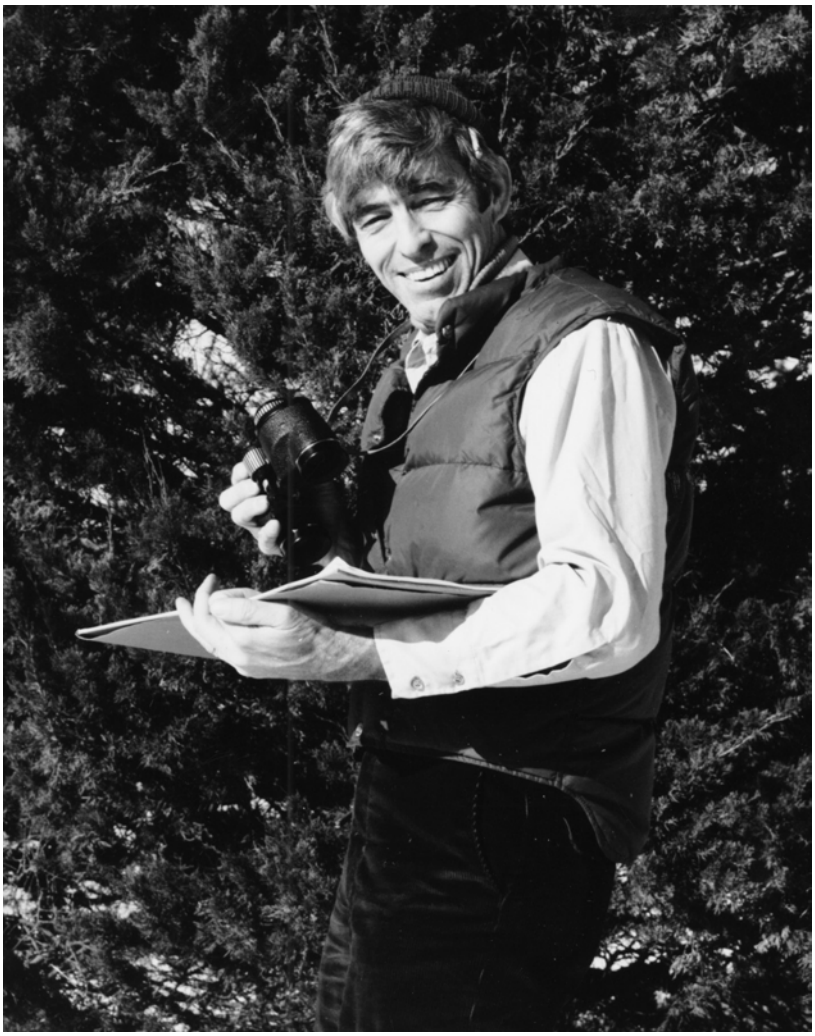
In Memoriam



Elmer Paul Catts
1930–1996

Elmer Paul Catts — 1930–1996

E. Paul Catts contributed greatly to the entomology profession by his efforts to teach students, implement entomological certifications, and guide college faculty with a unique combination of philosophy and cutting-edge science. However, none of the various disciplines in entomology have benefited greater from his efforts than the area of forensic entomology. Dr. Catts was first and foremost a generalist, possessing a broad spectrum of knowledge in scientific illustration, insect ecology, fly taxonomy, and insect behavior. It was from his base of well-rounded knowledge that he was able to delve into the specifics of insect behavior and ecology relating to the decomposition of human remains.

Paul was always available and eager to help, be it the student with little funding for research or the police department lacking a budget for insect analysis. The questions raised by these situations were what he thrived upon, and it was by helping others that he could find the answers he sought. Never did Dr. Catts refuse to aid a colleague who asked him for guidance with a question on forensic entomology. We forensic entomologists were all learning the science as we went, and Paul was always present to keep us on the proper course and keep us working as a team. He even held some of us in check so we did not travel down misguided avenues that we might eventually regret. We have all learned from his being our mentor and, on a few occasions, learned even more from his stern personal lectures as to what was proper and scientifically correct.

Now, with his passing we must carry on his ideals, philosophies, humor, and candor. To aid in his memory, the Department of Entomology at Washington State University, Paul's home for more than 16 years, has established the E. Paul Catts Memorial Lectureship Fund. With this effort we sincerely hope that his great contributions to each of the authors of this work, to the Department of Entomology at Washington State, and to entomology in general will not be forgotten. We have learned many lessons from him, and we miss him greatly. His memory will be forever etched in the science of forensic entomology.

Neal H. Haskell
Jason H. Byrd

Foreword

Entomology is a broad and varied field of study to the extent that insects interact with practically all human endeavors including the food we eat, our domiciles, our health, and our concern with the environment. Standards of food quality, structural pest control regulations, and public health necessitate laws and regulations that often require the interpretation and testimony of an entomologist. To the extent that the entomologist becomes involved with forensic questions, i.e., the practice of forensic entomology, this is well summarized by Robert D. Hall in the Introduction of this book.

As a busy forensic pathologist for over 4 decades, I have investigated deaths dealing with problems of structural pest control regulations, public sale of chemical pesticides, myiasis, envenomization, and taphonomy. The ubiquity of entomological problems involves many people other than entomologists.

In recent decades forensic entomology has focused on the field of medicolegal death investigation, the main theme of this book. Perhaps "recent decades" is not completely applicable. Interest in the effects of insects upon human remains has been with us since the dawn of human history. An early reference is found in the Old Testament, Job 21:26: "They shall lie down alike in the dust, and the worms shall cover them." Humans have been capable of observation and learning since creation. Unfortunately, vast amounts of traditional oral knowledge have been lost. Even after writing was invented, illiteracy and loss of written historical records have resulted in the paucity of our understanding of that knowledge. It is impossible to determine how much our knowledge is lacking due to the loss of written documents and the failure of oral history to record early events and advances in science. Accordingly, the earliest record of the use of entomological evidence in a medicolegal investigation is that of Sung Tz'u in the 13th century (see Introduction). We may state that forensic entomology, born within dim history and awaiting the invention of the microscope and the realization that manure piles do not result in "spontaneous generation" of flies, is off to a running start within the field of medicolegal death investigation during the last half of the 21st century.

The basic problem with utilization of entomological evidence has been lack of interest and fiscal support. Early in my career, I purchased *The Blowflies of North America* by D. G. Hall in hopes of utilizing such knowledge in a systematic fashion, but to no avail. Unschooled in the subject and lacking a local university entomology program, I interacted only with entomologists who managed structural pest control businesses and was unable to fully utilize such evidence. Fortunately, others within academic centers of entomology extended their talents into assisting local law enforcement and death investigation agencies. When enough of these forensically oriented centers arose, a critical mass of pragmatic information ensued. This aroused an interest and created a desire by death investigators for entomological support.

At that time, along came Dr. William Bass at the University of Tennessee with his "body farm" concept. Knowledge and interest accelerated, especially within the American Academy of Forensic Science (AAFS). The academy's section on Physical Anthropology was a perfect forum. For those not familiar with the AAFS, it is a multidisciplinary educational organization currently composed of 10 sections. Each section deals with a different facet of forensic science. The annual meetings follow the principle of "cross fertilization." A member may attend any one of the section meetings no matter what the specialty of that member. At the 1999 meeting five papers dealing with entomological evidence were presented, four in the Physical Anthropology Section and one in the Pathology and Biology Section. Accordingly, entomologists may appreciate potential applications to disciplines not oriented to entomology, while the nonentomologist may learn of entomology as it may relate to his or her field of scientific interest.

New information is constantly being discovered, and old information is often applied to new situations. Today we have DNA considerations, certainly a newcomer to the field of forensic science and still in its infancy in forensic entomology. Toxicological analytic methods are improving and are being applied to insect larvae, which may contain toxins derived from the decedent. I am intrigued by the included topics of entomological evidence as it relates to bodies in water and buried bodies, not the usual environment for consideration of insect activity in the taphonomic process. To me, one area for future research is what factors determine the variations in which insects become attracted to a nondecomposed corpse. In the warm climate of Miami, we have seen considerable outdoor variation — eyes, nose, and mouth encrusted with fly eggs 30 minutes after suicidal hanging and no flies on a bloodied gunshot victim several hours into the day. Over 3 decades ago the county government changed from a once per week garbage collection to twice a week. Almost overnight the house fly population plummeted. Much work needs to be done to map out the variations of insect response to a dead body in different geographic areas.

However, this text brings with it new and interesting variants on the theme of forensic entomology with the authors discussing their specific specialty and studies within the field of forensic entomology. The forensic pathologist should be specifically interested in the chapter concerned with insect alteration of bloodstains, as well as artifacts created by insect feeding on human skin (in South Florida ants wreak havoc upon the skin pattern of a gunshot wound). The protocol for the collection of entomological evidence at the scene and the latest information pertaining to insect succession and time of death estimates also should be of great interest. Additionally, forensic pathologists can learn what their fellow death scene investigators, the entomologists, are doing. The entomologist certainly would be interested in all of the above plus computer models of insect growth. In fact, a perusal of the Table of Contents should whet the intellectual appetite of forensic pathologists, crime scene investigators, entomologists, and even mystery fiction writers. This book is timely and a useful addition to one's library of forensic science.

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Preface

The area of forensic entomology has developed in recent years to become an increasingly important aspect of the forensic sciences. As a forensic anthropologist, I was associated with some of the early research directed at establishing the "time since death," including the use of insects in the estimation of the postmortem interval (Gilbert and Bass 1967). I taught at the University of Kansas from 1960 to 1971 where I identified skeletal remains for law enforcement agencies. I moved from Kansas to the University of Tennessee in June 1971. The Tennessee State Medical Examiner at that time was Dr. Jerry Francisco, who knew of my forensic anthropology experience and asked if I would serve as the state forensic anthropologist for Tennessee. In this position, I worked closely with the State Medical Examiner and had the opportunity to identify a much greater number of bodies that were in active stages of decomposition and colonized by insects.

In fact, over half of my first identification cases from Tennessee were maggot-covered bodies in an active stage of decomposition. The first question asked by the police, when called to the scene of a dead person, is: how long has he/she been dead? I often wondered why I was getting so many bodies from Tennessee that were maggot covered. I think that the answer is simply that Kansas has twice the amount of land as Tennessee and half the number of people. A dead person thrown out in Kansas is likely not to be found until he or she is a complete skeleton. Outside deaths in Tennessee, with half the amount of land and twice the population as Kansas, are much more likely to be found in the early stages of decay.

At that time I had only limited experience with the early stages of decomposition, and after researching the literature I found that only a few articles treating "time since death" had been published. The majority of these articles were based on studies done with pigs and dogs (Payne 1965; Payne et al. 1968, 1970, and 1972; Reed 1958). Since the University of Tennessee had taken part in these early studies, I proposed to continue this early work by conducting studies of human decomposition on actual cadavers (Rodriguez and Bass 1983). This resulted in the creation of the Anthropology Research Facility, which through the writings of author Patricia Cornwell has become better known as the "Body Farm."

The time after death is known as the postmortem period and can be divided into the immediate postmortem interval and the extended postmortem interval. Forensic investigations during the immediate postmortem interval may occur from minutes until 2 days after death and are usually carried out by a forensic pathologist. The determination of the postmortem interval is estimated primarily through observation and measurement of body conditions such as cooling, muscular flaccidity, rigor mortis, lividity, pallor of the skin, and others. The extended postmortem interval and the entomological factors used in its estimation, receive the emphasis of this book. This time period may cover from days to decades after death.

The present volume contains research by the leading forensic entomologists on the use of insects and arthropods in medicolegal investigations and in determining the length

of time since death. An overview of entomology and arthropod identification is presented along with those factors that affect the colonization of human remains. Much of the data and conclusions presented are based on actual case studies in which the contributors have participated. The scientific areas related to criminal investigations have expanded rapidly in the past 20 years, and, as you will see in the following chapters, forensic entomology has become a valuable tool in the solving of crimes. This book uses a comprehensive approach to provide current information and discusses recent technologies from experts in the field. I appreciate the opportunity to write the Preface for this pioneering volume in the field of forensic entomology.

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The Editors

Jason H. Byrd, Ph.D., is a board certified forensic entomologist and Diplomate of the American Board of Forensic Entomology. Currently, he is an Assistant Professor of Criminal Justice and Biology at Virginia Commonwealth University in Richmond, VA. Combining his formal academic training in entomology, forensic science, criminal justice, and law, Dr. Byrd regularly consults in both criminal and civil legal investigations. He also specializes in the education of traditional academic students, law enforcement officials, medical examiners, coroners, attorneys, and other death investigators on the use and applicability of arthropods in legal investigations.

Dr. Byrd has been involved in the collection and analysis of entomological evidence for over 10 years. His research has focused on the development and behavior of insects that have forensic importance as well as computer modeling of insect growth on human remains. The results of his research are currently being applied to postmortem interval estimations based upon entomological evidence recovered at the death scene. Dr. Byrd regularly conducts workshops and training lectures throughout the U.S. in an effort to educate death investigators in the proper processing of crime scenes for entomological evidence. He also has been involved in the publication of several other works dealing with the topic of forensic entomology. Dr. Byrd is a member of both the American Academy of Forensic Sciences and the Entomological Society of America.

James L. Castner, Ph.D., is an entomologist-photographer-writer with special interests in tropical biology and medical entomology. He is currently an Adjunct Professor in the Biology Department at Pittsburg State University in Pittsburg, Kansas. Combining photographic expertise with an academic background in entomology and biology, Dr. Castner has endeavored to create and publish works that bring scientific topics to both a professional and general audience. This includes several heavily illustrated works treating forensic entomology.

Dr. Castner has worked previously as a scientific photographer for a major university. His photographs have appeared in a variety of books and magazines, including almost every college-level biology textbook. His favorite topics are related to the insect world and the rainforest. Some of his writing and photo credits include: *National Geographic*, *Natural History*, *International Wildlife*, *GEO*, *GeoMundo*, *National Geographic World*, *Ranger Rick*, and *Kids Discover*. His book credits include: *Amazon Insects*, *Rainforests*, *A Field Guide to Medicinal and Useful Plants of the Upper Amazon*, and the secondary school learning center titled *The Amazon Rainforest*. Dr. Castner is currently working on a series of children's rainforest books, as well as graduate-level materials dealing with insect identification and arthropod taxonomy.

In 1997, Dr. Castner left his academic position to pursue writing and the development of educational materials full-time. He has been actively involved as an educator of second-

ary school students and their teachers for many years, often acting as a workshop leader or instructor of field courses. He designs and leads natural history tours for teachers, students, and naturalists to the Amazon Basin and often serves as a consultant in many capacities. He has traveled and photographed throughout South and Central America, including over 50 trips to Peru to study tropical insect biodiversity.

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Dedication

Firstly, this volume is dedicated to Shari who is a constant source of happiness in my life. Her patience with my nearly continuous antics is endless. It also is dedicated to my parents, Evelyn and Hugh, and to Justin and Juli, my brother and sister. A wonderful family is inspiring indeed.

J. H. Byrd

For my father, Chief of Detectives (Ret.) Franklyn Castner, who devoted his professional life to law enforcement. Thank you for everything.

J. L. Castner

Introduction: Perceptions and Status of Forensic Entomology

ROBERT D. HALL

Contents

Introduction

Forensic entomology is the broad field where arthropod science and the judicial system interact. It has been subdivided into three principal areas focused on those issues most often litigated (Lord and Stevenson, 1986). Therefore, *urban entomology* concentrates mainly on controversies involving termites, cockroaches, and other insect problems accruing to the human environment. The name "urban" is somewhat a misnomer, in that private or public nuisance actions involving pest insects, such as flies emanating from livestock or similar facilities, are categorized under this heading. There is currently much litigation involving insect nuisance as it relates to agricultural endeavors, especially cattle feedlots, poultry houses, and "corporate" hog facilities. That numerous lawsuits relate to termite damage, termite extermination, and the effect of termites and related structural pests on the value of real estate should not come as a surprise. However, other litigation under the "urban" rubric is not as easily foreseen by the uninitiated. Patients in hospitals and nursing homes occasionally suffer myiasis (infestation by fly larvae) and this usually results in actions claiming neglect. Negligence actions against mortuaries may result from maggot-infested corpses. To illustrate how diverse the field can be, the authenticity of figurines and other artifacts (Figures I.1a to I.1d) from west Mexican shaft tombs has been verified by insect evidence (Figures I.2a,b and I.3a,b) (Pickering et al., 1998).

The area of *stored products entomology* involves disputes over arthropods and arthropod parts in food and other products. Insect debris in breakfast cereal, caterpillars in cans of vegetables, and fly maggots in sandwiches from fast-food restaurants are good examples of commonly litigated cases in the stored products area (Figure I.4). Occasionally, a consumer will attempt to defraud a restaurant or other business by "planting" insects or insect

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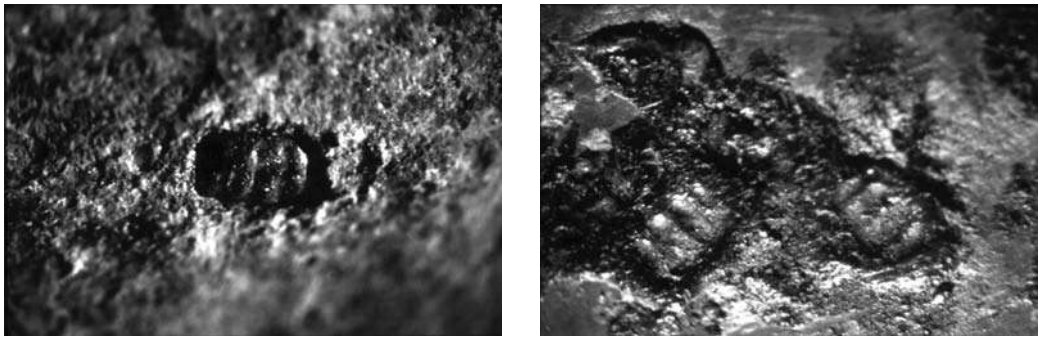
Figure I.1a This olla form pot in the "Colima red" style represents a shallow basket of fruit with a smaller olla in the middle. It is believed that these vessels were filled with drink offerings, and they were commonly placed in the shaft tombs approximately 2000 years ago. (Photo courtesy of Robert B. Pickering, Photo Archives, Denver Museum of Natural History.)

Figure I.1b This small standing figurine is characteristic of the Tuxcaceuso-Ortices style. Manganese stains can be seen over parts of the figure, some of which included mineralized puparia. (Photo courtesy of Robert B. Pickering, Photo Archives, Denver Museum of Natural History.)

Figure I.1c Solid ceramic figurine of a mother and child. On this figure, two widely separated impressions of puparia were discovered. (Photo courtesy of Robert B. Pickering, Photo Archives, Denver Museum of Natural History.)

Figure I.1d A detail of the back of a small solid ceramic figurine. A cluster of insect puparia that have been mineralized by manganese deposits can be seen in the middle of the photograph (black deposits), while unmineralized impressions can be seen adjacent and upper right. (Photo courtesy of Robert B. Pickering, Photo Archives, Denver Museum of Natural History.)

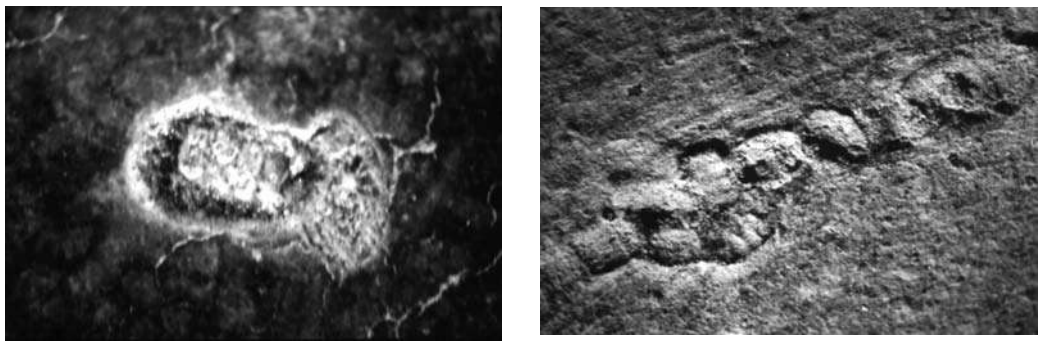


(a) (b)

Figures I.2a,b Photomicrographs of a single insect puparium (a) and a grouping of puparia (b) that have been mineralized by manganese (black deposits) on Colima dog figurines discovered in west Mexican shaft tombs. (Photos courtesy of Ephraim A. Cuevas, Photo Archives, Denver Museum of Natural History.)

parts in products purchased beforehand. The resolution of such cases requires a forensic entomologist.

The focus of the present text, the area often called "medicolegal" entomology or "forensic medical entomology" is now commonly known as *medicocriminal entomology* because of its emphasis on the utility of arthropod evidence in solving crimes, most often crimes of violence (Hall, 1990). Medicocriminal entomology usually involves all of those elements necessary to produce a fascinating story. It contains the intrigue surrounding human death, the decay process with its grisly aspects, the detective work necessary to bring perpetrators to trial, the adversarial criminal justice system with its arcane terminology, seeming inconsistencies, and the drama of the courtroom. Add to this the application of an impartial biological science and it is not surprising that medicocriminal entomology has been embraced by a broad spectrum of individuals. Those individuals include consumers (criminalists and detectives), advocates (prosecuting and defense attorneys), and future practitioners such as students in many colleges and universities. It also has been used to resolve questions about the death of animals other than humans, including livestock or protected species such as bears (Figure I.5) (see Anderson, 1998).



(a) (b)

Figures I.3a,b Photomicrographs of an unmineralized single insect puparium (a) from the outer surface of a pitcher discovered in a west Mexican shaft tomb. (b). An unmineralized grouping of insect puparia on the inside of a broken pot discovered in a west Mexican shaft tomb. (Photos courtesy of Ephraim A. Cuevas, Photo Archives, Denver Museum of Natural History.)

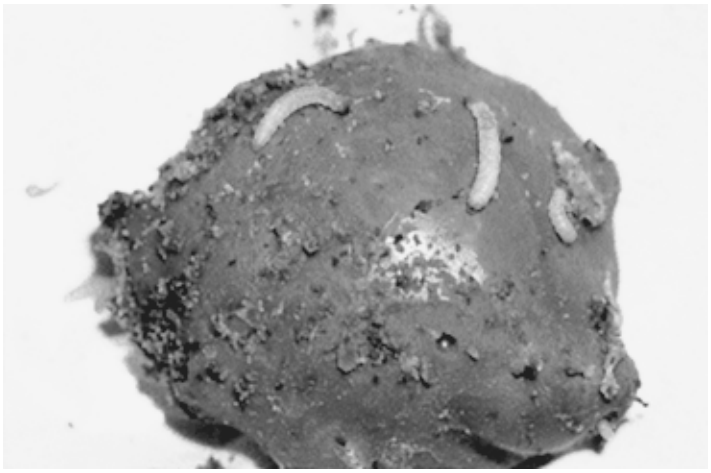


Figure I.4 Indian meal moth larvae (*Plodia interpunctella*) (Lepidoptera: Pyralidae) infesting a bite-sized chocolate candy. Often the civil litigation surrounding insect contamination of food products requires a forensic entomologist. (Photo courtesy of Dr. Jason H. Byrd.)

Although medicocriminal entomology may involve deliberate homicide or assault using insects, cases of unexplained sudden death (such as anaphylaxis from bee stings) or causation of traffic accidents (e.g., inattention to driving during frantic attempts to evade a wasp inside an automobile), the typical questions posed to the medicocriminal entomologist involve estimates of the time a decedent has been dead (the "postmortem interval" or PMI) and less frequently, the place (*situs*) where death occurred (Hall, 1990). For the

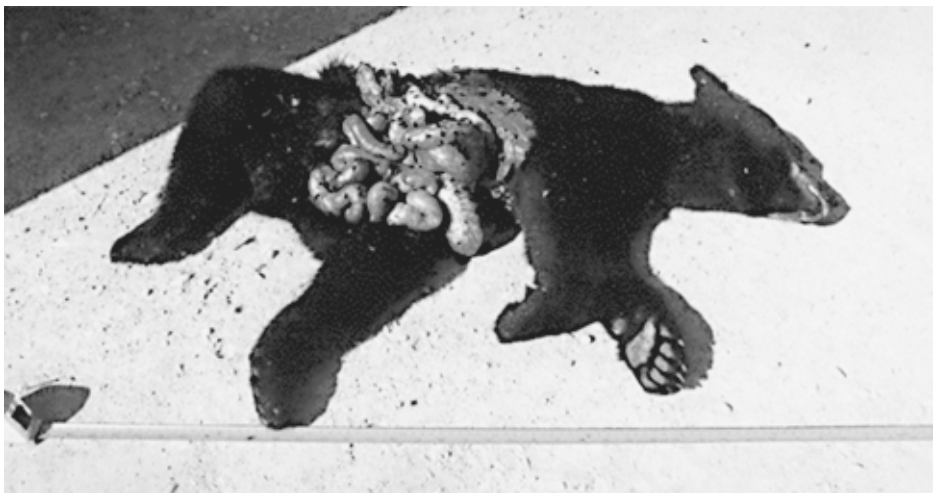


Figure I.5 Forensic entomologists are often involved in cases of wildlife poaching involving protected species in an effort to resolve questions as to the time of death as with this black bear cub found in the parking lot of a garbage dump. Recently, forensic entomologists have teamed with police and wildlife officials in Canada to solve cases in which bears have been poached to satisfy the market for their gall bladders, which are sold as supposed aphrodisiacs. (Photograph taken by W. Campbell, RCMP, Winnipeg, Manitoba, FIS. With permission of the Royal Canadian Mounted Police.)

former determination, two principal approaches are employed. The first involves application of the temperature-dependent development of insects (typically flies), and the second recognizes that a generally predictable succession of arthropods often facilitates decomposition of organic matter, including human corpses or animal cadavers, and that, by recognizing how a corpse's fauna relate to such pattern of colonization, an estimate of PMI may be made.

Because of its association with flies, which are of broad importance to human and animal health, and the ecology of decomposition, the field of medicocriminal entomology is rightly recognized as a specialty within medical entomology itself. The province of medical entomology, preventive medicine, in its broadest form involves the study of mosquitoes, ticks, mites, and all other arthropod species that can damage human or animal health directly or vector pathogenic organisms that in turn cause disease. Thus, medical entomology is an important biomedical science and the medical entomologist is no stranger to the medical community. For many years, medical entomologists have worked alongside physicians and veterinarians in fields including infectious diseases, pathology, and dermatology, elucidating the various direct and indirect ways that insects and their allies impact human and animal health.

The aforementioned phenomenon called *myiasis* is commonly noted in veterinary medicine and can occur in humans even in the West's sanitized society. Those species of flies producing facultative, or secondary, myiasis have long been recognized as major players in the decomposer biotic community. The natural ecological cycles returning dead plants and animals into raw material for future life frequently involve insects. In particular, the decomposition of human or livestock feces, or the physical remains of humans or other animals, depends heavily on insect involvement. The nuisance factors associated with flies from rural livestock facilities (Thomas and Skoda, 1993) or the beneficial effects of fly maggots in wounds (see Graner, 1997) directly included medical entomologists in these health-related issues. Therefore, it is not surprising that most medicocriminal entomologists currently operate under the broad rubric of medical entomology, which includes veterinary applications.

Medicocriminal Entomology as a Profession

Most modern progress in medical entomology has been recorded during the past century, especially since Pasteur's articulation of the "germ theory" in the latter part of the 1800s, and this progress correlates well with similar advances in medicocriminal entomology. The medicocriminal entomologist thus finds his or her professional status currently analogous to that of the medical entomologist in the broad sense. The status of medicocriminal entomology as a "profession," therefore, can be examined most efficiently in the context of a biomedical career track.

Although the data are now more than a decade old, a survey of forensic entomologists worldwide provided an interesting perspective (Lord and Stevenson, 1986). Of 62 entomologists responding, 33 indicated that they were associated solely with the medicocriminal specialty and five more were involved with both medicocriminal and other forensic entomology specialties. Most held full-time positions with universities or governmental agencies and about 35% indicated involvement with consulting work. Fewer than 40% of respondents were members of the American Registry of Professional Entomologists (ARPE), the

"professional association" of American entomologists, which has since been amalgamated into the Entomological Society of America as the Board Certified Entomologist (BCE) program. The majority of entomologists responding to the survey held the Ph.D. degree or equivalent, with the remainder possessing M.S. or, occasionally, M.D. degrees.

Bottlenecking

When compared to "licensed professions," medicocriminal entomology will be seen to suffer from several factors that may be viewed as shortcomings. The most important of these will center on the issues of quality assurance and career opportunity. Every career track exhibits some form of "bottleneck," perhaps known better to students or other aspirants as the "weeding out point." Once these bottlenecks are identified, it becomes more straightforward to succeed in the ultimate goal of career progression. In the professional curricula of medicine, veterinary medicine, and law, the initial bottleneck is highly visible; it is at the point of admission to the appropriate institution.

Although the well-known principle of supply-and-demand causes fluctuations, virtually every professional curriculum boasts many better-qualified applicants than are admitted annually. Most medical and law schools currently admit about 10% of applicants; those that are admitted usually succeed and progress on to graduation and subsequent professional careers. In contrast, most graduate curricula in the life sciences — and entomology is no exception — admit a higher proportion of qualified students who apply, even though all admitted might not receive financial support. What this means in practice is that a student with a reasonable undergraduate academic record will likely be accepted into a postgraduate program in the life sciences, including entomology.

The underlying reasons for this include, but are not limited to, the trend to measure productivity of academic curricula by easily calculated numbers such as students enrolled, graduated, and total student credit hours taught. A major pressure on most graduate life science faculties is to recruit and retain students. Coupled with this is the dependence of university research on the economical labor provided by highly motivated graduate students. After even a short period of enrollment, such students become quite valuable to both departments and advisors, in that they may have yet-unfinished research projects, done at considerable investment of resources, that will yield scientific publications (another easily counted measure of productivity) in the future. The pressure, therefore, trends toward retaining students who are making satisfactory progress.

Most of these students eventually earn their graduate degree. The point of admission and academic success, therefore, are not the principal bottlenecks in the entomology graduate career track. The major bottleneck occurs at the point of competition for the few professional positions available. A desirable faculty, governmental, or industry job may generate a hundred or more applications, and virtually all applicants will possess the requisite terminal degree. What separates the successful candidate from the rest is the remainder of his or her portfolio, including publications, involvement with grants (overhead-producing extramural support is another easily evaluated number), and ancillary professional involvement.

The bottleneck at the point of job competition is made more compelling when one notes that few professional positions exist for entomologists — and indeed most other life scientists — outside the "institution." Whether the institution in question is a college, university, government agency, or private corporation, the point is the same: there is little

professional life for the entomologist outside the framework of the employing entity. This is in direct contrast to the professional career tracks in medicine (including dentistry), veterinary medicine, and law. Those in the latter fields indeed have opportunities to compete for faculty, government, and industry positions, but they also have an option not typically available to entomologists: they can operate independently under their state-issued professional license. The "licensed" pest-control industry provides less opportunity for the graduate entomologist than one would think, because most of the actual control practice is conducted by technicians.

Taking all the above into account, one unexploited career path might include the M.D. degree and a subsequent residency in forensic pathology at an institution also offering a graduate program in forensic entomology. A collateral or consecutive doctoral degree in the latter area would give such an individual the requisite entomological credentials along with ready employability as a forensic pathologist.

Licensing and Quality Assurance

The issue of, and controversy about, professional licensing is not new to entomology, having been debated most enthusiastically since the advent of organic insecticides produced an easy analogy to prescription drugs in medicine (Hall and Hall, 1986). However, entomologists have been remarkably resistant to any sort of licensing effort (Perkins, 1982; Hall and Hall, 1986). There is a second "weeding out point" in the professional career tracks of physicians, veterinarians, and attorneys. This is the state licensing examination, which must be passed before one can practice medicine, veterinary medicine, or law. Far from being perfunctory or "ritual" exams, these tests serve as a major hurdle and by no means do all candidates pass, even though at the point of examination they may hold the terminal academic degree. As such professionals further specialize, there exists a vast array of board examinations in the medical field. The purpose for these excruciating tests is simple: quality assurance. As a society, we have agreed that we want those practicing medicine, veterinary medicine, and law to be fully qualified and to meet objective educational and performance minima. Curiously, we have not insisted on similar standards for other career fields, including medicocriminal entomology.

Perhaps the field of entomology is sufficiently arcane in and of itself to give anyone associated with it the aura of "expert," but in practice this is far from the truth. The fact that an individual possesses an earned Ph.D. with a major in entomology is no indication of competence in the medicocriminal field. Unfortunately, it is often no indication of general competence in entomology itself. The American Board of Forensic Entomology (ABFE) was formed to serve as a quality assurance tool in medicocriminal entomology work. Similar to other such boards associated with the American Academy of Forensic Sciences, the ABFE has educational and performance criteria that must be met before an applicant can be certified as Diplomate. An earned Ph.D. with a major in medical entomology is the educational minimum (similar to the general criterion for faculty appointments at the assistant professor level or higher), and applicants must demonstrate involvement with and contributions to both research and case work in medicocriminal entomology. Peer recommendations are solicited, and applicants are screened by committee and approved by the Board to sit for the certification examination. As with other professional certification bodies, a goal is to have annual continuing educational require-

ments for retention of certification. Because the ultimate application of medicocriminal entomology is in the context of the adversarial legal system, the ability to qualify as an expert witness is necessary to fulfill this professional function. As detailed in Chapter 14, expert testimony can be excluded if it can be shown that the witness offering it is unqualified. As more trial judges assume the role of "gatekeeper" under Daubert* standards, and as more pretrial motions to exclude are filed, there will assuredly be closer scrutiny of the entomologist's professional credentials. Membership in the ABFE is intended to reflect the professional status of fully qualified medicocriminal entomologists and the membership list of that organization is a good place to start when such expertise is needed. A current list of ABFE members is available on the Internet.

Development of Medicocriminal Entomology

The earliest record of medicocriminal entomology comes from 13th century China. The Chinese criminalist Sung Tz'u was recorded in the seminal *Washing Away of Wrongs* (translated by McKnight, 1981) to have investigated a murder-by-slashing in a village. When all villagers were required to bring their scythes to one spot, he noted that flies congregated on only one, ostensibly because of minute traces of blood and other tissue. Confronted by this evidence, the guilty villager reportedly confessed. In addition to concluding this earliest known case, Sung Tz'u also recorded the rapid appearance of maggots on a decedent during the warm season, and the potential utility of maggot infestation in recognizing antemortem wounds.

It was not until the mid-1800s that medicocriminal entomology saw recorded use in the West. Bergeret (1855) investigated the death of an infant near Paris, France, where the corpse had been discovered behind a mantle. By evaluating the insect fauna on the mummified remains, he concluded — perhaps incorrectly (see Greenberg, 1991) — that the baby had been dead about 2 years, thus exonerating the current inhabitants of the house who had resided there a shorter time. Despite any putative errors, this case represents the first application of insect succession data in forensic entomology and paved the way for later studies cited in Nuorteva (1977), Keh (1985), Smith (1986), and Catts and Goff (1992). J. P. Megnin is usually credited with focusing Western attention on the forensic utility of entomology, especially with publication of his famous capstone work, *Fauna of Cadavers* (Megnin, 1894). The eight stages of human decomposition described therein, which were followed by Leclercq (1969) and Easton and Smith (1970), and the insects associated with them, have perhaps served as much as an obstacle to understanding the entomology-associated decay process as illuminative of it (see Greenberg, 1991). An analysis of 11 insect faunal succession studies suggested that the phenomenon represented a continuum of decay rather than the tidy "seres" described by Megnin (Schoenly and Reid, 1987). This area continues as an active field of research in forensic entomology, and computer technology has been incorporated (Byrd, 1998; Schoenly et al., 1992). Perhaps the most frequent forensic use of successional insect colonization of corpses and cadavers has been in the Hawaiian Islands, likely because of the climatic stability and faunal predictability found there (see Goff et al., 1986; Goff et al., 1988; Goff and Flynn, 1991). Researchers in this geographic area also noted the possible effects of certain illicit drugs on fly development

* *Daubert v. Merrell Dow Pharmaceuticals, Inc.,* 113 S.Ct. 2786, 61 U.S.L.W. 4805 (1993).

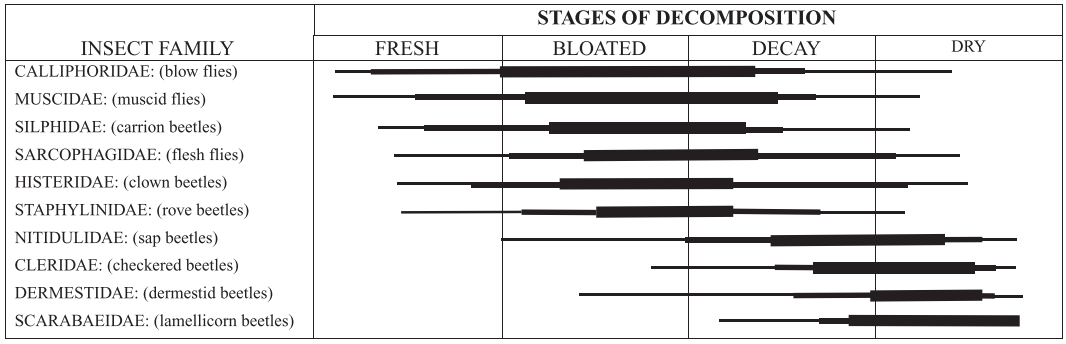
(Goff et al., 1989; Goff et al., 1991). Goff and Lord provide an extensive treatment of entomotoxicology in Chapter 11.

The foundation necessary for reliable application of forensic entomology was laid during the first half of the 20th century by taxonomists interested in those insect species of medicocriminal importance. These include two principal families: the Sarcophagidae (or "flesh flies," which is not descriptive because most sarcophagid species are not necrophilous) and the Calliphoridae ("bottle flies" or "blow flies"). The latter family contains the species of greatest medicocriminal interest. When J. M. Aldrich applied Boettcher's concepts regarding distinctive insect male genitalia to specific determinations of the flesh flies (Aldrich, 1916), he for the first time enabled reliable species identification of this forensically significant family. The *Blowflies of North America* (Hall, 1948) followed Aldrich's lead, was the first monographic treatment of Nearctic Calliphoridae, and laid the foundation for modern work in North American medicocriminal entomology. Hall chose this family at least in part as a response to requests by the FBI and Washington Metropolitan Police regarding the utility of insect evidence (Hall and Townsend, 1977). This monograph remains the standard reference work on American blow flies, although changes in nomenclature have been proposed (Shewell, 1987) and considerable work has been accomplished on the immature forms (e.g., Knipling, 1936, 1939; Liu and Greenberg, 1989; Greenberg and Singh, 1995).

Early research on immatures proceeded by collecting living female flies and garnering their eggs or larvae, which were then divided into subsamples with some preserved and described, and others reared to adults. When adult specimens included males that could be identified by their genitalia, the circle was complete. More recent work has employed scanning electron microscopy (Liu and Greenberg, 1989) and as continually refined tools are employed in taxonomic research it can be anticipated that more accurate identifications will be possible. In the future, molecular techniques employing DNA sequencing and polymerase chain reaction may facilitate identification of the immature stages of flies including eggs and early instars (for example, see Gleeson and Sarre, 1997; Roehrdanz and Johnson, 1996; Sperling et al., 1994; Stevens and Wall, 1997; Wallman and Adams, 1997; Wells and Sperling, in press). Sophisticated statistical analyses have been used to facilitate understanding of the relationships between blow fly species (Stevens and Wall, 1996).

In addition, several species of blow flies, heretofore confined to the Old World, have recently become established in the Americas (Baumgartner and Greenberg, 1984; Richard and Ahrens, 1983; Wells et al., 1999). Of special interest is *C. rufifacies*, the invading "hairy maggot blow fly," first noted in southern areas of the U.S. but recently established in the Knoxville, TN region (S. A. Shahid, personal communication) and which has been collected as far north as East Lansing, MI (R. W. Merritt, personal communication). Attention has been paid to the potential effect of such exotic species on the indigenous blow fly fauna (Wells and Greenberg, 1992).

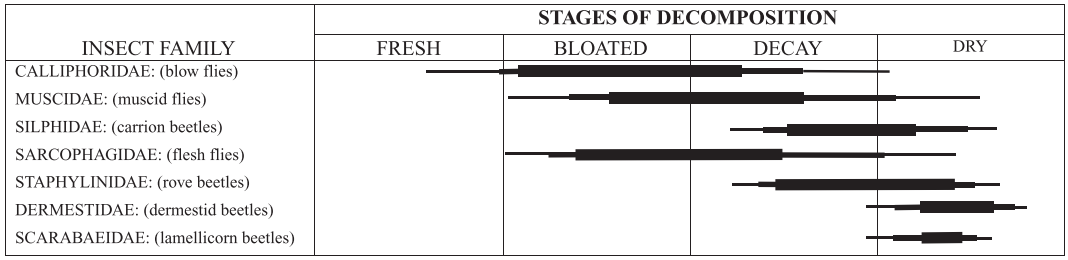
As mentioned earlier, the principal methodology used in medicocriminal entomology is application of the temperature-dependent development of insects, especially flies, in estimating a decedent's PMI. Other things being equal, insects (as poikilothermic animals) develop faster as temperatures increase over a thermal minimum threshold up to a lethal maximum (described by the well-known "S-shaped" biological curve) (Andrewartha and Birch, 1973; Davidson, 1944). Laboratory rearing of forensically important species at constant temperature (Kamal, 1958) permits calculation of the number of accumulated thermal units (such as degree-days or degree-hours) necessary for the insect to progress



*Each stage of decomposition is given the same amount of space in this table.

Indicates a small number of individuals present.
Indicates a moderate number of individuals present.
Indicates a large number of individuals present.

(a)



*Each stage of decomposition is given the same amount of space in this table.

Indicates a small number of individuals present.
Indicates a moderate number of individuals present.
Indicates a large number of individuals present.

(b)

Figures I.6a,b (a) Succession of adult arthropods on human cadavers in east Tennessee (during spring and summer). (b) Succession of arthropod larvae on human cadavers in east Tennessee (during spring and summer). (Adapted from Rodriguez and Bass, 1983. © ASTM. With permission.)

from the stage deposited on the decedent to the stage collected. Granted that the season is amenable to fly development and that flies had access to the decedent, the time required for such development under appropriate field conditions constitutes the minimum PMI. Confounding factors include delayed oviposition because the decedent was protected from flies (for example, in an automobile trunk, inside a house, or wrapped in plastic) or any phenomenon that might alter the thermal regimen, such as "maggot mass" effects (Greenberg, 1991). Because retrospective and generally remote temperature data are used for most medicocriminal entomology analyses, there are many other factors that may complicate the analysis.

Although a wide variety of food substrates have been used for this sort of research, especially beef or pork liver (Hall, 1948) or small animals (e.g., Payne, 1965), the ultimate medicocriminal application is human tissue. Establishment of the Anthropological Research Facility (ARF) in Knoxville, TN permitted research on insect colonization and succession on human corpses (Figures I.6a,b) (Rodriguez and Bass, 1983) and validated the use of swine cadavers as surrogates in other areas where research on human remains

is illegal. One objection voiced to ARF-generated data was theoretical "faunal enrichment" at that site, where "local populations of carrion insects are concentrated and abundant" (Catts and Goff, 1992). Comparisons of decomposition measured on replicate swine carcasses at the ADF with that at three progressively remote sites in the Knoxville area failed to substantiate differences in decay rates during 1998 (S. A. Shahid and N. H. Haskell, personal communication). Similarly, research on oviposition habits of blow flies has been conducted to test the general assertion that these insects usually do not lay eggs after dark (Greenberg, 1990; Hall, 1948; Nuorteva, 1977; Tessmer et al., 1995). Initial results in Missouri confirm that blow fly egg-laying ceases during hours of darkness in rural sites with no artificial lighting (C. Hempel, personal communication). The effect of time since death on resultant attractiveness of a carcass to blow and flesh flies also has been evaluated by Hall and Doisy (1993).

Current Perceptions

The expanding literature in medicocriminal entomology is still small by comparison to many other fields of science. A bibliography published in 1985 listed only 329 references through 1983 (Vincent et al., 1985). To supplement the one textbook on the subject (Smith, 1986), a guide was published to facilitate acquisition of entomological evidence by crime scene personnel (Catts and Haskell, 1990). Because of the increased use of medicocriminal entomology evidence in litigation, the subject was included in the 1995 iteration of *Forensic Sciences*, which contains an analysis of the substantive literature (Hall and Haskell, 1995). There are two recent review articles on medicocriminal entomology (Catts and Goff, 1992; Keh, 1985). The practical utility of insects as forensic indicators was recognized by Nuorteva, whose published case histories were largely responsible for rekindling interest in medicocriminal entomology during the 1970s (see Nuorteva, 1974; Nuorteva et al., 1967, 1974).

A commonly voiced concern regarding medicocriminal entomology is that it seldom links any particular suspect or defendant with a crime, providing instead mainly inferential data on postmortem interval. Occasionally, however, the science is able to provide incriminating evidence in the former sense. In one case, the presence of chigger bites on a murder suspect was used to link him to the crime scene (Lord, 1990). Molecular technology may in the future permit analysis of arthropod gut contents, with ingested tissue such as blood (from blood-feeding arthropods) or semen (in maggot intestinal tracts) possibly linking a suspect to a particular decedent or locale (see Introna et al., 1999).

Conclusions

Medicocriminal entomology is one of three areas in the broad field of entomology that routinely is involved in forensic applications. Although litigation involving urban entomology and stored products entomology typically occurs, it is the area of medicocriminal entomology that is directly utilized by law enforcement agencies in death investigations. Insect evidence can be paramount in establishing an accurate postmortem interval for a decedent, as well as providing additional information to those investigators who are capable of deciphering the entomological clues.

Insect attraction to and interaction with human remains has been known, and even used, for centuries, yet medicocriminal entomology is still considered to be in its infancy. The scientific literature available on this topic, although constantly growing, remains small when compared to the areas of entomology that deal with agriculture or disease vectors. Likewise, the number of qualified practicing forensic entomologists capable of fully utilizing insect evidence is currently very small.

Medicocriminal entomology has reached an exciting stage in its evolution as testimony based on the interpretation of insect evidence is now routinely provided in court by expert witnesses. The establishment of the ABFE is an attempt to provide courts and others with quality assurance regarding the individuals presenting such testimony. The increased acceptance and recognition of medicocriminal entomology as a forensic discipline, coupled with the increased reliance of courts on biological evidence, shall continue to present increased opportunities for qualified forensic entomologists. The availability of an accurate PMI can be responsible for the overall direction of an investigation, and the interpretation of entomological evidence may eventually be the deciding factor in the determination of guilt or innocence in a court of law.

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General Entomology and Arthropod Biology

1

JAMES L. CASTNER

Contents

Introduction

Arthropods are those invertebrate animals with jointed legs, including the insects, arachnids, centipedes, millipedes, and crustaceans. One of the largest groups of arthropods, the insects, is differentiated by having six legs and a body divided into three regions. The study of insects is termed entomology. It is good crime scene protocol to have an entomologist with forensic specialization as a member of the forensic science team, and that they be consulted for the accurate identification and interpretation of insect evidence. However, a degree of familiarity with basic insect anatomy, development, and behavior is extremely useful to all death scene investigators in order to understand the ecological roles insects play and to fully appreciate their forensic value in investigations. For example, the pupal shells of flies can easily be overlooked or mistaken for rodent droppings at a crime scene. Postmortem feeding by fire ants or cockroaches on a body may damage the tissue and produce artifacts similar in appearance to chemical scarring to the untrained eye. Knowledge of general entomology and basic arthropod biology is essential for the accurate interpretation of insect evidence. The ability to recognize which arthropods need to be collected as forensic evidence at a death scene is an invaluable skill and will be discussed in Chapter 2.

Insects are the most numerous and diverse organisms on the planet (Figure 1.1). While less than a million species have been described and named, research indicates that as many as 3 to 30 million may actually exist. They are found in almost all terrestrial habitats and in most aquatic ones as well, except for salt water. As a group, insects have evolved the presence of wings, a feature that distinguishes them from all other invertebrates. This enables them to travel considerable distances when foraging for food or attempting to locate a suitable habitat for laying their eggs. This is an extremely important factor in species of forensic importance that must quickly locate and utilize temporary resources such as carrion.

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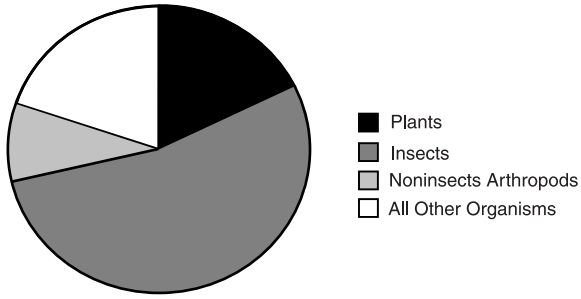

Figure 1.1 Insects comprise more than half of all the living species on Earth, exceeding all other groups of organisms combined. (Figure courtesy of Jason H. Byrd.)

Two major groups of insects are predictably attracted to cadavers and provide the majority of information in forensic investigations. They are the beetles and the flies. Both groups are diverse, although the beetles have far more known species than any animal group, insect or otherwise. There are approximately 300,000 described species in the world, of which 30,000 species occur in North America. Estimates of total beetle species in the world are at least 750,000, and they currently compose over one third of all known insects. Flies are much less numerous with only 86,000 described species worldwide, of which 16,300 occur in North America.

Many other types of arthropods are found in association with bodies, but these are typically opportunistic feeders taking advantage of the circumstances. They are made up of both scavengers feeding on decaying material and predators feeding on the species that have colonized the carrion. The beetles and flies have distinctive external structures and develop in significantly different ways. These body features and developmental cycles shall be discussed in the remainder of this chapter.

External Anatomy

When we look at insects, we see an almost bewildering array of shapes, sizes, and colors. However, all insects have evolved from a common ancestral form and retain certain diagnostic features about their external anatomy. Scientists believe that the insect predecessor was elongated, roughly cylindrical, and segmented with paired appendages on each segment. Through time, certain segments grouped together into functional regions on the insect's body. Three such regions have resulted. They are represented on the insect body by the head, thorax, and abdomen.

The body wall of an insect is called its exoskeleton and serves two functions. One is providing points of attachment for the muscles and the other is protection by means of the hard outer layer of the exoskeleton called the cuticle. This durable cuticle results in the bodies of insects remaining in the environment for extended periods of time. In this way, an insect can serve as forensic evidence long after the organism itself is dead. Fragments and parts of dead insects have been used successfully to link suspects to a crime scene or victim in numerous cases.

The insect skin is not one continuous hard shell, but rather is composed of a number of hardened plates. These plates (or sclerites) are separated from one another by seams or sutures, and by larger membranous areas such as between body segments (Figures 1.2a,b,c).

Figure 1.2a The bodies of insects must have the flexibility to expand. The membranous portion of this mosquito's abdomen is visible as a narrow region between the white spots on the top and bottom portions of the body. (Photo courtesy of James L. Castner.)

Figure 1.2b The same mosquito after finishing its blood meal shows a greatly distended abdomen where the membranous area has expanded. (Photo courtesy of James L. Castner.)

Figure 1.2c Hardened plates or sclerites are clearly visible as small darker areas with various shapes on the back of this carrion beetle larva. (Photo courtesy of James L. Castner.)

The degree of hardness varies. Extremely hard areas of the insect such as the jaws, or the wing covers in most beetles, are said to be heavily sclerotized. The more lightly sclerotized or membranous areas of the body permit the insect a degree of flexibility and greater range of movement.

A species' biology and behavior will greatly affect its morphology, including whether it is hard- or soft-bodied. The exoskeleton of a scarab beetle can be incredibly hard, making them difficult to prepare for display. Yet insects that live in protected situations (such as internal parasites) or the immature forms of bees, wasps, and ants may be completely soft and vulnerable. Even the body of the scarab beetle is soft when it is an immature grub underground. Thus, a great difference may exist from one life stage of an insect to the next.

Body Regions

As previously mentioned, through the course of evolution the segments of insects have fused to form three body regions: the head, thorax, and abdomen (Figures 1.3a,b). Each of these regions has specialized external and internal structures that perform certain functions. The head is the main area of sensory perception and the point of ingestion. The thorax is located directly behind the head and contains the segments with the legs and wings on adult insects. It is primarily responsible for locomotion. The abdomen is the

Figure 1.3a A queen carpenter ant clearly shows the three body regions of an insect: the head, the thorax, and the abdomen. (Photo courtesy of James L. Castner.)

Figure 1.3b A dorsal view of a blow fly allows the head, thorax, and abdomen to be easily identified. (Photo courtesy of James L. Castner.)

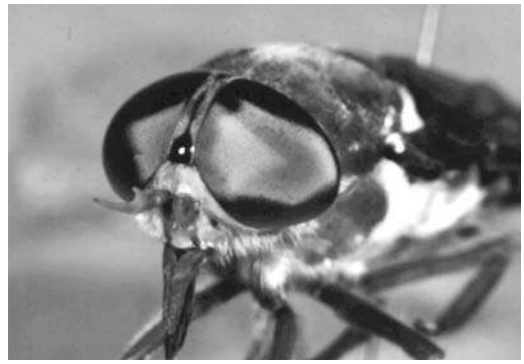

Figure 1.4a Compound eyes may make up the majority of the head in flying insects such as this horse fly. The individual facets are barely discernible at this level of magnification. (Photo courtesy of James L. Castner.)

Figure 1.4b The large red compound eyes of this oriental latrine fly are a helpful character in field identification. (Photo courtesy of James L. Castner.)

hindmost region and follows the thorax. It contains the genitalia, as well as other specialized external structures. Internally it contains many of the essential body systems.

The head of most adult insects is a hardened capsule. Internally it contains the brain, musculature for the mandibles and mouthparts, and structural supports. Its most noticeable external features are the eyes, antennae, and mouthparts. All of these are important characters used in the identification of insects.

Insects have evolved various mechanisms for processing the light information from the environment in which they live. In predatory insects, as in the beetles and adult flies that are associated with carrion, vision may be extremely acute. In other species that spend the majority of their life in the absence of light (such as underground beetle grubs) or that live immersed in their food source (such as the maggots of carrion flies), vision may be totally lacking. One of the most prominent features of the insect head is a pair of multifaceted compound eyes. In predators such as mantids or highly agile flying insects like dragonflies and horse flies, the compound eyes may make up the majority of the head (Figures 1.4a,b). The compound eye itself is made up of facets which may number from only a few to as many as several thousand. The shape of the compound eyes, their location on the head, and whether or not they touch are all characters sometimes used in identification.

Figure 1.5 Longhorned woodboring beetles (family Cerambycidae) get their common name from their extremely long antennae. (Photo courtesy of James L. Castner.)

There also may be from zero to three simple eyes found on the head of adult insects. These ocelli are composed merely of a single facet and can only detect changes in light intensity. Externally they appear like small jewels embedded in the surface of the insect head, often amber in color. Simple eyes or photoreceptors are found on the head of certain larval insects as well as on the adults of more primitive insect groups.

The antennae or "feelers" are often the most noticeable appendages on the insect head. There is a great variety of shapes and sizes from the long thread-like antennae of katydids that may be twice the body length, to the tiny bristle-like antennae of dragonflies that could be easily overlooked. The antenna type is one of the key taxonomic features that enable us to identify insects down to the family level.

Insect antennae are sensory organs and they are covered with chemical receptors that allow the insect to evaluate its environment. While all antennae serve the same basic function, a diversity of physical appearances and shapes have evolved throughout the insect world. For example, the antennae of beetles are usually long and obvious, composed of 10 to 20 individual segments (Figure 1.5). In the carrion beetles (family Silphidae), the tip of the antenna is enlarged into a broadened club. This antenna type is called clavate (Figure 1.6). A variation on this clubbed antenna is also found in the scarab beetles (family Scarabaeidae), another group often collected at remains. The terminal antenna segments

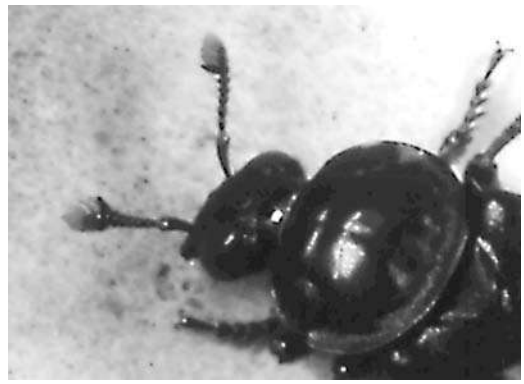

Figure 1.6 The expanded terminal segments of a carrion beetle's antennae form a club. This type of antenna is termed clavate. (Photo courtesy of James L. Castner.)

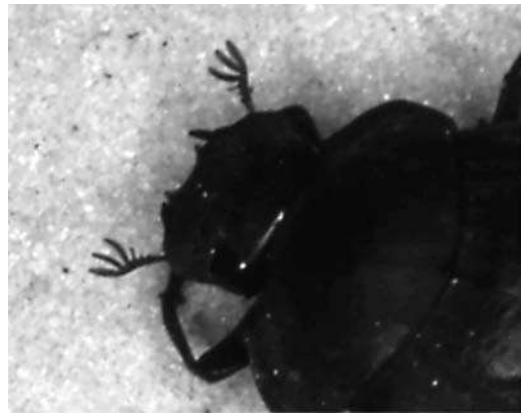

Figure 1.7 The lamellate antennae of this dung beetle have flattened parallel segments at the tip. These segments are shown widely spread in this photo, but they also can be held tightly together. (Photo courtesy of James L. Castner.)

that form the "club" of scarab beetles are enlarged parallel plates that stick out perpendicular to the rest of the antenna. This antennae structure is called lamellate (Figure 1.7).

Flies have very different antennae from beetles. Three of the most important groups of flies (blow flies, flesh flies, and house flies) attracted to human remains have an antenna type called aristate (Figure 1.8). This antenna is composed of three large, wide segments, the outermost of which has a long, slender hair protruding from it. This hair is called the arista. The antennae of these flies are much smaller in proportion than the beetle antennae discussed. They are difficult to see with the naked eye and must be viewed with a microscope. The twelve most common insect antennae types are listed and described in the Glossary at the end of the chapter.

The other significant features of the head are the mouthparts. In some species that do not feed as adults, these may be vestigial or absent. However, most adult insects will have distinctive mouthparts whose shape and morphology are indicative of the type of food it consumes. The chewing mouthtype is the most primitive and the most commonly observed

Figure 1.8 Aristate antennae are found on many flies of forensic importance. The name derives from a hair called an arista, which is found on the last antennal segment. The arista shown above is lined with many smaller hairs and, therefore, termed plumose. (Photo courtesy of James L. Castner.)

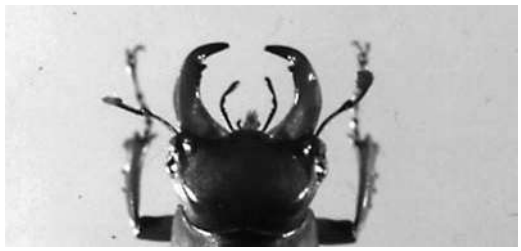

Figure 1.9 The large mandibles of this stag beetle are indicative of the chewing insect mouthtype. (Photo courtesy of James L. Castner.)

in the insect world (Figure 1.9). It is found on nearly all of the adult beetles of forensic importance, as well as the majority of larval insects associated with carrion. The chewing mouthtype also is called mandibulate, due to the presence of mandibles.

The mandibles are paired structures and occur on both sides of the mouth and meet in the middle. They are responsible for tearing or biting off chunks of food and grinding or chewing it to a consistency that the insect can ingest. The darkest portions of the mandibles are the hardest or most heavily sclerotized. Looking at a mandible under the microscope will show areas that are sharp for cutting and others that are blunt for grinding. These areas are comparable to the incisors and molars of humans.

Many groups of insects have chewing mouthparts similar to those described above. In some groups, however, slight modifications of the design have occurred. Fly larvae or maggots have paired, hardened mouth hooks that tear and cut into the carrion upon which they feed (Figures 1.10a,b). These mouth hooks often have a distinctive shape and can be used in the identification of fly larvae to species. Adaptations that allowed one insect group to exploit a food source not used by the majority of other groups would have decreased competition and given a survival advantage. Thus, we see a variety of mouthtypes that enable insects to feed on different kinds of food in vastly different ways.

Many insects feed on liquid rather than solid food. The nourishing liquid may take the form of nectar from flowers, or plant juices withdrawn directly from plugging in to the plant's vascular system, or even blood that is sucked out of an animal's vascular system.

Figure 1.10a The tiny mouth hooks of this secondary screwworm fly larva provide it with the tools for feeding on flesh. This scanning electron micrograph shows the head of the maggot 80× life size. (Photo courtesy of Jason H. Byrd.)

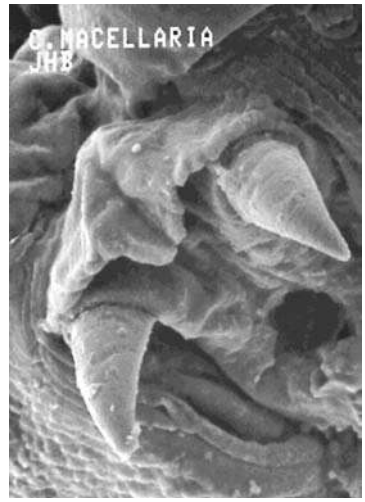

Figure 1.10b The paired mouth hooks of maggots are distinct and can be used with other larval characters to make a species identification. This scanning electron micrograph shows the mouth hooks at 350× life size. (Photo courtesy of Jason H. Byrd.)

A piercing-sucking mouthtype is often responsible for the removal of a liquid food source and the mouthparts themselves are generally referred to as a beak (Figure 1.11). These beaks can be formidable as evidenced by the predatory assassin bug that feeds on other insects. Such bugs are sometimes found preying on the insects that have colonized a body. An intermediate-sized beak that most of us are all too familiar with is found on mosquitoes.

The majority of species of flies attracted to human remains have a sponging mouthtype (Figures 1.12a,b). Instead of a piercing beak, the mouthparts have evolved into a mechanism that permits the noninvasive sucking of liquids. In most sponging mouthtypes, the tip consists of a fleshy lobe or lobes that are crossed with grooves. Liquid is drawn into these grooves via capillary action and then on up into a food channel. The secretion of

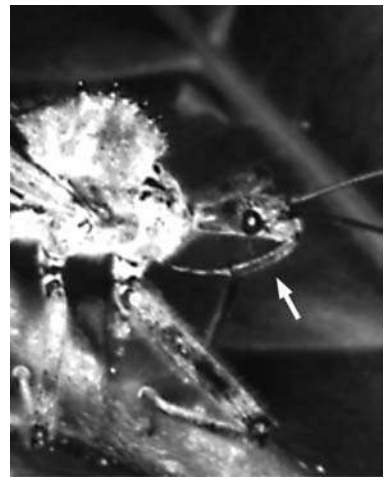

Figure 1.11 The wheel bug is a common predatory insect. Beneath its head is a stout, curving, three-segmented beak that exemplifies a piercing–sucking mouthtype. (Photo courtesy of James L. Castner.)

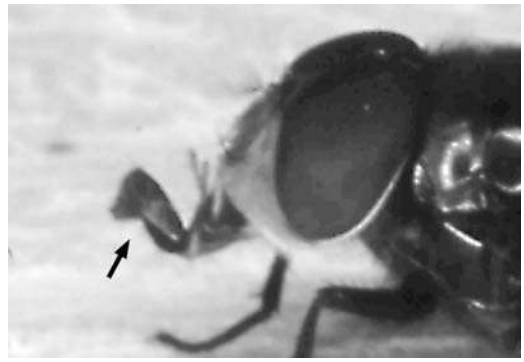

Figure 1.12a The sponging mouthparts of a fly are usually kept retracted and close to the head unless it is actually feeding. Here, the mouthparts of an oriental latrine fly are extended. (Photo courtesy of James L. Castner.)

Figure 1.12b A bronzebottle fly extends its proboscis to sponge up liquid on the surface of a rotting fruit. (Photo courtesy of James L. Castner.)

saliva by the fly onto the food material helps to break it down and liquefy it so that it can be sucked up easily.

Other mouthtypes exist and are often adapted for special diets. Variations on the sponging mouthtype are found in a number of fly species that suck blood. Butterflies and moths have a tongue that is a long, coiled tube that functions like a straw (Figure 1.13). The honey bee has an unusual combination of chewing–lapping mouthtypes. It can suck nectar through its tongue, but also has mandibles that are used for molding wax and chewing its way out of its pupal case. The larval stages of lacewings and antlions have long, grooved, sickle-like jaws. They impale their victims and feed on the body juices that flow down the grooved mandibles.

Directly behind the head, we encounter a region composed of three fused segments called the thorax. The obvious external structures (legs and wings) found associated with the thorax of most adult insects make it clear that it is the center of locomotion. The individual segments making up the thorax, from front to back, have been named the prothorax, mesothorax, and metathorax. The prefixes pro-, meso-, and meta- are commonly used when discussing thoracic structures to specifically identify the segment on which they are located (Figure 1.14). Therefore, a leg on the second thoracic segment would be a mesothoracic leg. A wing on the third and last thoracic segment would be a metathoracic wing.

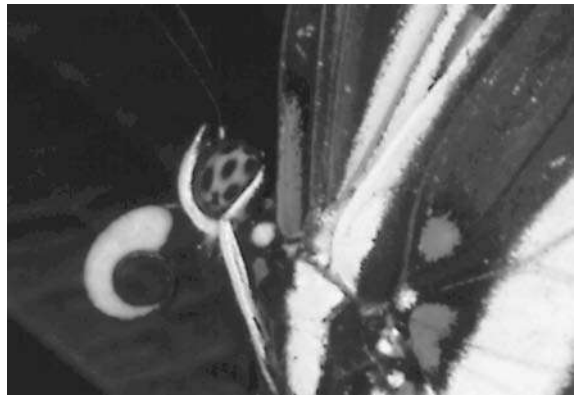

Figure 1.13 The tongue of butterflies and moths can be uncoiled and extended to reach the nectar in flowers. The coiled mouthparts of this heliconian butterfly are covered with pollen. (Photo courtesy of James L. Castner.)

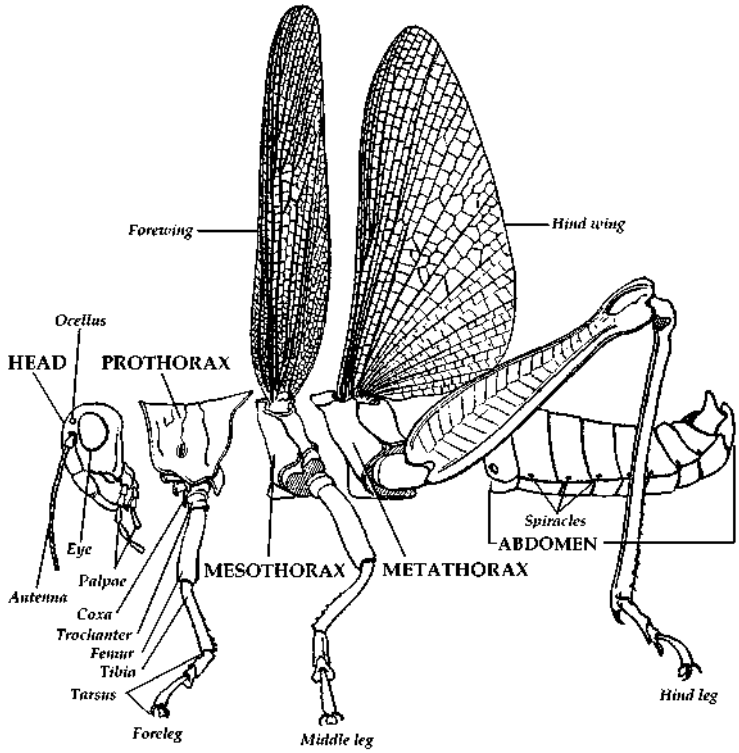

Figure 1.14 This diagrammatic illustration shows the prothorax, mesothorax, and metathroax of an insect. (Figure courtesy of the U.S. Department of Agriculture.)

Figure 1.15 The predatory praying mantis catches its prey by grasping. The raptorial front legs are covered with sharp spines to make such captures easier. (Photo courtesy of James L. Castner.)

The thoracic region is usually heavily sclerotized to provide support and bracing for the movement of the legs and wings. The top portion of each thoracic segment is called a notum. The pronotum is often prominent and conspicuous, and in insects like beetles and cockroaches it may cover the entire top part of the thorax.

Most adult insects have six legs, which is why the current Class Insecta was formerly called the Class Hexapoda. One pair of legs originates from each thoracic segment. The typical adult insect leg has five major parts or segments. The femur and tibia are usually the longest and most evident portions. The terminal portion of the leg is the tarsus, which may be composed of from one to five segments. The last tarsal segment often has a tarsal claw.

An insect living underground that is forced to contend with moving through a heavy medium such as soil or sand will show morphological adaptations extremely different from species that run on the soil surface. Insects living in an aquatic environment will exhibit anatomical structures that facilitate living in or under water. While the insect body, as a whole, will reflect modifications for living within a certain physical habitat, some of the most severe and noticeable morphological changes have occurred on the legs.

Raptorial legs are characterized by sharp teeth or spines that impale and cling to prey organisms. The praying mantis is probably the most commonly recognized insect with raptorial legs (Figure 1.15), but variations on this leg type are seen throughout the insect world. Many hunting species have evolved legs for grasping prey, including assassin bugs, ambush bugs, diving beetles, and dragonflies. Life underground or beneath the leaf litter is termed fossorial, as is the leg type evolved for locomotion and movement underground. The front or prothoracic legs are usually the most affected. Shoveling through the soil requires wide, flattened structures for scooping and moving earth (Figure 1.16). Some of the larger beetles found with bodies, such as species of sexton and scarab beetles, have exactly these types of adaptations on their front legs. Other insect leg types include cursorial (evidenced by cockroaches and used for running), saltatorial (bulky and used for jumping as in grasshoppers), and natatorial (flattened and used for swimming) (Figure 1.17).

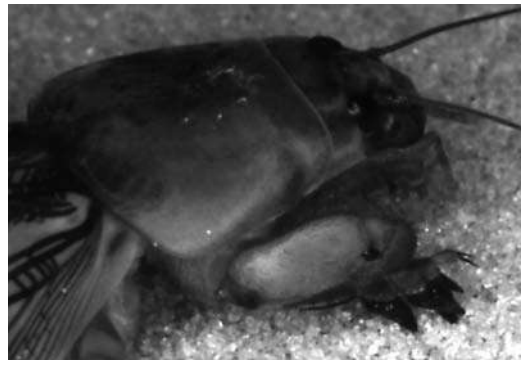

Figure 1.16 The front leg of a mole cricket possesses large digging claws well adapted for a subterranean existence. (Photo courtesy of James L. Castner.)

The final structures associated with the thorax that we shall discuss are the wings. An important feature in the success of insects as a group, functional wings are almost always found only on adult insects. However, not all insects have wings. Some of the most primitive groups are "apterous" or wingless. Other more advanced groups of insects have "lost" their wings evolutionarily as a survival adaptation to a specialized way of life. For example, both the fleas and the sucking lice are apterous, probably because wings would be a hindrance to their movements among the fur and between the hairs of their hosts. Most winged insects have two pairs of wings. Some only have one pair of wings (flies), while in others winged individuals serve a reproductive purpose and are produced only at certain times of the year (ants and termites).

Dark thickened "lines" appear to radiate out and crisscross throughout the wing (Figures 1.18a,b). These are wing veins and are very important characters for identification in some groups. In other groups, venation is greatly reduced or almost nonexistent. The veins connect to the insect body and circulatory system. The movement of blood through these veins is essential in expanding the wings to their full size after the insect has shed its skin for the last time.

Adult flies are unique in having a single pair of wings. The hindwings are absent and each has been replaced by a small, knob-like structure called a haltere (Figure 1.19). In

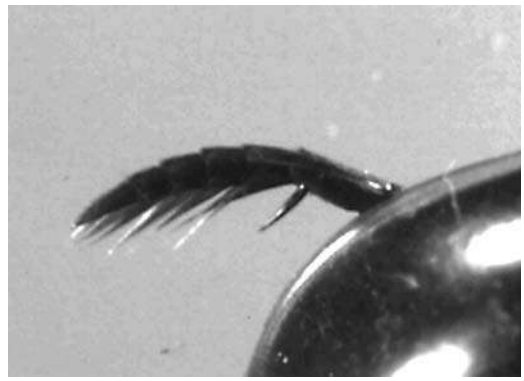

Figure 1.17 The hind leg of this aquatic beetle is covered with long stiff hairs. These increase its surface area and allow the leg to function as an oar in propelling the beetle through the water. (Photo courtesy of James L. Castner.)

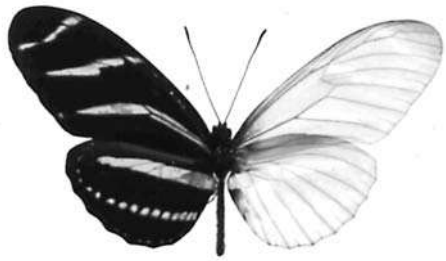

Figure 1.18a The wings of butterflies are usually covered with scales that obscure the venation. Scales have been removed from the wings on the right side of this butterfly to show the underlying wing veins. (Photo courtesy of James L. Castner.)

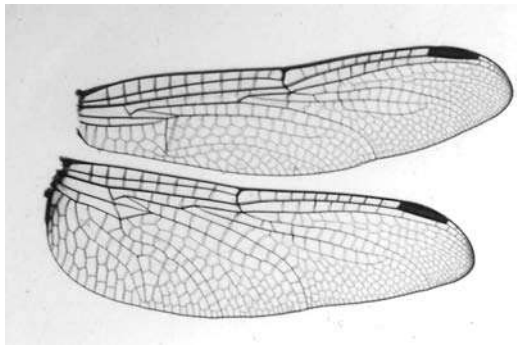

Figure 1.18b Wing veins are easily visible on the wings of a dragonfly. This reticulate venation strengthens the wings in these active flyers. (Photo courtesy of James L. Castner.)

Figure 1.19 This papaya fruit fly is positioned so that its left haltere is clearly visible as a small yellow knob between the base of the wing and the abdomen. (Photo courtesy of James L. Castner.)

Figure 1.20 A scarab beetle shows the hard, brown wing covers called elytra, which normally conceal the larger membranous rear wings used for flying. (Photo courtesy of James L. Castner.)

beetles, the forewings have undergone extreme modification into hard, shell-like covers that protect the membranous hindwings that remain folded beneath (except during flight). These hardened forewings are called elytra, and their color, shape, and texture can be very important in species identification (Figure 1.20).

The abdomen is the third and final region and forms the posterior portion of the insect's body. It contains organs whose functions deal mainly with reproduction, digestion, circulation, and respiration. There are typically 11 abdominal segments although a reduction in number has occurred in some groups. Each segment appears to be divided into a top and bottom plate with a membranous area between. This membranous intersegmental area permits flexibility and expansion or extension of the body (Figure 1.21).

One of the most obvious abdominal features in most insects occurs at the tip of the abdomen. These are the paired, feeler-like structures called cerci (Figure 1.22). The cerci vary in size among different groups of insects, and sometimes are not present. They appear to be sensory organs that are useful in detecting vibrations and disturbances in air currents. However, in some insects like the earwigs, they have evolved into sclerotized pincers that are used for both defense and prey capture (Figure 1.23). The cerci of silverfish are extremely long and tail-like.

Another feature found at the tip of the abdomen, but only in female insects, is the ovipositor. The female uses this structure when laying her eggs. It allows for careful and

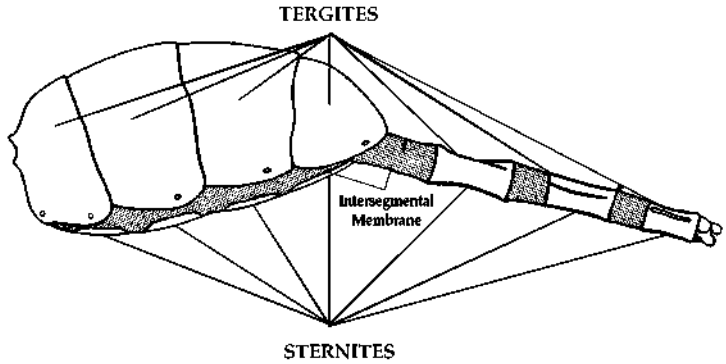

Figure 1.21 The flexible intersegmental membrane between the insect's sclerites allows for flexibility and movement. (From Contemporary Publishing Company of Raleigh, Inc. With permission.)

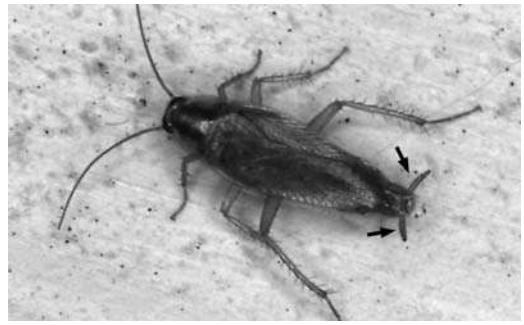

Figure 1.22 A pair of segmented cerci protrude from the tip of the abdomen of this German cockroach, looking like small thick antennae. (Photo courtesy of James L. Castner.)

exact placement of the eggs, and sometimes provides the mechanical means for introducing the eggs into a specific material such as the ground or a plant stem. It may be large and obvious as in some katydids, or it may be withdrawn and hidden within the body as in female house flies and blow flies (Figure 1.24a). The latter type often has segments that telescope one within the other. Some insects have extremely long ovipositors used to deposit eggs in other insects, plants, or soil (Figures 1.24b,c). Other wasps and bees have ovipositors that are modified for defense. These ovipositors are called stingers and can be extruded to puncture intruders, often with the injection of venom. Female honey bees, bumble bees, yellowjackets, paper wasps, and hornets protect themselves and their nests in this way.

A series of small oval spots, one pair per segment, also may be present along each side of the abdomen. They are easily visible on most caterpillars and are called spiracles (Figures 1.25a,b). They represent the external openings of the insect's respiratory system. The spiracles connect to tiny tubes that get progressively smaller as they ramify throughout the insect's body (Figure 1.26). By coordinating muscular movement with the opening and closing of the spiracles, the insect can effectively ventilate air through its body. Maggots have a thoracic spiracle on each side of the body as well as a pair of posterior spiracles at the tip of the abdomen (Figures 1.27a,b). The shape and coloration of these structures are useful in determining the species.

Figure 1.23 The cerci found at the tip of an earwig's abdomen are pincer-like. They are used both in defense and to help catch food items. (Photo courtesy of James L. Castner.)

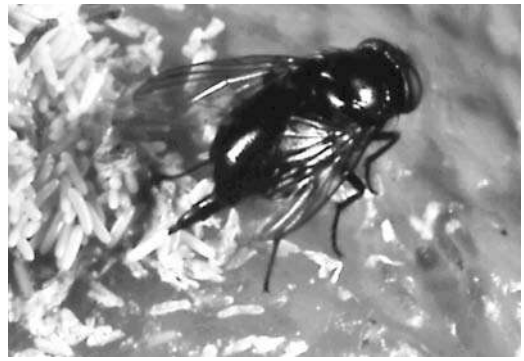

Figure 1.24a A greenbottle fly adds an egg to an existing mass. The narrowing tip of her abdomen serves as an ovipositor. (Photo courtesy of James L. Castner.)

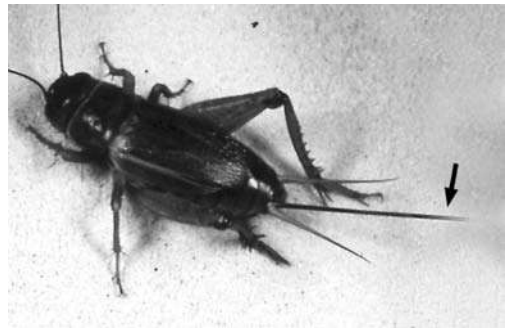

Figure 1.24b The ovipositor of a field cricket is used to place her eggs in the soil. It is long and needle-like, extending straight back from the abdomen between the long slender cerci. (Photo courtesy of James L. Castner.)

Figure 1.24c The female papaya fruit fly uses her ovipositor to penetrate the skin of fruits to deposit her eggs in the center. (Photo courtesy of James L. Castner.)

Figure 1.25a The spiracles of the tobacco hornworm caterpillar are visible here as eight white-rimmed oval black spots along the side of the body. (Photo courtesy of James L. Castner.)

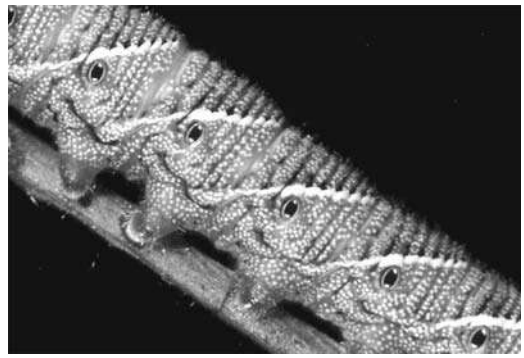

Figure 1.25b A close-up view of the spiracles of a tobacco hornworm caterpillar. (Photo courtesy of James L. Castner.)

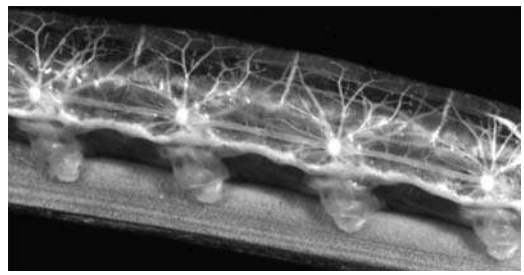

Figure 1.26 The white, thread-like tracheae of a skipper butterfly caterpillar are clearly visible through its nearly transparent skin. They branch out from the oval white spiracle seen above each leg. (Photo courtesy of James L. Castner.)

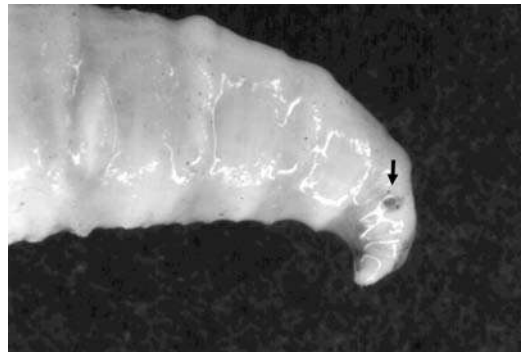

Figure 1.27a The thoracic spiracle of this flesh fly maggot is visible as a darkened spot near the larva's head on the right. (Photo courtesy of James L. Castner.)

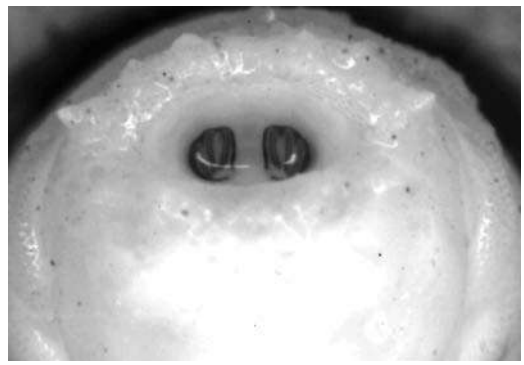

Figure 1.27b The two posterior spiracles of fly larvae are spaced close together at the tip of the abdomen such as in this flesh fly maggot. (Photo courtesy of James L. Castner.)

Copulatory structures also are present at the tip of the abdomen. In male insects, the penis or aedeagus is often withdrawn into the body. Accessory structures such as claspers, used to help hold the female during mating, are often present as well. In most insects sperm transfer is direct from the male to the female during copulation or mating.

Insect Development

Insects pass through a series of stages when developing from egg to adult. The appearance of these stages and the time spent within each varies with the species and with the environmental conditions that are present. For example, the developmental cycle is usually accelerated as temperature increases, which is why accurate climatic data are of utmost importance in the calculation and estimation of the postmortem interval based on insect evidence. The process of undergoing physical changes from one life stage to the next is known as metamorphosis. This is accomplished by means of the insect "shedding its skin" or undergoing ecdysis at certain points as it grows. The skin that is left behind is called an exuvium (Figures 1.28a,b). The time period spent in any particular life stage is referred to as a stadium. Finally, the insect itself also may be called an instar, especially during larval development. For example, a second larval instar has shed its skin once to proceed to its second stage since hatching from an egg.

Figure 1.28a A cicada sheds its nymphal skin during the process of molting. (Photo courtesy of James L. Castner.)

Figure 1.28b The shed skin (exuvium) of a cicada. (Photo courtesy of James L. Castner.)

The simplest form of development occurs when the only differences between the immature and adult forms are size and sexual maturity. Very primitive insects such as silverfish and springtails show this development, which is called ametabolous. The young hatch from an egg and they are essentially a small version of the adult, since there is no outward morphological difference between immatures and adults except size. The insects that undergo ametabolous development are all wingless.

A slightly more complex form of growth is hemimetabolous development or gradual metamorphosis. We see this growth pattern in the true bugs, such as the assassin bugs. With this type of development, eggs are laid and hatch out into immature forms that are called nymphs. These nymphs are somewhat similar to the adults, but have no wings. With

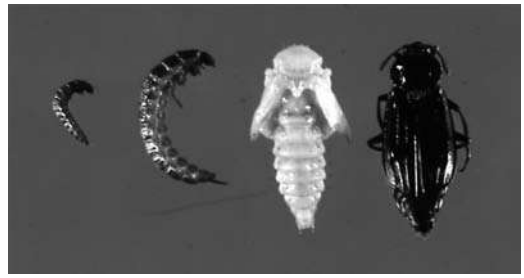

Figure 1.29 The stages in the life cycle of a carrion beetle. From left to right: a young larva, an older larva, a pupa, an adult. The egg is not shown. (Photo courtesy of James L. Castner.)

each shedding of the skin, the nymph becomes a little bigger. After ecdysis occurs several times, small wing pads begin to appear on the outside of the nymph. These pads get larger after the skin is shed each time until the adult stage is reached. At this point, the wings have developed to full size. This development also is seen in crickets, grasshoppers, katydids, and cockroaches.

The most involved and complex growth pattern is called holometabolous or complete metamorphosis. Most people are probably familiar with the life cycle of a butterfly that starts with an egg, progresses through caterpillar stages, forms a pupa, then eventually emerges as a butterfly. The same type of development also is seen in the two groups of insects with greatest value as forensic indicators of postmortem interval. Beetles undergo complete metamorphosis, but their egg and pupal stages are often hidden in protected areas. The larvae are often underground or under the body in contact with the exudate-soaked ground beneath and not readily visible. The same distinct four stages of egg, larva, pupa, and adult are part of each beetle life cycle (Figure 1.29). In some cases such as the rove beetles (family Staphylinidae), the larvae are highly mobile and capable of foraging on their own for food. In some species of carrion beetles (family Silphidae), parental care has evolved and the adult beetle actually regurgitates food into the mouths of the young larvae until they are mature. Beetles usually pass the pupal stage underground or in a protected cavity or area. They tend to be white or yellowish in color, becoming increasingly darker as they mature. The legs and appendages are easily visible and free from the rest of the body, characteristic of the pupal type referred to as exarate (Figure 1.30).

Flies also undergo holometabolous development, but with a distinct variation. Although it is still complete metamorphosis, the chain of events and appearance of the

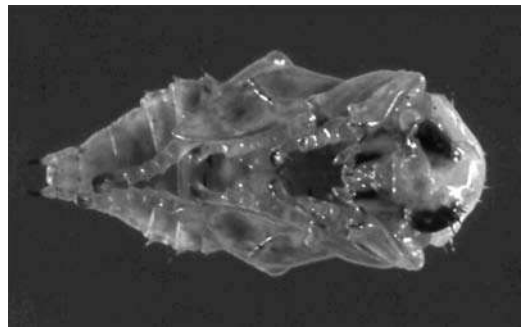

Figure 1.30 The exarate pupa of a sexton beetle demonstrates how the legs and wings develop externally to the rest of the body. (Photo courtesy of James L. Castner.)

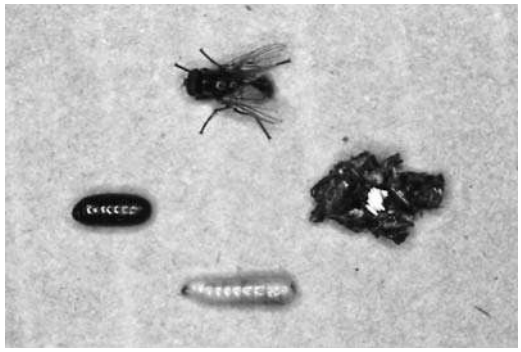

Figure 1.31 The life stages of a fly consist of the egg (a cluster of eggs is shown here), larva or maggot, pupa, and adult. (Photo courtesy of James L. Castner.)

life stages are quite different (Figure 1.31). Those families of flies that are both most commonly encountered and most useful as forensic evidence is the Calliphoridae (blow flies), Sarcophagidae (flesh flies), and the Muscidae (house flies). All are among the most evolutionarily advanced of the flies and belong to the suborder Cyclorrapha.

These flies begin life as eggs that are usually laid in large numbers on carrion, feces, or decaying material. In the flesh flies, the eggs are retained by the female until hatching. The small, first-instar larvae are then deposited directly on the food source. The young or maggots pass through three larva stages and are then ready to pupate. At this point, the mature maggots usually migrate away from the remains or food source to pupate in the soil (Figure 1.32). Sometimes, however, fly puparia can be found in the clothing of the deceased (Figure 1.33) or under carpets or in furniture when in a house environment.

Blow flies, flesh flies, and house flies all molt or shed their skin to form an exarate pupa, but they do it inside the old skin of the mature larva which then shrinks and hardens. This outer skin is called the puparium, and this pupal type is referred to as coarctate (Figure 1.34). The puparium darkens with time to brown, reddish brown, or almost black depending on the species (Figure 1.35). Morphological characteristics of the larvae are retained on the outer surface of the puparium and are often distinctive enough to permit a species identification to be made.

Figure 1.32 Maggots pupate in the soil, typically after migrating away from their food source. These puparia were uncovered by removing the soil surface debris. (Photo courtesy of James L. Castner.)

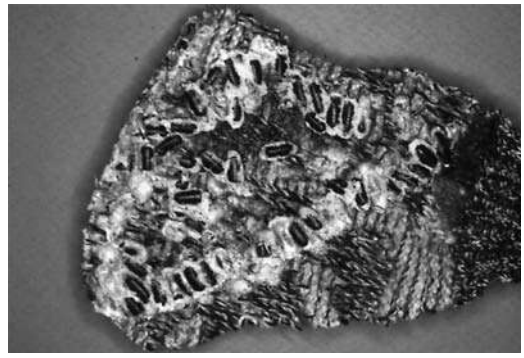

Figure 1.33 Under certain circumstances fly puparia may be found attached or embedded in the clothes of the decedent. (Photo courtesy of James L. Castner.)

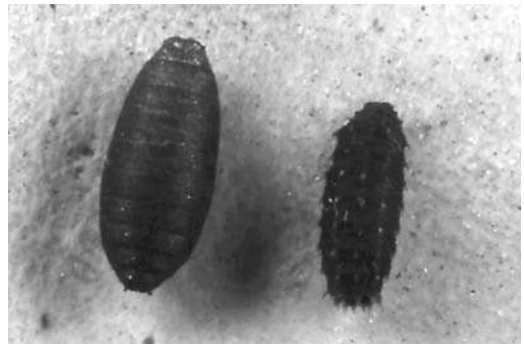

Figure 1.34 The coarctate pupae of many flies of forensic importance form when the last larval skin shrinks and hardens such as in these flesh fly (left) and blow fly (right) puparia. (Photo courtesy of James L. Castner.)

Figure 1.35 As most fly puparia mature, they darken and harden. In this image, the youngest flesh fly puparium is at the left and the oldest is at the right. (Photo courtesy of James L. Castner.)

Insects in aquatic environments also undergo gradual or complete metamorphosis. For example, mayflies (order Ephemeroptera) have hemimetabolous development just as true bugs do. The immature forms of aquatic insects with gradual metamorphosis are called naiads rather than nymphs. Caddisflies (order Trichoptera) are aquatic insects that are moth-like as adults and undergo complete metamorphosis. Their immature forms are still referred to as larvae.

Conclusions

Insects and other arthropods are found in almost every conceivable type of habitat. As a result, human remains are often found colonized by carrion-feeding invertebrates or in association with species that are scavengers or opportunistic feeders. The ability of true carrion-feeding insects to rapidly locate a body enables them to colonize remains that have even been wrapped, buried, or otherwise protected. The ubiquitous nature of insects makes their eventual appearance at a death scene a near certainty.

To take advantage of the potential forensic value of arthropods, evidence must be systematically collected and processed. For an investigator or crime scene technician to collect such material, they must first know what to look for. A basic understanding of insect biology and anatomy, especially with regards to the flies and beetles, shall facilitate the search, recognition, and collection of insect specimens for evidence. Recognition of insects and other arthropods to a greater taxonomic level, such as that of family or species, is covered in Chapter 2.

Suggested Readings

Borror, D. J., C. A. Triplehorn, and N. F. Johnson. 1997. *An Introduction to the Study of Insects*, 6th ed., Saunders College Publishing, Fort Worth, TX.

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Romoser, W. S. and J. G. Stoffolano. 1997. *The Science of Entomology*, 4th ed., Macmillan Publishing Co., New York.

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Glossary

Insect Antennae

Recognition of the different types of antennae is essential for identifying insects. A brief description is given below for each of the major antenna types, along with selected insect groups that serve as examples.

Aristate A distinctive three-segmented antenna found on certain flies where the third segment bears a protruding hair called an **arista.** Blow flies, house flies, flesh flies, and tachinid flies all have aristate antennae.

Capitate The tip of the antenna is enlarged into a rounded knob. Examples are found on butterflies, antlions, and owlflies.

Clavate The tip of the antenna is enlarged into a broadened club. Carrion beetles and carpet beetles both have distinctly clubbed antennae.

Filiform Thread-like or hair-like. Composed of a series of cylindrical or flattened segments. Examples are cockroaches, crickets, grasshoppers, true bugs, bark lice.

Geniculate Elbowed. Abruptly bent such as in a knee joint or elbow joint. Examples of such antennae occur on ants, on bees, and on weevils.

Lamellate A form of clubbed antenna where the terminal segments are enlarged parallel plates that stick out perpendicular to the rest of the antenna. These plates are close fitting and may not show any obvious space between them on dead specimens. Scarab beetles typically have lamellate antennae.

Moniliform Bead-like and composed of a series of rounded segments like a pearl necklace. Examples include termites and some beetles.

Pectinate Lateral processes stick out from the antenna at regular intervals like the teeth of a comb. Comb-like. Examples are glow-worms and some fireflies.

Plumose Feather-like, or with whorls or clumps of hairs. Mosquitoes and silk moths both exhibit plumose antennae which are more pronounced in the males of each.

Serrate A combination of roughly triangular segments that give a saw-tooth appearance. Found on click beetles and others.

Setaceous Slender, bristle-like, and gradually tapering to a tip. Examples include dragonflies, damselflies, and cicadas.

Stylate Antenna that terminates in a long slender point called a **style.** The style may be hair-like and similar to an arista, but is found at the tip of the antenna rather than projecting from the side. Stylate antennae are found on robber flies and bee flies.

Insects of Forensic Importance

2

JASON H. BYRD
JAMES L. CASTNER

Contents

Introduction

The proper identification of the insect and arthropod species of forensic importance is the most crucial element in the field of forensic entomology. It is the species identification that allows the proper developmental data and distribution ranges to be applied to an investigation. If the species determination is incorrect, or otherwise in error, the estimated postmortem interval is invalid. A few insect species exist that have growth and development rates so similar that one can be applied to the other with little or no error in the resulting postmortem interval determination. However, some species that appear similar to the naked

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eye have drastically different growth rates, behaviors, and habitat preferences. It is not possible to make comparisons between insect appearance and developmental physiology. Therefore, a qualified forensic entomologist should always make the species determinations.

The purpose of this chapter is not to encourage forensic investigators or other individuals to make definitive species identifications, but to show general taxonomic characteristics, present behavioral information, and to describe the biology of species when known. While some species of insects can be readily identified by simply viewing a photograph, such instances are in the minority. This is particularly true with the insects of forensic importance. However, familiarization with the appearance and habits of selected species will enable crime scene technicians and law enforcement personnel to make informed decisions regarding the collection of insect evidence.

This chapter features color photos of the most common species of insects and other arthropods of forensic importance in the U.S. and Canada. Although this work is not inclusive of every possible species that an investigator might recover at a death scene, it does represent the most commonplace and useful fauna. Cases of insect-induced postmortem artifacts that could be confused with premortem trauma to remains also have been included.

It is important to note that insects may play various roles in the process of a death investigation. The ones included in this chapter are species whose growth rate and developmental history, behavior, and geographic distribution are frequently utilized by forensic entomologists. There are situations when any insect can be of potential forensic importance. For example, a moth larva recovered from a plant seed attached to a blanket used in a rape or sexual assault may provide evidence linking the crime to a particular location. Insect evidence also has other valuable uses beyond aiding in the estimation of a postmortem interval. Sometimes entomological evidence is found as broken and fragmented insect parts in the clothing or personal belongings of the suspect or victim. These fragmentary remains are sometimes utilized successfully to create a link between the victim, suspect, and scene. Many species of insects could serve in this role, but it is beyond the scope of this work to illustrate fragmentary arthropod remains.

Investigators that become familiar with the appearance of the arthropods associated with human remains, and that follow the collection methodologies as described in Chapter 3, will be able to provide the forensic entomologist with entomological evidence that will yield the maximum amount of information possible. Additionally, procedures at the crime scene will be more efficient, as the investigator will spend less time collecting transient and incidental insects and be able to focus on the insects with the most evidentiary value.

Flies (Order Diptera)

This insect order is composed of the flies and has over 86,000 known species. Over 16,000 of these species occur in North America. This is one of the largest insect orders. Flies can be found in almost any habitat and are characterized by having only one pair of wings. The second pair of wings is reduced to only knob-like organs called the halteres, which are used to stabilize the insect in flight. Flies have large compound eyes with mouthparts of various types (see Chapter 1). However, most flies associated with a corpse have sponging mouthparts. The larvae of flies are called maggots and most are cream colored, soft, legless, and lack a visible head (Figure 2.1). Aquatic larvae (such as those of midges and mosquitoes) are slender and have a recognizable head (Figure 2.2). Flies are important as scaven-

gers, removing decomposing plant and animal material from the environment. Some are predators and parasites of other insect species, and others aid in the pollination of plants (Figures 2.3a,b).* This section focuses on the species of diptera that are important decomposers and scavengers.

Blow Flies (Family Calliphoridae)

This is an extremely large family of medium-sized flies that contains more than 1000 species. Blow flies are found throughout the world. They, along with the sarcophagid and muscid flies, are the most important species that provide information relating to the accurate estimation of the postmortem interval. Calliphorid flies are attracted to carrion and excrement, with some species exploiting open wounds. This family includes the familiar green bottle flies (genus *Phaenicia*) and blue bottle flies (genus *Calliphora*), as well as the screwworm flies (genus *Cochliomyia*). In addition to their forensic importance, this family is extremely valuable in nutrient recycling and community ecology based on their removal and breakdown of vertebrate carcasses.

Adult calliphorids usually range from 6 to 14 mm in length. The majority of species are metallic in appearance, with colors ranging from green, blue, bronze, or black. In some species, a covering of fine powder or dust masks the bright metallic coloration of the fly body. This results in only a dull metallic sheen remaining. Adults have three segmented antennae with a hair or arista on the last segment (see Figure 1.8). This arista is plumose or hairy throughout its entire length.

The mature larvae of blow flies range from 8 to 23 mm in length. They are usually white or cream colored. The terminal segment of the larval body typically has six or more cone-shaped tubercles about its perimeter (Figure 2.4). This segment also contains the posterior spiracles, which are the primary breathing apparatus of the larva (see Figure 1.27b). The slits within each spiracle slant towards the center of the larva, as opposed to those of sarcophagid maggots that slant more outward or downward.

Blow flies are among the first insects to discover and colonize human remains. In experimental studies, calliphorid flies have been recorded arriving at carcasses within minutes of their exposure. The telescoping segments of the tip of the female's abdomen extend to form an ovipositor, which is used for egg laying. Large numbers of eggs are commonly placed in the nose and mouth, as well as other natural body openings that are exposed. Areas with open wounds also are selected for egg placement. Thus, subsequent maggot mass formations and uneven defleshing of bodies can be indicative of premortem or perimortem trauma. It is a good practice to have both a forensic pathologist and a forensic anthropologist closely examine these sites. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hall and Doisy, 1993; Hogue, 1993; James and Harwood, 1969; Liu and Greenberg, 1989; Peterson, 1979; Shewell, 1987.)

Calliphora vicina Robineau-Desvoidy (= *C. erythrocephala* Meigen)

Common Name: European Blue Bottle Fly (Figures 2.5a,b,c)

This is one of several species with the common name of blue bottle fly. It is nearly worldwide in distribution. In the U.S., it is most abundant in the northern half of the

* See color insert following page 78.

country. *C. vicina* is a large fly, usually ranging from 10 to 14 mm long. The head is black in color, with the lower part of the bucca or "cheeks" appearing red to yellow. The cheeks are black in *C. vomitoria,* a very similar species. The thorax is black, but coated with grayish powder giving it the overall appearance of a grayish-blue. The thorax also has dark longitudinal stripes on the dorsal surface between the bases of the wings. The abdomen is a noticeable metallic blue, patterned with silver. Overall the body appears very bristly.

The adults are attracted to most types of decaying matter and frequent rotting fruit, decaying meat, and feces. However, the larvae are found primarily on carrion. *C. vicina* is extremely common on human corpses throughout the U.S. and Europe in temperate regions. In the southern U.S. this is considered a winter species, while a spring and fall species in the temperate zones, and a summer fly in the subpolar regions. This species primarily favors shady situations and urban habitats, where it is often the dominant species on human cadavers. This species is another that has been known to produce myiasis. (Erzinclioglu, 1985; Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; James, 1947; Nuorteva, 1977; Payne, 1965; Smith, 1986.)

Calliphora vomitoria (Linnaeus)

Common Name: Holarctic Blue Blow Fly (Figure 2.6)

This blow fly species is Holarctic in distribution, as the common name implies. In the U.S. it is most common from Virginia to California and northward to Alaska. It also is found throughout southern Canada. The Holarctic blue blow fly ranges from 7 to 13 mm in length. The thorax is dark blue to black, with a light gray dusty coating. Depending on the condition of the specimen, darker blue longitudinal stripes also may be visible on the dorsum or upper surface of the thorax. The abdomen is metallic blue, but appears to have a light coating of silver-gray powder as well. This pollen-like coating masks much of the metallic nature of the color, but a glint of metallic sheen is still visible when specimens are examined closely. The legs of this species are black.

This species is similar in appearance to *C. vicina*, except the head appears almost entirely black and has only a few red to orange hairs near the posterior margin. Overall the body appears very stocky and bristly. This species is common in wooded rural as well as suburban areas where it prefers shaded locations. The Holarctic blue blow fly is slow flying and makes a loud buzzing sound during flight. The biology is much the same as described for *C. vicina*, but *C. vomitoria* is not as common. (Erzinclioglu, 1985; Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; James, 1947; Smith, 1986.)

Chrysomya megacephala (Fabricius)

Common Name: Oriental Latrine Fly (Figures 2.7a,b,c)

This blow fly is widely distributed throughout the Asian regions, South Africa, and South America. It also is now well established in the southern U.S. The adults have short stout bodies similar in appearance to *C. rufifacies*, but with a noticeably larger head. The eyes are unusually large and a very prominent shade of red, making this fly easily recognizable in the field.

The adult flies are attracted to carrion and sweet foods as well as to urine and excrement; hence, the common name. Although *C. megacephala* has a pronounced activity peak during the heat of the afternoon, this species is one of the first species to become active in the early morning hours and is one of the last species to depart carrion at nightfall.

Once the adults have settled on carrion, they are not easily disturbed. The adults also have a habit of entering dwellings in search of suitable oviposition sites. The larvae are primarily carrion feeders, and the adult oriental latrine fly shows a preference for fresh remains. Dry, decaying carrion has little attraction for this species. This calliphorid readily enters dwellings in search of food and egg-laying sites. (Bohart and Gressitt, 1951; Gagne, 1981; Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; James, 1947; Prins, 1982; Smith, 1986; von Zuben et al., 1993.)

Chrysomya rufifacies (Macquart)

Common Name: Hairy Maggot Blow Fly, Hairy Sheep Maggot (Figures 2.8a,b,c)
The hairy maggot blow fly is indigenous to the Australian and Asian regions of the Old World tropics. It was introduced into the continental U.S. in 1981 and is now well established in Louisiana, Texas, California, and Florida. During the summer months it ranges as far north in the U.S. as Michigan. The adults of *C. rufifacies* have stout bodies and are brilliant blue-green in appearance. The terminal edge of the abdominal segments are noticeably tinted a dark purple to blue.

The adults of this species are usually the first to arrive on carrion (often within hours after death) in the southeastern U.S. Unlike *C. megacephala*, this species rarely enters dwellings and the larvae only develop on carrion, not excrement. The larvae of this species are readily distinguished from other larvae in the family Calliphoridae that commonly occur in the U.S. by the presence of prominent fleshy protrusions along their body. The larvae are both predacious and cannibalistic and, therefore, should be separated from other species when live collections are made for shipment to a forensic entomologist. If the food supply becomes depleted, the larvae will consume, and often totally eliminate, other species from the carcass. The larvae also are able to burrow several inches into the soil to colonize buried remains. This species is rapidly expanding its range throughout the U.S., and due to its predatory nature it is likely that forensic entomologists will encounter the hairy maggot blow fly with increasing frequency. (Baumgartner, 1986, 1993; Baumgartner and Greenberg, 1984; Gagne, 1981; Greenberg, 1971, 1988; Hall, 1948; Hall and Townsend, 1977; James, 1947; Oldroyd and Smith, 1973; Richard and Ahrens, 1983; Smith, 1986; Wells and Greenberg, 1992; Zumpt, 1965.)

Cochliomyia macellaria (Fabricius)

Common Name: Secondary Screwworm Fly (Figures 2.9a,b,c)
This species is very abundant throughout the New World. It can be found from the American tropics throughout the U.S. to the Canadian border. Adults are a metallic greenish-blue with three dark green longitudinal stripes on the dorsal surface of the thorax (between the base of the wings). These stripes do not extend onto the abdomen. On close observation, the head appears orange and the legs may vary from a reddish brown to dark brown in color. The larvae have readily visible respiratory tracheae on their posterior end. The tracheae appear as swirling black lines easily visible against the white background of the maggot's body. These characters make this species readily identifiable in the field.

The secondary screwworm fly prefers warm humid weather. It occurs throughout the southern U.S. and is most abundant during rainy periods. This species frequents carrion in both sunny and shaded locations, but is rarely recovered from indoor habitats. *C. macellaria* is not cold-tolerant and, therefore, it is not usually abundant in the northern

U.S. during the winter months. (Denno and Cothran, 1975; Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; James, 1947; Rodriguez and Bass, 1983; Smith, 1986; Wells and Greenberg, 1992.)

Cynomyopsis (= Cynomya) cadaverina (Robineau-Desvoidy)

Common Name: Shiny Blue Bottle Fly (Figures 2.10a,b)
This species ranges throughout the Nearctic region. It is most commonly found in the northern U.S. and southern Canada, but also can occasionally be found as far south as Texas and northern Florida. The shiny blue bottle fly is a large species with the thorax being blue to blue-black and covered with a silvery powder, which causes it to sometimes appear gray. The abdomen is a shiny metallic blue. Three darker blue stripes are present on the dorsum behind the head.

This species is attracted to both carrion and feces. They are most abundant during the spring and fall months, and during the winter the adults may enter houses in large numbers. The adults are slow flying and easily captured when making collections at the death scene. The larvae are typically found on carrion during the advanced stages of decomposition, and the adults are not usually attracted to fresh remains. (Erzinclioglu, 1985; Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; James, 1947; Smith, 1986.)

Lucilia illustris (Meigen)

Common Name: Green Bottle Fly (Figure 2.11)
This species is one of several that have been given the common name of green bottle fly. *L. illustris* is distributed throughout Holarctic region. In North America this is a very common species that ranges from Mexico to southern Canada and throughout the American Midwest.

The adults are approximately 6 to 8 mm in length. The thorax and abdomen are a shining greenish-blue, while the legs are black. This is considered a "warm weather species," as it is most abundant in open woodlands during the summer months. The adults are attracted primarily to fresh carrion, but they can occasionally be collected on excrement. The larvae feed on both carrion and fecal material, but they also are most common on carrion. (Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; Smith, 1986.)

Phaenicia cluvia (Walker)

Common Name: Green Bottle Fly (Figure 2.12)
This fly is Nearctic to Neotropical in distribution and is most common in the southeastern U.S. during the late summer and fall. The thorax and abdomen are a bright metallic green, with legs a dark brown to black in color. The biology and habits of this fly are nearly identical to that of *P. coeruleiviridis*. The adults are attracted to both carrion and rotten fruits. (Hall, 1948.)

Phaenicia coeruleiviridis (Macquart)

Common Name: Green Bottle Fly (Figures 2.13a,b,c,d)
The green bottle fly has a Nearctic distribution and is very common in the southern U.S. The thorax and abdomen are a shiny, metallic blue-green, which may be tinted with purple or bronze. The legs are dark brown to black. The adults of this species are attracted to

almost all decaying animal matter and is one of the most common species attracted to fresh carrion. This is probably the predominate species of blow fly in the southeastern U.S. during the spring and fall. It remains active during mild winters and is commonly recovered on human corpses. (Hall, 1948; Hall and Townsend, 1977.)

Phaenicia cuprina (Wiedemann) (= *Phaencia pallescens* Shannon)

Common Name: Australian Sheep Maggot or Bronze Bottle Fly (Figures 2.14a,b,c,d)
This species is found in Australia, Africa, and the Americas. In the U.S. it occurs primarily in the southern region. The bronze bottle fly is 6 to 8 mm in length and is a metallic yellow-green to dull copper in color, which may be tinted with green. This species is often confused with the sheep blow fly (*P. sericata*), but the front femora are metallic green. The adults seem to prefer excrement to carrion, but are commonly attracted to both as well as to decaying fruit. They often alight on the ground or vegetation in proximity to a food source and take flight readily when disturbed, thus making them difficult to collect. The larvae of this species are most frequently found on carrion.

The bronze bottle fly is abundant from spring through fall and can be collected year-round in Florida. It is often found near dwellings and readily enters homes. This species has been known to produce myiasis (infest living tissue) in both man and animals. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; Hogue, 1993; James and Harwood, 1969; Peterson, 1979; Smit, 1931; Smith, 1986.)

Phaenicia eximia (Wiedemann)

Common Name: Green Bottle Fly (Figure 2.15)
This green bottle species is found in the southern U.S., including Texas, Louisiana, and Florida. It is similar in appearance to *P. sericata* and *P. coeruleiviridis*. The adult is bright metallic blue-green to entirely blue or purple with legs that are black to dark brown. Adult flies are attracted to both carrion and decaying fruits, with the larvae developing on the same substances. The larvae of *P. eximia* are typically found during the early stages of decomposition. (Hall, 1948; Hall and Townsend, 1977.)

Phaenicia sericata (Meigen)

Common Name: Sheep Blow Fly (Figure 2.16)
Historically, this species has been Holarctic in distribution, but now it is nearly cosmopolitan in range. The sheep blow fly is most common in the temperate zone of the Northern Hemisphere. In the U.S. it is most common in the western regions, but it can be collected throughout both the U.S. and southern Canada.

Adult sheep blow flies are 6 to 9 mm in length. This fly is a brilliant metallic blue-green, yellow-green, green, or golden bronze. The thorax has three prominent transverse grooves on its dorsal surface and the front femora are black or deep blue, a useful character in identification. The larvae of this species can successfully develop in a wide variety of food substrates, but they are best suited to carrion. This is one of the earliest arriving fly species on remains, with oviposition occurring typically only a few hours after death. The adults prefer carcasses located in bright sunshine and open habitats; however, they will seek shaded areas of the body in which to deposit their eggs. There have been reports of this species anticipating death and ovipositing on the wounds of the dying. However, there

also are reports that the larvae develop most rapidly on decomposing (not fresh) carrion. The larvae of *P. sericata* have been employed in maggot therapy for the removal of necrotic tissue from wounds. (Cragg, 1956; Cragg and Cole, 1952; Cragg and Hobart, 1955; Davis, 1928; Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; Hudson, 1914; James, 1947; Ratcliffe, 1935; Smith, 1986; Zumpt, 1965.)

Phormia regina (Meigen)

Common Name: Black Blow Fly (Figures 2.17a,b,c)
The black blow fly is Holarctic in distribution and can be found throughout the U.S., with the exception of southern Florida. The adult flies typically range from 7 to 9 mm in length. This species has a dark green to olive-colored thorax and abdomen, with black legs. However, the entire body may appear black depending on lighting conditions. The anterior thoracic spiracle is surrounded by distinctive bright orange hairs, which serve readily in the identification of this species.

This species is typically considered a cold weather fly because throughout most of the U.S. it is most abundant in the spring and fall, and can be found throughout the winter months. In the southern U.S., it is predominant during the winter months and is not typically found during the hot summers. The larvae develop mainly in carrion, and are well known myiasis producers. This species has historically been used to cleanse wounds in maggot therapy. (Denno and Cothran, 1975; Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; James, 1947; Kamal, 1958; Smith, 1986; Zumpt, 1965.)

Protophormia terraenovae (Robineau-Desvoidy)

Common Name: Bird's Nest Screwworm Fly, Holarctic Blow Fly (Figures 2.18a,b)
This species of blow fly is Holarctic in distribution and typically found from Mexico to Alaska. It is most common in the northern portions of the U.S. and in southern Canada. It is infrequent to rare in Florida during the winter and early spring.

Adult Holarctic blow flies are 7 to 12 mm in length. This species has a dark blue to black body coated with a silver-gray powder. The abdomen is a greenish-blue to blue, but, with its powdery coating, appears tessellated. The legs of this fly are black. Depending on the condition of the specimen, darker black longitudinal stripes may be visible on the dorsal surface of the thorax, starting immediately behind the head. This species is rather large in size and appears more hairy than most of the Calliphoridae (with the exception of *Calliphora*).

Although most common during the spring months, *P. terraenovae* can be collected throughout the summer in the higher elevations of the U.S. Cool weather favors development and this species is the most cold tolerant of all calliphorid species. In the northern U.S. it may become more common than *P. regina* during the winter and spring months. In Canada it is an early spring species and it is most abundant in July. The larvae develop primarily on carrion. (Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; James, 1947; Kamal, 1958; Nuorteva, 1977, 1987; Smith, 1986.)

Flesh Flies (Family Sarcophagidae)

Flesh flies comprise a large family with over 2000 species, approximately 327 of which occur in the U.S. and Canada. Representatives of this family are found throughout the

world, with most species occurring either in tropical or warm temperate regions. Adults are common and often found on flowers where they are attracted to nectar. The adult flies feed on other sweet substances as well, including sap and honeydew. This family's Latin name means "flesh eating" and apparently refers to the larvae or maggots that typically feed on some sort of animal material. In addition to carrion, they also may feed on excrement or exposed meats. They have been known to cause myiasis and may be involved in the mechanical transmission of diseases. Many species of sarcophagid or flesh flies are parasitic on other insects, especially bees and wasps. At least one species is beneficial to man, however, and serves as a major natural control of the forest tent caterpillar.

Flesh flies are medium-sized and range in length from 2 to 14 mm. The adults commonly have gray and black longitudinal stripes on the thorax and have a tessellated (checkerboard) pattern on the abdomen. Although they are roughly the same size as the blow flies and the bottle flies (Family Calliphoridae), flesh fly adults never have a metallic coloration like the others. Also, the arista of the antennae of flesh flies is plumose only at the base, while in calliphorids it is plumose throughout the length. The bodies of sarcophagids tend to be bristly and the eyes are fairly widely separated in both sexes. In some species the eyes are bright red in color, as are the highly visible genitalia at the tip of the abdomen.

The larvae of flesh flies have the posterior spiracles located in a pit or depression at the tip of the abdomen, which is edged with fleshy tubercles. This characteristic can be used to differentiate between flesh fly and blow fly larvae. Species of sarcophagids are similar to one another in both the adult and larval stages, and are notoriously difficult to identify to the species level. Maggots should always be reared to the adult stage to facilitate positive species identification.

Flesh flies are attracted to carrion under most conditions, including sun, shade, dry, wet, indoors, and outdoors. They can be found associated with carcasses throughout both the early and late stages of decomposition. Female flies in this family deposit living first instar larvae on decomposing remains. They do not lay eggs and, thus, fly egg masses associated with human remains cannot be attributed to sarcophagids. The time period necessary for egg development also must be eliminated when calculating a postmortem interval (PMI) based on flesh fly evidence.

Flies of the genus *Sarcophaga* arrive on human remains concurrently, or slightly after, the blow flies. They are known to fly under inclement conditions that would prevent the flight of most other flies and, therefore, may actually be the first species to arrive on carrion. The adults commonly enter indoor habitats and, thus, the larvae are often recovered from human remains located in homes. This is the predominant fly recovered on human bodies located in indoor habitats during the summer months in the southeastern U.S. (Aldrich, 1916; Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Greenberg, 1971; Hall and Doisy, 1993; Hogue, 1993; James, 1947; James and Harwood, 1969; Knipling, 1936; Payne, 1965; Peterson, 1978; Shewell, 1987; Smith, 1956, 1975, 1986.)

Sarcophaga bullata (Park)

(Figures 2.19a,b,c)

This species occurs only in the Nearctic region or that part of North America north of Mexico. Although it can be found from Canada south to Florida and west to California and Washington, it is most common in the southern U.S. Adults of *S. bullata* range from

8 to 14 mm in length. They appear very much like the adults of *S. haemorrhoidalis,* to which they are closely related. They also are very similar in behavior and habitat preferences. (Aldrich, 1916; James, 1947.)

Sarcophaga haemorrhoidalis (Fallen)

Common Name: Red-Tailed Flesh Fly (Figures 2.20a,b,c,d)
The red-tailed flesh fly is cosmopolitan in distribution, occurring worldwide. Investigators may find it associated with human remains throughout the U.S. and Canada. It is a large species that ranges from 8 to 14 mm in size. This fly appears very similar to flies in the family Muscidae. However, it is typically larger and its abdomen terminates in a red tip, which is actually the external genitalia. Although named the red-tailed flesh fly, it shares this characteristic with other sarcophagid species as well. The body is black, but covered in whitish powder that gives it an ash gray appearance. It has three dark longitudinal stripes on the thorax between the wing bases. These stripes do not continue on the abdomen, which has a black and gray tessellated (checkerboard) pattern.

The adults of this species are attracted to both excrement and carrion. Females commonly enter dwellings to deposit their larvae, which are frequently found on human corpses located indoors, particularly during the summer months. Eggs are not laid, as they hatch within the body of the female. Therefore, it is the living first instar larva that is deposited on carrion or excrement. This species can infect the wounds or digestive tracts of living animals, particularly mammals. *S. haemorrhoidalis* has been recorded as even completing development entirely within the human digestive tract. (Aldrich, 1916; Greenberg, 1971; James, 1947; Smith, 1975, 1986; Zumpt, 1952.)

Muscid Flies (Family Muscidae)

Muscid flies belong to a large family that is worldwide in distribution, with over 700 species in North America alone. Many species are ubiquitous and synanthropic (found closely associated with man). This habit often has resulted in the unintentional transportation and introduction of species to areas outside their natural ranges. The propensity with which muscid flies are found in domestic situations contributes to both their medical and forensic importance. Common members of this family are the house fly, stable fly, horn fly, and latrine fly. The tsetse fly, which vectors African sleeping sickness, is also a muscid fly.

The biology and habits of muscid flies are quite varied as the adults may feed on decaying plant and animal material, dung or excrement, pollen, or even blood. In the latter case, stable flies and horn flies can become severe nuisances of both humans and livestock. Other species that breed and feed on garbage, sewage, and human waste can be responsible for the mechanical transmission of diseases. These include species such as the house flies which regurgitate digestive fluid directly onto food materials as part of their feeding process. They have been implicated in the transmission of typhoid, anthrax, dysentery, and yaws.

Muscid flies are small to medium-sized, typically ranging from 3 to 10 mm in length. They tend to be dull gray to dark in color, although a few species have a metallic sheen. These species can be separated from the bottle flies and blow flies (family Calliphoridae) by taxonomic characters of the wings and head. Adult muscids are generally not as bristly as the sarcophagid and calliphorid flies. The arista of the antennae of muscids is plumose throughout its length, a character shared by the family Calliphoridae.

Most muscid larvae conform to the typical cylindrical shape associated with a maggot, tapering from the tail end towards the head and mouth. Mature larvae range from 5 to 12 mm in length and are white, yellow, or cream-colored. The surface of the maggot is smooth in most species, although members of the genus *Fannia* are flattened and have many ornate projections. This genus, which includes the latrine fly and the lesser house fly, is sometimes placed in the family Anthomyiidae or in its own family (the Faniidae).

Muscid flies are of great forensic importance due to their wide distribution, ubiquitous nature, and close association with man. They tend to arrive at bodies after the flesh flies and blow flies. They often lay their eggs in natural body openings, at wound sites, or in fluid-soaked clothing. Larvae feed directly on carrion, but in some species exhibit predacious behavior as they mature. In such cases, muscid larvae may affect the faunal composition on a set of remains by preying on the eggs and larvae of other carrion-feeding flies. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; Huckett and Vockeroth, 1987; James and Harwood, 1969; Peterson, 1979.)

Fannia canicularis (Linnaeus)

Common Name: Lesser House Fly (Figures 2.21a,b,c,d)

The lesser house fly (also called the little house fly) is cosmopolitan in distribution. This species is a small, slender fly usually attaining a length of 6 to 7 mm. It has a black to dark brown thorax and an abdomen coated with silvery-gray powder. The legs appear yellowish in color. The thorax has three longitudinal brown stripes that do not continue onto the abdomen.

The lesser house fly is most abundant during warm summer months and it is the species of *Fannia* most commonly involved in myiasis. They are most often found on excrement, decomposing vegetable matter (such as fruits), dairy products, and materials saturated with urine. They are often recovered on decomposing human remains when excrement or gut contents have been exposed.

The maggots develop well on decomposing substances that are liquefying. As an adaptation to this type of a habitat, the larvae are flattened with branched protuberances that apparently aid in flotation in semiliquid environments. The larvae of *F. canicularis* are similar in appearance to those of *F. scalaris*, but the protuberances are not as branched. They also can grow longer than *F. scalaris* (the latrine fly). The larvae will migrate to drier habitats in order to pupate. The adults are prone to enter indoor habitats where they may be seen hovering in mid-air or flying about in an erratic manner. They are often considered nuisances as they can aggregate in relatively large numbers indoors during certain periods of the year. The adults are strongly attracted to both carrion and excrement. (Greenberg, 1971; James, 1947; Nuorteva, 1974; Payne, 1965; Smith, 1986; Wasti, 1972.)

Fannia scalaris (Fabricius)

Common Name: Latrine Fly (Figures 2.22a,b,c)

The latrine fly is worldwide or cosmopolitan in distribution. It is very similar to the lesser house fly in appearance. Adults are typically 6 to 7 mm long. This species is a black fly with the thorax and abdomen having a dusty silver-gray coat that makes it appear superficially like a small housefly. The larvae are small and flattened, usually reaching a length of no more than 6 mm. The immatures possess a pair of lateral processes (tubercles) on

each body segment. These protuberances are feathered and may aid in flotation in their preferred habitat of semiliquid fecal masses.

Eggs are typically laid in human or animal dung, as well as decaying vegetable material. The larvae can develop in anything from dung, carrion, the nests of birds and other insects, to human cadavers. The latter is especially true when excrement or exposed gut contents are present. The larvae of this species are likely to be encountered on urine soaked clothes and on other such soiled materials. This species is not as prone to enter indoor habitats as is *F. canicularis*. The presence of the latrine fly indoors is usually indicative of unsanitary conditions. (Greenberg, 1971; James, 1947; Nuorteva, 1974; Smith, 1986.)

Hydrotaea (= Ophyra) aenescens (Wiedemann)

Common Name: Bronze Dump Fly (Figures 2.23a,b,c)
In the U.S., the bronze dump fly is distributed from Oregon to Arizona, Illinois to Florida, and along the Atlantic Coast. It also is found throughout Central and South America. This is a small, shining, blue-black species with a bronze tint. Like *H. leucostoma*, it readily enters dwellings seeking suitable oviposition sites and food sources. Although the adults are attracted to both carrion and excrement, the maggots develop primarily in feces. The larvae of *H. aenescens* are predatory. They may be recovered on human cadavers during the late or active decay stage. Larvae are often found in body exudates that have soaked into the soil beneath remains, and are commonly associated with exposed gut contents. The bronze dump fly is most abundant during the summer months in the U.S. (Greenberg, 1971; James, 1947; Smith, 1986.)

Hydrotaea (= Ophyra) leucostoma (Wiedemann)

Common Name: Black Dump Fly (Figures 2.24a,b,c)
The black dump fly is found throughout the U.S. Although Holarctic in distribution, it is restricted to the area north of Mexico in North America. This is a species with shining blue-black to black adults. The flies are attracted to both carrion and excrement, where they frequently deposit their eggs. The adults may be noticed hovering either above or near decomposing carcasses on days with little to no wind. The larvae develop primarily in feces and are predatory in nature. They frequently attack the larvae of *M. domestica* when they occur together. The larvae usually appear during the late or active decay stages on human cadavers and are often found in the fluid-soaked soil underneath the body. They are most abundant during the summer months. This is one of several species that is frequently recovered from remains found in indoor habitats. (Greenberg, 1971; James, 1947; Smith, 1986.)

Musca domestica (Linnaeus)

Common Name: House Fly (Figures 2.25a,b,c)
The house fly is worldwide in distribution. Although it is found throughout the U.S. and Canada, it may not be the predominant fly species in all geographical areas. The adults are 6 to 7 mm in length. They are gray in color with black longitudinal stripes on the thorax that continue onto the abdomen. This species is almost never found away from man and his dwellings, and it is a major nuisance due to its movements from garbage, carrion, and excrement to human food. It is a vector of over a hundred disease-causing

pathogens. Both adults and larvae prefer excrement and decomposing vegetable matter. The adults also are attracted to sweet foods and meat, and their larvae can adequately develop in these materials.

The adults will readily enter dwellings in order to colonize decomposing remains. They are among the first flies attracted to excrement and also are attracted to carrion, usually following the blow flies. This species can be found year-round throughout its range, but it is most abundant during the summer months. In temperate zones, the population reaches its peak in spring and late summer. In the tropics and subtropics, house flies are most abundant during the hot summer months. The presence of this species on a fresh corpse is rare, unless excrement is present or gut contents are exposed. (Chapman, 1944; Greenberg, 1971; James, 1947; Smith, 1986.)

Synthesiomyia nudiseta (Van Der Wulp)

(Figures 2.26a,b,c,d)
This species is found throughout the tropical and subtropical regions of the world. In the U.S. it has been collected primarily from California to Texas and from North Carolina to Florida. This fly is one of the largest muscids, approximately 7 to 10 mm in length. It has a gray color with a tessellated or checkerboard pattern on the abdomen, closely resembling that found on the flesh flies. However, it is easily separated from sarcophagids because the thorax is patterned with four distinct longitudinal stripes instead of three, and the terminal segment of the abdomen appears yellow (or bright orange) instead of red. The antennae and palpi also are yellowish to orange in color. The larvae are commonly found in animal and human feces as well as in decaying vegetable materials, refuse, and garbage. However, they have been reported to prefer carrion as the food source of choice.

Although this species has been noted to arrive along with flies in the genus *Sarcophaga* to deposit their eggs, their larvae develop more slowly and pupate with the larvae of the later arriving fly species. The larvae of this species are predacious and are one of the few species that is noted to consume the larvae of *Chrysomya rufifacies*. The puparia of this species may be difficult to recognize as the larvae often secrete a silky white substance in which the puparium forms. Additionally, soil particles also may be cemented to the outer surface to help form this protective "cocoon." (Greenberg, 1971; James, 1947; Lord et al., 1992; Rabinovich, 1970.)

Skipper Flies (Family Piophilidae)

The skipper flies comprise a small family of only 69 species, but are found worldwide where their greatest diversity is found in temperate regions. Adults are metallic blue or black in color and typically range from 2.5 to 4.5 mm in length. Piophilid or skipper flies are found in a variety of habitats that may include carrion, human waste, bones, skin, and fur. They are common and usually associated with protein-rich food sources that are dry in nature.

The common name of "skipper" comes from an unusual behavior exhibited by the larvae of some species, including the cheese skipper (*Piophila casei*). The larvae will grasp small protrusions on the anal segment with the mouth hooks and suddenly release their grip (see Figure 2.27D). This action flings the larva into the air 3 to 4 inches, and laterally over a distance of 6 to 8 inches. This "jumping" behavior is used as an effective "escape"

mechanism and it also is utilized extensively during larval migration. However, they also move in the more traditional creeping manner exhibited by most fly larvae.

Skipper fly larvae are primarily scavengers and feed on the same kinds of decaying food materials on which the adults are found. One group specializes on rotting fungi, while in Europe there is a species whose larva is a blood-sucking ectoparasite of young birds. The maggots of skipper flies can be considerably larger than the adults and usually range from 5 to 10 mm in length. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; McAlpine, 1987; Peterson, 1979.)

Piophila casei (Linnaeus)

Common Name: Cheese Skipper, Jumping Maggot (Figures 2.27a,b,c,d,e)
Cheese skippers are cosmopolitan in distribution and found throughout the world. They are small (2.5 to 4.5 mm) shiny black flies with yellow legs. The adults are found on carrion, excrement, and garbage. Observations show that they prefer darker, shaded areas and hold their wings flat over the back when at rest. Almost any protein-rich food source is a suitable substrate for the developing larvae.

Cheese skippers are a serious pest of the food industry. The adults lay eggs on cheeses, bacon, smoked fish, and cured meats. This preference for some of the same food items used as man has resulted in numerous cases of immature cheese skippers being ingested. Surprisingly, this species is able to survive and pass through the digestive system of humans, and their presence results in a condition referred to as enteric pseudomyiasis.

The larvae are often recovered on human bodies after active decay, as the body begins to dry. Adults have been recovered at remains only 3 to 4 days after death; however, it is important to note that the presence of an adult insect does not necessarily mean that oviposition (colonization) is occurring. Although skipper flies in general seem to prefer a food substrate that is dry, they are often recovered from bodies found in aquatic situations. The puparia of this species have even been recovered on human organs inside of a 2000-year-old Egyptian mummy (Cockburn et al., 1975; Simmons, 1927).

Dung Flies (Family Scathophagidae)

The dung flies are treated here as a separate family, although other references may include them as a subfamily under the family Muscidae or family Anthomyiidae. The Latin name for this family means "dung-eating," and although "Scathophagidae" is most widely used, some authors refer to the family as the "Scatophagidae." There are over 250 species, almost all of which are found in the Holarctic region. Approximately 150 species occur in temperate North America with the greatest diversity found in southern Canada.

The most common species of dung flies are red or yellow and densely hairy. Some species are attracted to excrement or decaying plant material, and their larvae are most commonly found in excrement. Other species are dark as adults and have larvae that are leaf miners, pests of flower heads, parasitic, predacious, and aquatic. The larvae of the genus *Scatophaga* feed on dung, but also are often found on decaying seaweed. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Peterson, 1978; Vockeroth, 1987.)

Scatophaga stercoraria Linnaeus

Common Name: Common Dung Fly (Figure 2.28)
The common dung fly is found throughout the U.S. and Canada. The adult is slender and reaches a length of 7 to 10 mm. Although the body itself is black, it is covered with a dense coat of yellow or yellow-brown hair, thus making the entire insect appear bright yellow. The thorax has several long, black hairs protruding from it and the wings are typically held flat over the back when at rest. The adults are present from spring until late fall. The larvae occur in a variety of habitats, but can be found on cadavers when excrement is present or the gut contents have been exposed.

Black Scavenger Flies (Family Sepsidae)

Black scavenger flies or sepsids are worldwide in distribution and represented by at least 240 species. The adults are small, shining black, purple, or red flies that are usually no more than 3.5 mm in length. They have a characteristic shape due to a head that is noticeably rounded and a constriction or narrowing of the abdomen at the base. Despite their small size, they are easily identified by the behavioral characteristic of flicking their wings outward as they walk. This habit of wing waving also has given them the common name of "waggle flies." These flies often occur in large numbers and are very common on dung.

Sepsid fly larvae develop in a variety of decomposing organic matter, including carrion and excrement. Some species are found in decaying seaweed. Mature larvae are small, ranging from 3 to 6 mm in length. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Streyskal, 1987; Peterson, 1979.)

Sepsis sp.

Common Name: Black Scavenger Flies (Figures 2.29a,b)
Black scavenger flies of the genus *Sepsis* spp. are worldwide in distribution. They are commonly found throughout the U.S. and Canada. The adults are shiny black and show all the other features described previously under the family Sepsidae. In addition, adult species of *Sepsis* also have a dark spot near the wing tip along the front margin.

These flies are attracted to carrion, excrement, and other types of decaying matter. They are sometimes mistaken for ants due to their small size and narrow "waist" (constricted abdomen). Black scavenger flies can be abundant on carcasses, especially during the advanced stages of decay, as well as on excrement and other types of decaying matter. (Greenberg, 1971; James, 1947; Smith, 1975, 1986.)

Small Dung Flies, Minute Scavenger Flies (Family Sphaeroceridae)

The small dung flies are a group with cosmopolitan distribution and over 240 species in North America. They can be found throughout the U.S. and Canada. They are small flies, ranging from 1 to 5 mm in length and typically a dull black or brown color. Taxonomic characteristics of the hind legs and the wing venation are used in their identification to the family level.

Sphaerocerid fly adults and larvae are found in association with dung and excrement. They are a common part of the insect fauna that inhabits cow dung, and the transport of

which is probably largely responsible for the spread of certain species. They are often found with the larger dung flies (family Scathophagidae) and the black scavenger flies (family Sepsidae).

In addition to animal waste, the small dung flies also may be found on carrion, refuse, seaweed, fungi, and other types of decaying organic matter. The presence of excrement or soiled clothing on human remains probably greatly increases the chance of encountering small dung flies in association with human remains. Unfortunately, little research has been done on the developmental times of these flies. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Marshall and Richards, 1987; Peterson, 1979.)

Poecilosomella angulata (Thomson)

Common Name: Small Dung Fly (Figure 2.30)
This species is small, only 2 to 3 mm long. It is not often recovered from human remains unless excrement or excrement-soiled clothing is present. On occasion, they can be attracted as well when gut contents are exposed, or to the soil that has become saturated with gut contents. They are common at very large vertebrate carcasses (i.e., bovines) where large amounts of gut contents are exposed during the process of decomposition. This species has been responsible for cases of intestinal myiasis. (Greenberg, 1971; McAlpine, 1987.)

Soldier Flies (Family Stratiomyidae)

Soldier flies encompass more than 250 described species that range in size from 5 to 20 mm. The color of adults varies, although many of the most common species are wasp-like in appearance. The antennae of soldier flies consist of three segments, the last of which is either elongated or rounded with a long hair protruding from it. Adults are often found at flowers and occur near woods and on vegetation in wet areas, as well as on almost any decomposing plant or animal tissue. The common name of this family derives from the spines found on the adults of certain species, which led some observers to consider them "armed" like soldiers.

Mature larvae vary greatly in length from 4 to 40 mm, depending upon the species. Some are purely aquatic, while others are found in terrestrial habitats. The terrestrial larvae usually develop in decomposing plant or animal material, although in some cases they may be predacious. Rotting wood, compost piles, and excrement are typical habitats. The aquatic forms sometimes can be found in surprisingly hostile environments such as hot springs or extremely saline waters. Soldier fly larvae occasionally are ingested through the consumption of infested fruit, subsequently appearing in stools. Several cases of such enteric pseudomyiasis have been reported. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970, Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James, 1981; James and Harwood, 1969; Peterson, 1979.)

Hermetia illucens (Linnaeus)

Common Name: Black Soldier Fly (Figures 2.31a,b)
This species is Neotropical in origin, but now found in almost all temperate and tropical areas of the world where it has probably been transported via contaminated food. The black soldier fly is found throughout the U.S. and Canada. It is very common in the

southeastern U.S., and least common in the Northwest. Adults are very wasp-like in appearance and are even sometimes referred to as the "wasp fly."

Adult flies are approximately 15 to 20 mm long. The females are entirely blue-black, while the males have an abdomen that is more brown in color. The tips of the legs of both sexes are white. The wings appear smokey-black and are held flat over the back (wasp-like) when at rest. The abdomen is elongate and narrow at the base, with the first two segments bearing translucent areas. This feature contributes to their narrow waist appearance and aids in the resemblance of a wasp. In addition, the flies also will behave in a wasp-like manner, moving their antennae rapidly, running about in an agitated manner, and sometimes even pretending to sting.

The larvae of the black soldier fly are broad, flattened, and distinctly segmented with a narrow prominent "head." Each body segment has a row of broad stout hairs. Larvae are gray to brown in color and have a hard, thick surface. The body has a cobblestone texture when viewed under magnification and this type of surface and texture is described as "shagreened." An outer layer of calcium carbonate deposited on the skin and serving as protection from desiccation during dry periods causes their unusual external appearance. The larvae develop on many different types of decaying organic material. Under some circumstances, the black soldier fly is considered a beneficial species because it suppresses house fly populations through competition for the same food source. Larvae are commonly found on rotting fruits and vegetables, in compost and refuse, in excrement, and on carrion. When associated with human remains, the larvae usually occur during the advanced to dry stages of decay. (Bohart and Gressitt, 1951; Dunn, 1916; Greenberg, 1971; James, 1947; Lord et al., 1994; Payne and King, 1970; Reed, 1958; Smith, 1975, 1986.)

Humpbacked Flies or Scuttle Flies (Family Phoridae)

The humpbacked flies are a large family with more than 2500 species worldwide. Nearly half of these species belong to the single genus *Megaselia*. Approximately 226 species of phorid flies are currently recognized in the U.S., and a total of approximately 350 in North America.

Phorid flies are small, ranging in length from about 1.5 to 6 mm. They are easily recognized by their humpbacked appearance that is especially noticeable when the flies are viewed in profile. Other taxonomic characteristics helpful in their identification are the flattened femora of the hind legs and the heavy veins found at the base of the anterior wing margin. Humpbacked flies may be black, brown, or yellow in color.

Adult phorid flies are found in a variety of situations. They are commonly associated with decaying plant matter and are often unwanted yet ubiquitous pests where live insect colonies are maintained. The adult insect will run in a very characteristic swift and erratic manner, which has earned them the common name of "scuttle flies." The larvae typically develop in any decomposing organic matter, whether human, animal, or vegetative in origin. The puparia are easily recognized as they are dorsoventrally flattened, with a pair of horns or "breathing trumpets" emerging from the anterior end. Some species are predacious while others are parasitic. A few are commensals in the nests of ants and termites, while other larvae develop in fungi. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Peterson, 1979, 1987.)

Megaselia scalaris (Loew)

Common Name: Humpbacked Fly (Figures 2.32a,b,c)
This species is distributed throughout the world and its members are generally similar in appearance to the familiar fruit or vinegar flies (genus *Drosophila*). The thorax is yellow to yellowish-brown, and on close inspection the abdomen is black above with yellow to white sections both laterally and ventrally. The legs are yellow and the abdomen is yellowish with brown bands. The larvae of *M. scalaris* can develop in vegetation, carrion, or excrement. This species has been recorded as causing cutaneous, intestinal, and ophthalmic myiasis. (Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; James, 1947; Smith, 1986.)

Conicera tibialis (Schmitz)

Common Name: Coffin Fly
This is a very small black species (1.5 to 2.5 mm) often found associated with buried remains. Eggs are laid on the soil surface and the larvae of the coffin fly burrow down through holes and fissures in order to colonize bodies that have been buried. Research has shown that this and other species of phorid flies can reach remains that are buried from 30 to 100 cm deep. Material of this species recovered during exhumations has shown that apparently the coffin fly can develop and progress through many generations on buried human cadavers once they are colonized.

Large numbers of adults have been reported on the soil surface of gravesites. This behavior can be used to aid in the location of buried human remains. It should be noted, however, that adult flies tend to be associated with remains that have been buried for at least a period of months and, in some cases, for years. (Colyer, 1954a, 1954b, 1954c; Greenberg, 1971; Hall, 1948; Hall and Townsend, 1977; James, 1947; Payne et al., 1968; Smith, 1986.)

Moth Flies, Sand Flies, and Owl Midges (Family Psychodidae)

Moth flies are found throughout the world, with more than 90 species in the U.S. and Canada. They are very small as adults, usually no more than 3 to 4 mm in length. The body and wings are covered with scales (hairs) that give them a moth-like appearance. The wings come to a point at the tip and have straight, prominent, longitudinal veins. The larvae develop in moist environments and may be up to 10 mm long.

Two groups (or subfamilies) within the family Psychodidae have medical importance. Members of the subfamily *Phlebotominae* are referred to as sand flies, and are characterized by nonaquatic larvae and adults that do not hold their wings roof-like over the body. They occur in the southern U.S. and in tropical areas of the world. Female sand flies suck blood and are responsible for vectoring the disease leishmaniasis, as well as several others.

Members of the subfamily *Psychodinae* are called moth flies or owl midges. The larvae are usually aquatic and typically develop in the slime that accumulates in sewers and drains. The adult flies tend to hold their wings roof-like over the body. Species in this subfamily do not take blood, but are often considered pests due to their sheer numbers. They are common in warm, damp places and are often seen indoors on the walls of restrooms or showers. Adults run about and fly very quickly in an erratic, haphazard, and very distinctive manner.

Psychodid flies seldom constitute more than a minor portion of the arthropod fauna that colonizes human remains. However, adult moth flies are occasionally attracted to carrion and are a commonplace insect found indoors and associated with man. (Arnett and Jacques, 1981; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Peterson, 1979; Quate and Vockeroth, 1981.)

Psychoda alternata (Say)

Common Name: Moth Fly, Drain Fly, Sewage Fly, Trickling Filter Fly
(Figures 2.33a,b)
This species is found distributed throughout North America. The adults of these small, moth-like flies are gray to brown in color and range from 2 to 4 mm in length. Their wings and body are densely covered with hair. Their wings are noticeably pointed and may have black spots giving them a mottled appearance. When at rest, the wings are held spread and at a slight angle over the body.

Adults occur throughout the summer and prefer damp places. They are usually seen walking or crawling on walls or other vertical surfaces, and they fly only very short distances. Their characteristic flight is very jerky and erratic. The larvae are long, slender, and grayish white with a noticeable head and dark colored "tail," which is their respiratory horn or breathing apparatus. The larvae develop in liquid or semiliquid decaying matter, including excrement. They are commonly found in foul water, sewage, and in the filter beds of sewage plants. The latter has resulted in their common name of the trickling filter fly. Any wet, decomposing organic matter is suitable larval habitat, however, and they are particularly common in drain pipes and bathrooms.

The larvae of this fly will most likely not inhabit carrion itself, the but may be found in the excrement occasionally associated with decomposing remains. Since they have been recovered from both floating and buried bodies, their occurrence on human remains should not be taken casually. Although this species is not considered of major forensic importance, its presence may contribute information when deriving postmortem interval estimations. This species has been reported as causing human myiasis. (Bohart and Gressitt, 1951; Greenberg, 1971; James, 1947; Payne et al., 1968; Smith, 1986.)

Beetles (Order Coleoptera)

The Coleoptera is the largest order containing about a third of all known insects, which is about 300,000 species. About 30,000 of those species are found in North America. This order is of tremendous economic importance. Members of this order are characterized by having hard wing covers (called elytra) that cover and protect the membranous hind wings used for flight. Adult beetles possess chewing mouthparts and most have the ability to fly. Their feeding habits vary greatly. They can be predacious, scavengers, or vegetarians, with a few being parasitic. The larvae are called grubs, and they vary widely in appearance (Figure 2.34). However, all grubs have a noticeable head and possess six legs. Beetles are known to attack plants, infest stored foods, act as important scavengers and decomposers, and some species serve as pollinators of plants.

Carrion Beetles (Family Silphidae)

The carrion beetles comprise a large family that is nearly worldwide in distribution and has more than 1500 species. In North America there are approximately 46 species of silphid beetles, which are widely distributed. Certain members of this family also are known as sexton or burying beetles, derived from the fact that some species bury their carrion or carcass food source.

Silphid beetles are usually medium to large in size, typically ranging from 10 to 35 mm. Although adults vary greatly in size and shape, certain reliable physical characters can be used in their identification. The antennae are clubbed and either knob-like or broaden gradually. The wing covers (or elytra) that cover the back are often short and leave several abdominal segments exposed. The body also tends to be broader towards the posterior end rather than the anterior. The tarsi (feet) or terminal portion of each leg has five segments. The body is usually black, but marked with orange, yellow, or red patches of color.

Carrion beetle larvae also vary in size and shape, but are generally from 15 to 30 mm long. Most tend to be flattened, with some species (e.g., *Oiceoptoma inaequale*) almost trilobite-like in appearance (see Figure 2.44). All larvae seem capable of mobility even when they remain in the same place throughout larval development.

The habits of silphid beetles are unusual and have not been entirely observed. While some larvae develop in rotting vegetable material, others are predacious. However, most species are attracted to and feed on decaying animal carcasses. In the case of the sexton or burying beetles (genus *Nicrophorous*), adults bury small animal carcasses upon which they lay their eggs. In some species, a depression is made in the decaying flesh to house a group of developing larvae that the parents feed and protect. The burying of the food source may be a means of eliminating competition with other carrion feeding insects such as flies. In both the burying beetle group and the carrion beetle group (genus *Silpha*) of the silphids, the larvae apparently feed on carrion while the adults consume primarily maggots. (Abbott, 1937; Anderson and Peck, 1985; Arnett and Jacques, 1981; Arnett et al., 1980; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Dorsey, 1940; Hogue, 1993; Illingworth, 1927; James and Harwood, 1969; Payne and King, 1970; Peterson, 1979; Steele, 1927; White, 1985.)

Heterosilpha ramosa (Say) (= *Silpha ramosa*)

Common Name: Garden Carrion Beetle (Figure 2.35)
The garden carrion beetle is found throughout the western U.S. from California east to New Mexico and north to Nebraska and Montana. It also occurs in the southern and western half of Canada, as well as northern portions of Mexico. The adult ranges from 14 to 18 mm in length. The pronotum and elytra are a velvety black to dark brown. The pronotum is smooth and contrasts with the rough elytra, which have noticeable ridges. The elytra are short, exposing the last two abdominal segments. The larvae are dark brown to black and have a light brown stripe along the dorsal surface.

Garden carrion beetle adults are most active in spring and remain active throughout the summer months. They are found on fresh remains and throughout the later stages of decomposition. The eggs are typically laid in the soil around a carcass rather than on the remains themselves. The adults and larvae feed on carrion and other insects, particularly maggots. (Anderson and Peck, 1985; Brewer and Bacon, 1975.)

Necrodes surinamensis (Fabricius)

Common Name: Suriname Carrion Beetle (Figures 2.36a,b,c)
This species is commonly found throughout the U.S. (except in the Southwest) and throughout central and southern Canada. The pronotum is shiny black, while the elytra are dull black with red markings near the tips. However, the patterns on the wing covers vary greatly from completely black to having two transverse red bands (or rows) of red spots near the tip. There are pronounced lengthwise ridges along the elytra, which are rounded at the tip and not truncate as in *Nicrophorus*. The elytra do not quite cover the entire abdomen and leave the tip exposed. The larvae are a dark reddish-brown with a light brown dorsal stripe.

Adult Suriname carrion beetles are primarily nocturnal and emerge in early spring after overwintering in the adult stage. Both the adults and larvae are most common on large carcasses such as bear, deer, and human. The adults can secrete an offensive odor as a mode of defense when disturbed. (Anderson, 1982a; Anderson and Peck, 1985; Dorsey, 1940; Ratcliffe, 1972.)

Necrophilia americana (Linnaeus) (= *Silpha Americana*)

Common Name: American Carrion Beetle (Figures 2.37a,b)
The American carrion beetle is found east of the Rocky Mountains from eastern Texas north to Minnesota, and from Maine to Florida in the U.S. They also are present in southeastern Canada. The adults are 12 to 22 mm in length. The adult body is oval in shape and has a large yellow pronotum with a black center. The elytra are dull brownish-black to black with three raised ridges connected by smaller cross ridges. In the northern extent of its range, the elytra have a yellow tip, and in the southern portions the elytra are entirely black. The elytra are shortened, exposing the tip of the abdomen. The head, legs, antennae, and underside of the beetle are black. The larvae of this species are black and appear armored. A light brown dorsal stripe may be visible on some specimens.

Adult and larval American carrion beetles feed on carrion, fly larvae, and the larvae of other beetles. They are found from spring to fall, and overwinter in the adult stage. The adults of this species are diurnal (active during the daylight hours). (Anderson and Peck, 1985.)

Nicrophorus americanus (Olivier)

Common Name: American Burying Beetle (Figure 2.38)
This species is most common in the eastern portions of the U.S., from Florida to eastern Texas and north to Michigan. They are collected only rarely in southeastern Canada. This is the largest member of the genus *Nicrophorus*, with the adults ranging up to 30 to 35 mm. However, this species is rarely collected due to their nocturnal behavior and the fact that habitat destruction has restricted their distribution and abundance. *N. americanus* is officially considered an endangered species. The pronotum is slightly oblong and is a distinctive reddish orange, while the elytra have orange and black markings. (Anderson, 1982b; Anderson and Peck, 1985.)

Nicrophorus carolinus (Linnaeus)

Common Name: Burying Beetle (Figures 2.39a,b,c)
This burying beetle can be distinguished from other species in its genus by the extremely narrow pronotal margin. The pronotum appears slightly dull and punctate in the anterior

half, and more smooth and shiny in the posterior portion. This beetle is typically 12 to 18 mm in length and can be found in the Atlantic coastal states from Virginia to southern Georgia, throughout Florida, and along the gulf coastal states from southern Alabama to east and northern Texas. This species also ranges north of Texas into eastern Colorado and southeastern Wyoming. It has been recovered from isolated localities in Arizona. Although little is know about the biology and natural history of this species, it is restricted to open, sandy habitats in sparsely wooded areas. Although most beetles display the common orange and black color pattern found in other burying beetles, some individuals of this species are entirely black in populations of the desert Southwest. (Anderson and Peck, 1985.)

Nicrophorus investigator (Zetterstedt)

Common Name: Burying Beetle (Figures 2.40a,b)
Unlike *Nicrophorus carolinus*, the margin of the pronotum is very wide and pronounced. This is a relatively large beetle, reaching 22 mm in length, and has a variable color pattern on the elytra. This species is found primarily in the northwestern U.S.; however, individuals have been collected as far south as Arizona and New Mexico. They also are commonly collected along the U.S. and Canadian border in the east and throughout Canada. *N. investigator* overwinters in the pupal stage, and the adults appear in early summer. The adults have been shown to be both diurnal and nocturnal and, therefore, able to seek carcasses both during light and dark hours. Variations in color and elytral patterns are extensive in this species depending on geographic habitat. (Anderson and Peck, 1985.)

Nicrophorus marginatus (Fabricius)

Common Name: Margined Burying Beetle, Margined Sexton Beetle (Figure 2.41)
This colorful silphid beetle is found in southern Canada and throughout the U.S., except for southern Georgia and Florida. It is 15 to 22 mm in length with shortened or truncated elytra that expose the last couple of abdominal segments. Their bodies are shiny black with two orange bands on the elytra. The pronotum is a shiny black disk with a distinctive row of orange hairs behind the head.

The adults are mainly nocturnal and are most active during the summer months. The larvae and the adults are predacious on fly larvae. This species can be found at fresh remains as well as throughout the later stages of decay. *N. marginatus* overwinters as adults. Pairs of adults often bury small animal carcasses, mold them into a ball, and deposit their eggs on them. The larvae are cared for and fed periodically once they are hatched. (Anderson and Peck, 1985.)

Nicrophorus orbicollis (Say)

Common Name: Sexton Beetle (Figures 2.42a,b,c,d)
This sexton beetle is found in central-southern and southeastern Canada, as well as in eastern Texas north to the Dakotas. The pronotum is black and slightly oblong in appearance on the adults. The elytra are also black with two rows of orange spots, and they are short exposing the last few abdominal segments.

N. orbicollis is most abundant during the midsummer months and they overwinter as adults. They are mainly nocturnal and most commonly found in wooded habitats. (Anderson and Peck, 1985.)

Nicrophorus tomentosus (Weber)

Common Name: Gold-Necked Carrion Beetle (Figure 2.43)
Nicrophorus tomentosus is easily recognized by the dense covering of gold hairs on the pronotum, which gives this species its common name. This species is smaller than other species in its genus, generally only about 15 mm long. *N. tomentosus* is found throughout the eastern and central U.S. (primarily east of the Rocky Mountains), with the exception of Florida and southern Texas. It also is found in southeastern and southcentral Canada. As with most other silphid beetles, they are active in the summer; however, unlike other species in its genus, the adults do not actually bury a carcass. Instead, they remove soil from under the carcass so that it sinks into the excavation. They then cover the freshly dug pit with leaf litter and surface debris. The larvae feed on the carcass and pupate in the adjacent soil. There is no known geographic variation in color or elytral pattern with this species, and it occurs in almost any geographic habitat throughout its range. (Anderson and Peck, 1985; Pirone, 1974; Shubeck, 1976.)

Oiceoptoma inaequale (Fabricius)

Common Name: Carrion Beetle (Figure 2.44)
This carrion beetle is found throughout the eastern U.S. from Texas to Florida and north to New York. It also is found in the extreme southeastern portions of Canada. Adults range from 13 to 15 mm in length. The pronotum and the elytra are black in color. The larvae vary from light brown to a dark reddish-brown.

O. inaequale is most common in forested areas where it arrives at carcasses before *O. noveboracense* and *N. americana*. The adults are diurnal and are most abundant in the spring through fall. They are very similar in appearance to *O. rugulosum*. (Anderson, 1982a; Anderson and Peck, 1985; Dorsey, 1940; Reed, 1958.)

Oiceoptoma noveboracense (Forster)

Common Name: Carrion Beetle (Figure 2.45)
This silphid beetle is found in the northeastern and central U.S. as far west as Wyoming and as far south as Texas. It also occurs in southern Canada. The adults are 13 to 15 mm in length. The center of the pronotum is black to dark brown with distinctive orange-red margins along the outer margins. The elytra vary from reddish brown to brownish-black to black in color with prominent lengthwise ridges. The larvae are light brown to a dark reddish-brown.

This species is most abundant in the early spring and it overwinters in the adult stage. The adults are diurnal and are most common in forested habitats. (Anderson and Peck, 1985.)

Oiceoptoma rugulosum (Portevin)

Common Name: Carrion Beetle (Figures 2.46a,b)
This carrion beetle is found in Florida, Louisiana, and Texas. It replaces *O. noveboracense* and *O. inaequale* in these localities. This is a dull black species with an oval body, and the elytra have three to four distinct longitudinal ridges. *O. rugulosum* can be found on human remains in most any stage of decomposition where both the adults and larvae are predatory on fly larvae. (Anderson and Peck, 1985.)

Thanatophilus lapponicus (= *Silpha lapponica*) (Herbst)

Common Name: Lapland Carrion Beetle, Northern Carrion Beetle, Common Carrion Beetle (Figure 2.47)

The Lapland carrion beetle sometimes also is referred to as the northern or common carrion beetle. In the eastern U.S., it is found chiefly in the northernmost portion, but in the western U.S. it ranges south to California, New Mexico, Arizona, and then north to Alaska. It also is distributed throughout Canada. Adult beetles are 10 to 14 mm in length. The pronotum is black and has gray to gold pubescence. The elytra are brownish-black and appear very bumpy. The body is gray to black, but it is covered with golden yellowish hair that sometimes appears gray. The body is slightly oblong in shape and appears very punctate when view from above.

This species prefers open habitats where it is predominant in the summer. Lapland carrion beetles can be found at both fresh remains and during the advanced stages of decay. The adults and larvae are predacious on other insect larvae. This species is very cold tolerant and overwinters in the adult stage. (Anderson, 1982a; Anderson and Peck, 1985; Dorsey, 1940.)

Skin Beetles, Leather Beetles, Hide Beetles, Carpet Beetles, and Larder Beetles (Family Dermestidae)

Members of this family also have been given the common names of hide beetles, carpet beetles, and larder beetles, based on their food preferences. Dermestid beetles are worldwide in distribution with over 500 species, approximately 123 of which are found throughout North America. Dermestids are generally small beetles, ranging from 2 to 12 mm in length. They are rounded to oval in shape and covered with scales that may form distinctive and colorful patterns. The larvae range from 5 to 15 mm and are usually covered with tufts of long, dense hair.

This family represents one of the most economically important groups of beetles in the world. The carpet beetle species damage rugs, clothing, and furniture. Others like the khapra beetle, infest grains and inflict serious losses on stored products. Still others, like the hide beetles, may ruin leather goods or destroy irreplaceable museum specimens, especially mounted insects. Almost all species are scavengers and feed on various types of dried animal tissue.

Skin beetles, especially in the genus *Dermestes*, can be of considerable forensic importance. In sufficient numbers, they have been reported as reducing a human body to a skeleton in only 24 days. Due to this uncanny ability, beetles have been employed for decades in the removal of flesh from the bones of museum specimens. The larvae are typically found on human corpses during the dry and skeletal stages of decomposition. They move away from light and will hide in any cavity or recess that is available. A close and detailed examination of remains may be required to collect the small, young larvae.

The adults are cannibalistic and will eat young larvae and pupae. For this reason, adult dermestid beetles should be put in separate containers from the immature stages. These beetles can be found in indoor situations on bodies throughout the year, but species of the genus *Dermestes* are most active during the warmer months. The presence of dermestid beetles or their sawdust-like frass (fecal material) is often an indication that considerable time has elapsed since death. The mere presence of their frass is proof that dermestid beetles have fed extensively on the tissues. In some cases where remains are mummified,

living dermestid adults and larvae may still be associated with the remains after a period of years. (Arnett and Jacques, 1981; Arnett et al., 1980; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Peterson, 1979; Smith, 1989; Voigt, 1965; White, 1985.)

Dermestes ater (DeGeer)

Common Name: Black Larder Beetle or Incinerator Beetle (Figures 2.48a,b,c)
The black larder beetle is cosmopolitan in distribution and appears similar to *D. maculatus*, but the elytra are not serrated. The elytra appear a dark to light brown in color and have scattered yellow hairs. This species also is distinctive in that the ventral pattern is yellowish and not white. The larvae can be easily distinguished from others by the two spines near the posterior end that extend backward and are not strongly curved. This beetle is a serious pest of dried fish, mushrooms, and cheese, and it is particularly attracted to the protein-rich tissues of decaying vertebrates. This beetle is attracted to dried pet food as well and can sometimes be found associated with the waste materials burned in incinerators.

Dermestes caninus (Germar)

(Figures 2.49a,b)
This species of dermestid beetle is distributed throughout the U.S. except for the Pacific Northwest. Like all dermestid beetles, they are attracted to stored food products and carrion of all types. This species has been found in the nests of predatory birds, apparently attracted by the prey remains. *D. caninus* and other members of this family overwinter in the adult stage and become active during the early summer months when the females readily enter dwellings in search of egg-laying sites.

Dermestes maculatus (DeGeer)

Common Name: Hide or Leather Beetle (Figures 2.50a,b)
This species of skin beetle is worldwide in distribution. The adults are 5 to 10 mm in length and black to reddish brown on the dorsum or upper surface. They also have characteristic black and white markings on the ventral surface. The apex of the elytra are serrated or saw-toothed and end in a terminal spine where they come together. The larvae of *D. maculatus* are dark brown and have a broad light brown to yellow stripe extending lengthwise along the body. They are easily identified by the fact that the spines near the tip of the tail curve forward, towards the head of the larvae. This and other species of dermestid larvae are covered with tufts of long dark hairs.

Dermestid Frass (Peritrophic Membrane)

(Figures 2.51a,b)
Beetles in the family Dermestidae produce a protective membrane that lines their gut and surrounds their food meal. This lining protects the gut walls from abrasion during the digestive process. As the digested food is passed from the beetle's body, the fecal material is wrapped in the protective membrane, which passes in an unbroken chain. This frass material is light brown, stringy in appearance, and quite dry. It crumbles very easily when disturbed. Dermestid beetles generally prefer dry tissues on which to feed, and it is not uncommon to find these beetles associated with decomposed and mummified human

remains found in indoor locations. Although generally indicative of an extended PMI, peritrophic membrane has been found associated with remains from as little as 4 months to 10 years. Due to this variability, a qualified forensic entomologist should be consulted before a PMI determination, based on the simple presence of peritrophic membrane, is attempted. A trained forensic entomologist is likely to find additional entomological evidence to help support the PMI estimation. However, in itself, dermestid frass and the associated peritrophic membrane is valuable entomological evidence since it indicates the conditions that were likely present throughout the decomposition process.

Dermestid Damage to Human Skin

(Figures 2.52a,b)
In many cases that involve extensively decomposed or mummified human remains, skin tissue often remains intact. The remaining tissues often contain many holes and they are sometimes mistaken for gunshot wounds. The feeding maggots usually produce symmetrically round holes that are of a uniform size. Dermestid adults and larvae often create the same symmetrical artifacts. However, they also produce holes that are irregular in size, as well as tears that resemble lacerations and abrasions. The edges of these artifacts are often irregular and jagged, not smooth as with those produced by feeding maggots.

Rove Beetles (Family Staphylinidae)

The rove beetles are the largest family of insects in North America north of Mexico with over 4100 species. They are widely distributed and found in various habitats. The adult beetles vary greatly in size, ranging from 1 to 25 mm. The characteristic shape of many species attracted to carrion, however, makes them easy to identify to the family level. Others members of this family do not have the typical shape, but they are not frequently found at carrion.

Typical rove or staphylinid beetle adults are slender, elongate, and have very short wing covers or elytra. The elytra typically appear square and are approximately as long as they are wide. Although the membranous hind wings remain folded beneath and completely concealed (except during flight), six to seven abdominal segments are exposed. This makes the rove beetle appear to be divided into four sections. The head, thorax, and wing covers make up the first three sections and are approximately equal to each other in size. The fourth section is the exposed abdomen, which is roughly equal to all of the first three together.

Although earwigs (order Dermaptera) (see Figure 1.23A) are vastly fewer in number of species than are the rove beetles, they seem better known to the general public. Because earwigs are elongate insects with short elytra, people often mistake staphylinid adults for earwigs, although staphylinds do not have pincer-like cerci at the tip of the abdomen.

Staphylinid larvae are typically long, slender, pale in color, and may have a darker head. Many larvae and adults have mandibles that are long and curved, which may cross over in front of the head. Larvae and adults are typically quick-moving and predacious on smaller insects. Some, however, eat fungi or diatoms. Even among those that are predacious, some have specialized diets whereas others are generalists. The species attracted to carrion feed on maggots and the larvae of other insects. The adults are strong flyers and often run about with the abdomen raised in the air as if they were capable of stinging, although they

are unable to do so. (Abbott, 1938; Arnett, 1961; Arnett and Jacques, 1981; Arnett et al., 1980; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Mank, 1923; Peterson, 1979; Stehr, 1991; Voris, 1939; White, 1985.)

Creophilus maxillosus (Gravenhorst)

Common Name: Hairy Rove Beetle (Figures 2.53a,b,c)
Adults range in length from 12 to 18 mm, but all are black bodied and covered with patches of pale yellow hairs. This species is found throughout the eastern U.S. The body is very slender and is typical of rove beetles, appearing to be divided into four sections. Both the adults and larvae are predacious on maggots. The adults may be found on carcasses only hours after death as well as during the advanced stages of decomposition. When threatened or disturbed, they often hold their abdomen curved upward and forward above the head as if to sting. Although they are unable to sting, they can emit an offensive odor as a defense mechanism.

Platydracus comes (LeConte)

Common Name: Brown Rove Beetle (Figure 2.54)
The adults of this species range in length from 10 to 15 mm and are brown with black markings. The body is very slender and appears divided into four sections. Very little is known about this species, but it is commonly attracted to carrion and frequently recovered from bodies in the southeastern U.S.

Platydracus fossator (Gravenhorst)

Common Name: Spotted Rove Beetle (Figure 2.55)
The adults of this rove beetle range from 12 to 18 mm in length. The body is dark blue (and may appear black depending on lighting conditions) with two red spots on the elytra. Some specimens may have a faint band of orange hair near the tip of the abdomen. Although detailed information is lacking on the habits of this species, it is often encountered on carrion and frequently found in association with human remains in the southeastern U.S.

Platydracus maculosus (Gravenhorst)

Common Name: Rove Beetle (Figure 2.56)
Adults are shaped like other *Platydracus* adults and are mottled dark brown with golden hairs. Little is known about the biology of this species, but it is frequently recovered on decomposing remains where it is a predator on fly larvae. The adults and larvae of this species should be collected as evidence whenever encountered at a crime scene.

Platydracus tomentosus (Gravenhorst)

Common Name: Rove Beetle (Figure 2.57)
This rove beetle is 7 to 13 mm in length and dull brown in color. The body has the same general shape as all other *Platydracus* adults, and this species is frequently found at carrion.

Clown Beetles (Family Histeridae)

This is a large family of over 3000 species, more than 500 of which are widely distributed throughout North America. Clown beetles are usually small, seldom getting beyond 10 mm in length. They are rounded, shiny beetles that are black or sometimes metallic green. The elytra are short and squared at their apex, exposing the last two abdominal segments. Therefore, the adults appear to be divided into three longitudinal sections, with the central section bearing a line down the middle. The antennae of clown beetles are both elbowed and clubbed.

Clown beetles are very common on carrion and excrement, as well as on fungi and decaying plant material. When on carcasses, they tend to stay concealed in the soil underneath during the daylight hours, becoming active at night. Both the larvae and adults are predacious and feed readily on maggots and fly puparia. They also have been observed feeding on the larvae of dermestid beetles. As with other predatory species that are collected from a crime scene, to be maintained alive, clown beetles should be isolated into separate containers. (Arnett and Jacques, 1981; Arnett et al., 1980; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Peterson, 1979; White, 1985.)

Hister sp.

Common Name: Clown Beetle (Figures 2.58a,b,c)
Species in this genus are commonly 4 to 5 mm in length and are worldwide in distribution. Their body color is most frequently a shiny jet black, but in some species can be brown, red, or metallic green. The body shape is very convex in profile, and the elytra are short and cut square at the apex exposing the last two abdominal segments.

Hister species are found on cadavers from bloat through the dry stages of decomposition. The adults and larvae are chiefly nocturnal feeding on carrion, maggots, and other insect eggs and larvae. The adults fly very well and can run swiftly. When disturbed, they pull their legs and antennae tightly against their body and lie motionless feigning death. Due to this feigning behavior they are often overlooked and not collected. (Arnett and Jacques, 1981; Arnett et al., 1980; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; Holdaway and Evans, 1930; James and Harwood, 1969; Nuorteva, 1970; Peterson, 1978; White 1985.)

Saprinus pennsylvanicus (Paykull)

Common Name: Clown Beetle (Figure 2.59)
The body of this clown beetle is oval, convex, and a shiny metallic green. This species, like other clown beetles, feigns death when disturbed by pulling the legs close to the body and lying motionless. *S. pennsylvanicus* feeds on fly eggs and maggots, and is often found underneath the body. This species is worldwide in distribution and is noted to occur from the fresh throughout the later stages of decomposition.

Checkered Beetles (Family Cleridae)

There are approximately 3500 checkered beetle species worldwide, more than 500 of which occur in North America. The bodies of most species are covered with bristly hairs and are

often brightly colored. Adults range from 3 to 12 mm in length. The head is often wider than the pronotum (neck area) which is narrower than the wing bases. This gives the appearance of a narrowing between the head and the point where the wings start. The antenna types found in this family are variable.

Both the larval and adult clerid beetles are predacious. Most species prey on the immature stages of various wood-boring beetles, while others feed on maggots. They are common visitors to decomposing animal matter in the later, drier stages of decomposition. Adult checkered beetles are frequently found on flowers. (Arnett and Jacques, 1981; Arnett et al., 1980; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Peterson, 1979; White, 1985.)

Necrobia rufipes (DeGeer)

Common Name: Red-Legged Ham Beetle (Figures 2.60a,b,c)
This species is worldwide in distribution and can be recovered throughout the U.S. This is a small beetle ranging from 3 to 7 mm in size. It is very distinctive with a shiny, metallic blue body and red legs. The larvae are brightly colored with purple specks on the body and a reddish-brown head and tail.

The common name originates from the habit of this species of infecting cured meat products. The adults and larvae also are predacious on eggs and maggots, and will sometimes feed on carrion. For most of the U.S., they are commonly found throughout the year on human corpses in outdoor habitats during the drier stages of decomposition. The adults may be found on carrion earlier in the sequence of decomposition, but colonization does not usually occur until much later. The adults are slow fliers and typically run instead of flying. However, their small size, relatively low numbers, and rapid movement can make them difficult to collect despite the fact that they do not often take flight. (Clark, 1895; Payne and King, 1970.)

Hide Beetles (Family Trogidae)

The trogid hide beetles are a small family of which approximately 50 species are widely distributed throughout North America. This group is sometimes considered or treated as a subfamily (Troginae) of the extremely large and cosmopolitan family of beetles known as the scarabs (family Scarabaeidae).

Trogids range from 5 to 20 mm in length and are quite distinctive in appearance. They are usually oblong to oval in shape and are basically similar in form to scarabs such as the June beetle. However, trogids tend to be brown in color and have the back or dorsum of the body rough and covered with ridges.

Trogid hide beetles are often present on carrion and carcasses. As many as eight different species have been collected from a single set of animal remains. However, adults often become covered with mud and animal tissue that becomes encrusted on the body. This results in the beetle looking very much like a small piece of debris which can be easily overlooked. Trogids are typically attracted to dry remains and are found during the advanced stages of decay. (Abbott, 1937; Arnett and Jacques, 1981; Arnett et al., 1980; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hogue, 1993; James and Harwood, 1969; Payne and King, 1970; Peterson, 1979; Spector, 1943; White, 1985.)

Trox suberosus Fabricius

Common Name: Hide Beetle (Figures 2.61a,b)
This species can be found throughout the U.S. and Canada. The dorsal surface of the body is very rough, convex, and light brown in color. When alive, they are often covered with dirt and debris and, thus, are often overlooked. *T. suberosus* is one of the last in the succession of insects on decomposing remains. They are carrion feeders and, like most trogids, occur on carcasses from advanced decomposition through the dry decay stages. When disturbed, they draw in their legs and lie motionless, resembling dirt or rubbish. This behavior increases the chances that they go unnoticed and uncollected by forensic investigators. *T. suberosus* overwinters in the adult stage becoming most active during the summer months. (Payne and King, 1970.)

Scarab Beetles (Family Scarabaeidae)

Scarab beetles represent one of the largest beetle families with over 19,000 species worldwide and with 1400 species in North America alone. The members of this family vary greatly in size, shape, and color. They also are generally elongate, robust beetles that are usually convex in profile, and they exhibit great differences in biology, ecology, and behavior. Most beetles in this family feed on dung, carrion, or decomposing plant materials, while others feed on flowers, fruits, and foliage of living plants. Some are serious pests of ornamental plants and agricultural crops. A few feed on fungi, and some live in the nests and burrows of vertebrate animals.

Dung beetles, or tumblebugs, belong to the subfamily Scarabaeinae and are found associated with dung and carrion. The adults of those species that are most likely to be encountered in association with cadavers are dull-colored, rounded, and less than an inch in length (Figure 2.62). At least 14 species of scarabs have been recorded on vertebrate carcasses in the U.S. The larvae of this family are C-shaped and are commonly referred to as "grubs" (Figure 2.63). The larvae of dung beetles develop from an egg laid in a ball of dung that is collected by the adults. These larvae are generally white with a brown head. Occasionally, these larvae can be found buried in the soil underneath the body. (Woodruff, 1973.)

Deltochilum gibbosum gibbosum (Fabricius)

Common Name: Tumblebug (Figure 2.64)
These are large and robust beetles that generally attain a length of 30 mm, making them one of the largest species of dung beetles in North America. The beetles are dull black in color, with pronounced ridges (striae) on the elytra. They occur in Alabama, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, and Texas. Although it occurs throughout the year in Florida, it is primarily nocturnal, and as a result few behavioral observations have been recorded. The adults roll almost any decaying plant or animal substance into a tightly packed ball, which is then buried. The eggs are deposited within the rolled ball and it serves as the larval food source. Although this species is attracted to many decomposing substances, it is particularly attracted to chicken. It is also commonly found on decomposing vertebrate carcasses and human dung. (Woodruff, 1973.)

Phanaeus vindex (MacLeay)

Common Name: Dung Beetle (Figure 2.65)
This prominent beetle is widely distributed from Massachusetts to Florida (except in the Everglades and the Florida Keys), west to Texas, and north to South Dakota. The male has a rhinoceros-like horn not found on the female. Both sexes have dark green elytra that are very iridescent. The head and pronotum are a reddish to coppery-gold color that has a metallic luster. Both sexes are very conspicuous on sunny days in open pastures and fields. This well-known and widely distributed beetle has a preference for dung and, in particular, human dung. *P. vindex* rolls dung into tightly packed balls which are stored in vertical burrows a few inches beneath the dung source. The beetle's eggs are then deposited in the dung ball. (Woodruff, 1973.)

Sap Beetles (Family Nitidulidae)

This is a large cosmopolitan family with more than 2500 species worldwide. In North America, approximately 183 species are found. Sap beetles are considerably variable in appearance, most species being dark in color and from 4 to 12 mm long. All have distinctly clubbed antennae, with the club portion rounded and ball-like. Many species are oval in shape, but some are very similar to rove beetles (family Staphylinidae) in appearance. These species have short elytra that leave from 1 to 3 abdominal segments exposed. Rove beetles, however, do not have clubbed antennae.

Most sap beetles are attracted to rotting fruit and decaying or fermenting vegetable matter. Some also are associated with fungi or flowers. A few species are attracted to carrion and decomposing animal remains, and are usually found during the more advanced stages of decay. One such species is *Omosita colon,* a very small, dark brown sap beetle that is only 2 to 4 mm in length (Figure 2.66). Although commonly found at the same time as skin beetles (family Dermestidae), sap beetles appear to prefer a moister environment. Little work has been done to establish the value of nitidulid beetles in forensic investigations. (Arnett and Jacques, 1981; Arnett et al., 1980; Bland and Jacques, 1978; Borror and White, 1970; Borror et al., 1989; Castner et al., 1995; Hinton and Corbet, 1975; Hogue, 1993; James and Harwood, 1969; Payne and King, 1970; Peterson, 1979; White, 1985.)

Other Arthropods of Forensic Importance

Venomous Arthropods

Forensic investigators often consider insects and other arthropods solely for their importance and value in determining the time of death, forgetting that they may be the causal agents of death themselves. Venomous arthropods cause only a small number of deaths each year in the U.S., yet still should be mentioned since the possibility of involvement in such cases exists. There are a number of circumstances where arthropod envenomization can lead to fatalities.

Spiders such as the brown recluse (*Loxosceles reclusa*) (Figure 2.67) and the widow group including the black widow (*Latrodectus mactans*) (Figure 2.68), red widow (*Latrodectus bishopi*), and brown widow (*Latrodectus geometricus*) (Figure 2.69), all have potent venom, which are introduced via fangs when the spiders bite. The bite of the brown recluse

causes extreme destruction of flesh, often requiring the removal of significant amounts of tissue. Widow bites are usually less severe, but more frequent due to their tendency to invade dwellings and take refuge in dark, enclosed areas. In the most common scenario, a person comes in contact with the spider without seeing it by sitting on it or disturbing it in some similar manner. Scorpions (Figure 2.70) inject venom through a stinger on the tail, often quickly penetrating the skin in several different spots. Fortunately, few scorpions in the U.S. are deadly. Centipedes (Figure 2.71) are found throughout most of the U.S. Although many are capable of breaking the skin and inflicting a severe bite, the pain typically diminishes quickly and gradually disappears. The bite of a centipede has been compared in severity to that of a wasp sting. Only one fatality due to the bite of a centipede has been recorded.

Bees (Figure 2.72), wasps (Figure 2.73), hornets, and yellowjackets (Figure 2.74) all belong to the insect order Hymenoptera. They are social insects and live in colonies that can sometimes grow to proportions where sheer numbers are dangerous even though the amount of venom carried by an individual is small. In a worst case scenario, the insects are both dangerous and aggressive as seen with the Africanized or killer bees (*Apis mellifera scutellata*) that have recently invaded the U.S. Even when social insects are not naturally aggressive, most will vigorously defend their nest against any intruder. Problems occur when the nests are constructed in close proximity to human habitations and activities. The inadvertent disturbance of a nest can lead to dozens of stings for the unfortunate individual, or in some cases even death. In the semitropical areas of Florida, yellowjacket (*Vespula squamosa*) nests (Figure 2.75) have been removed which contained over 200,000 adult wasps.

It should be noted that certain individuals are allergic to arthropod venom in any amount. Such people upon being stung or bitten can experience anaphylactic shock within minutes. Swift and immediate treatment, usually via the administration of epinephrine, is necessary to save the life of those allergic to insect stings. In cases where death results, an examination of internal organs may be necessary to confirm the cause, as external signs of trauma can be minimal.

People allergic to bees and wasps (or merely believing themselves to be allergic) will sometimes act in an uncontrollable and irrational manner in such an insect's presence. Although comical under certain circumstances, all too often this occurs during the operation of a motor vehicle. A certain amount of traffic deaths every year result from the strenuous efforts and overreaction of drivers attempting to rid themselves of bees or wasps that have entered their car.

Crime scene technicians and death investigators often receive stings while attempting to process death scenes since wasps are attracted by the large number of flies on which they feed. Accidentally collecting or crushing a wasp during scene processing often results in stings.

Scavengers

Many scavengers and predatory insects that do not specialize in feeding on carrion will utilize it opportunistically as a food source when it is available. For example, paper wasps and yellowjacket wasps (both family Vespidae) may sometimes be observed tearing off chunks of tissue and flying away with them. While the actual amount of flesh removed is usually minimal, the feeding activities of insects, as with much larger vertebrate scavengers and predators, alter the remains and may leave artifacts that are difficult to interpret. For

example, ants will often feed on human skin and body tissues when the remains are left outside and exposed. In the southeastern U.S., at least one species of fire ant (*Solenopsis invicta*) is very common and aggressive in their foraging habits. The feeding of these tiny, 3 to 4 mm long ants often leave postmortem damage to tissue that appears to be premortem burns (Figures 2.76a,b,c). Acrobat ants also affect the fauna of primary colonizers on remains by feeding on fly eggs and maggots (Figure 2.77). In some instances the predation rate of ants on fly eggs may be so great that initial colonization may be delayed 2 to 3 days.

In indoor conditions, cockroaches may alter a body in a similar manner as to that of fire ants in outdoor conditions. Cockroaches are scavengers on filth and refuse, but some species will feed on carrion when available, or even on living animals as well as the skin of living humans. Often babies that are left unattended in tenements and housing where unsanitary conditions prevail have been found with wounds indicating cockroach feeding. Cockroaches belong to the insect order Blattaria and are cosmopolitan in distribution. The species typically found indoors in the U.S. are the German cockroach (*Blatella germanica*) (Figure 2.78), the American cockroach *(Periplaneta americana*) (Figure 2.79), and the Australian cockroach (*Periplaneta australasiae*) (Figure 2.80).

Several other outdoor scavengers are frequently found in association with bodies and animal carcasses. Among the most common are pillbugs and sowbugs, sometimes called isopods (Figures 2.81a,b). These small, gray armored-looking creatures represent the only terrestrial crustaceans known. Some are capable of rolling up into a tight ball for protection, at which time they are about the size of a small pea. They are usually found in mulch, under rocks, or in damp earth, but sometimes occur in large numbers at carrion. Like other scavenging soil organisms such as millipedes (Class Diplopoda) (Figure 2.82), they are often in the protected area underneath the remains where the body comes in contact with the soil.

Conclusion

This chapter has featured color photography of some of the most common species of insects of forensic importance in the U.S. and Canada. Although this work is not inclusive of every insect species that an investigator might recover at a death scene, it concisely represents their general appearance. It is hoped that investigators will be able to improve their collection techniques by learning the general appearance of forensically important insects, and thereby have a higher and more successful recovery rate of these specimens. If the investigator becomes familiar with the appearance of the insects listed in this chapter and follows the collection methodologies as described in Chapter 3, he/she will be able to provide the forensic entomologist with entomological evidence that will yield the maximum amount of information possible. Additionally, procedures at the crime scene will be optimized as the investigator will spend less time collecting transient and incidental insects and focus their collection efforts on the insects with the most evidentiary value.

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Collection of Entomological Evidence during Death Investigations

3

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Contents

Introduction

Acceptance of insects as indicators of a variety of critical forensic parameters has steadily increased within the U.S. over the past several years. Analysis and expert opinion of forensic entomologists are now routinely solicited in both criminal and civil investigations. The widespread acceptance of forensic entomology within the criminal justice system and the resulting demand for entomological services by the law enforcement community, has made it necessary for death investigation professionals to become increasingly involved in the collection, preservation, and shipment of insect evidence to qualified forensic entomologists. Additionally, this need has caused nonforensically trained entomologists to become involved in the identification and analysis of insect and related evidence.

Central to the utilization of insects as forensic indicators is the proper recognition, collection, preservation, and shipment of the entomological samples. Accurate determination of specimens to the species level can be attained only if morphological characteristics are intact and specimens are properly preserved for study. In addition, thorough and proper documentation of circumstances, scene characteristics, and other supplemental data must

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be accurately recorded. The judicial system mandates the utilization of practices and proceedings that ensure that proper custody is maintained and a proper chain of custody should be established for entomological evidence as soon as it is collected from the scene.

Entomological Procedures

Given the frequency and diversity of untimely death scenarios across North America, forensic practitioners encounter numerous and varied habitats and environments when called to death scenes. Important insect evidence may be recovered from enclosed structures in urban areas such as houses, apartments, abandoned buildings, car trunks, or in dumpsters sometimes filled with an almost bewildering array of refuse. Contrasting environments such as forests, mountain slopes, deserts, swamps, irrigation ditches, riverbanks, lakes, ponds, or agricultural fields also are frequently the site of human remains recovery. Therefore, the following procedures are applicable to most geographic areas or habitats. Other works detail procedural instructions and may be consulted as well (Lord and Burger, 1983; Smith, 1986; Catts and Haskell, 1990; Wecht, 1995; Haglund and Sorg, 1997).

Working the Death Scene

When the forensic entomologist arrives at the death scene, a detailed overview of the physical surroundings, location and placement of the remains, and routes of ingress and egress must be developed. From an entomological viewpoint, it is desirable for the remains to be undisturbed and that limited numbers of individuals have entered the confines of the scene. Excessive activity can impact the presence of both flying and crawling insects on and around the remains. Therefore, immediate consultation and coordination with the ranking investigator as to who and how many individuals have already been in close proximity to the remains is crucial. Also, the forensic entomologist must be advised of the routes to and from the body, what physical evidence is in place, what has been recovered, and what not to disturb. All such information is critical during the initial stages of the entomological assessment. Entomologists work as a part of a forensic team and may be required to coordinate all collection and preservation activities with the assistance of either the primary investigator or a designated subordinate. In either case, the forensic entomologist should contact the primary investigator immediately upon arriving at the scene.

Protection of the scene is required to preserve the integrity of all types of evidence. Sampling of entomological evidence is somewhat intrusive and can result in minor unavoidable disturbance to the remains. Utmost caution, care, and coordination with other crime scene personnel is required in this endeavor. By effectively coordinating with the investigator in charge before proceeding with the entomological collection, any disturbance can be documented by written and photographic record. Crime scene personnel should thoroughly brief the forensic entomologist as to the circumstances surrounding the death scene, and provide all available information regarding past events at the scene. Likewise, the forensic entomologist should be in constant communication with other crime scene personnel about the entomological collection procedures that need to take place. In many cases crime scene personnel assist with the entomological collection procedures (Figure 3.1). Once proper briefings have been obtained and appropriate coordination and approval granted, the forensic entomologist can begin the entomological portion of the investigation (Catts and Haskell, 1990).

Figure 3.1 In many cases a forensic entomologist is unable to respond to the scene to assist in the collection of entomological evidence. In these instances, crime scene personnel properly trained in the collection and preservation of entomological evidence can effectively recover the insect samples. (Photo courtesy of James L. Castner.)

The initial approach to the remains is very important. Slow movement should be employed to minimize the disturbance of flying adult insects. Observations should begin several feet from the body (Figure 3.2) to determine what insects are present, where the major areas of colonization are (as evidenced by the presence of eggs, maggots, or puparia), and the location of any insect activity on the ground or substrate near the body. Distances from the body to remote insect activity sites should be measured and recorded, and other appropriate notations written. Many of these observations can be recorded on the Forensic Entomology Data Form and the Entomological Sample Log Sheet (see Appendix).

The entomological investigation at the death scene can be broken into several major stages:

1. Assessment and notation of general scene characteristics.
2. Visual observation and notation of insect infestations at the scene.
3. Initiation of climatological data collection.
4. Collection of adult flies and beetles.
5. Collection of eggs, larvae, and puparia.

Figure 3.2 Many adult insect species will fly away from the remains in response to increased human activity at the death scene. Therefore, visual and written observations of the scene and the patterns of insect activity should begin at a distance. Crime scene technicians should do much of the entomological collection preparation away from the remains so that the insects will not be disturbed. (Photo courtesy of Jason H. Byrd.)

6. Collection of specimens from the surrounding area [up to 20 ft (6 m)] from the body.
7. Collection of specimens from directly under and in close proximity to the remains [3 ft (1 m) or less] after the body has been removed.
8. Documentation of historical climatological data.
9. Assessment of the ecological characteristics (soil, plant, water, etc.) at the recovery site.

The observations and notes of the forensic entomologist can provide information valuable to the overall death scene investigation and substantiating data for entomological evidence evaluation. Recording detailed observations during the collection of entomological evidence may assist the overall investigation by providing insights into the probable cause and manner of death, and presence of other types of nonentomological evidence. Crime scene investigators have a tremendous challenge and responsibility. Attempting to identify the circumstances of a crime and to locate, document, and collect all evidence related to the crime scene can be an arduous task. A wide diversity of evidence may be present and many different procedures may be required to recover this evidence. Often, only one person is available to work the scene, which requires prioritization of the procedures that can be handled within the time constraints allowed. Given the pressures of the media, administrators, and the public for immediate answers regarding a death scene, important but obscure evidence may be passed over and not collected or noted. Forensic entomologists, by utilizing thorough and detailed observations, can foster effective crime scene management efforts. It is evident that entomologists are searching for very small evidence that is easily overlooked by the untrained eye. As a result, other types of physical evidence are often discovered by the detailed searches conducted by forensic entomologists in and around the body (Hall and Haskell, 1995).

Some of the insects may be seen when first observing the remains, but shortly afterwards these insects may not be found. Disturbances caused by investigators close to the remains can cause insects to run, fly, or otherwise disappear. Many species of adult flies may fly away when a body is approached, and will not return until the human activity has subsided. Beetles and maggots beneath the remains often bury themselves quickly when the body is removed. If these insects cannot be collected at the time they are first observed, then a written (or if possible a photographic) record of them noting their approximate numbers should be made. Photographs including a size reference scale can assist the entomologist in assessing type, abundance, and age of insects present. However, collection of specimens is best done early in the evidence recovery process before disturbance of the body becomes problematic (Figure 3.3).

Visual Observations and Notations of the Scene

Visual observations and notations of the insects active in and around the remains should generally be made at a distance before the remains are examined. When first coming into a crime scene, watch and observe where the major sites of insect activity are located on the remains. Provide some distance between you and the remains so as not to disturb flying adult insects and begin to record where the insects are, what types are present, and in what approximate numbers they are observed. The following is a guideline for the collection and recording of entomological evidence:

1. Enter records on a form such as the "Forensic Entomology Data Form" (see Appendix).
2. Approximate the number and kinds of insects observed.

Figure 3.3 Once a route of ingress has been cleared and established so that the body can be approached, entomological collection procedures can begin. Here crime scene personnel from the Tallahassee Police Department (Tallahassee, FL) complete final preparations prior to collecting the entomological evidence. (Photo courtesy of James L. Castner.)

3. Note locations of major insect infestations. (These infestations may include insect eggs, larval, pupal, or adult stages in combination or by themselves.)
4. Note immature stages of particular insects observed. (These stages can include eggs, larvae, pupae. Include notations on empty (eclosed) pupal cases, cast larval skins, fecal material (frass), and exit holes or feeding marks on the remains.)
5. Note any insect predators such as carrion and rove beetles (silphids and staphylinids), ants and wasps (e.g., formicids and vespids), or insect parasites (e.g., ichneumonid and chalcid wasps).
6. Note the exact position of the body, including the compass direction of the main axis, position of the extremities, position of the head and face; parts in contact with substrate, areas in sunlight and shade.
7. Note insect activity within 10 to 20 feet (3 to 6 m) of the body. Observe flying, resting, or crawling insect adults and larvae or pupae.
8. Note any unusual naturally occurring, man-made, or scavenger-caused alterations that could modify the decomposition of the remains (e.g., trauma or mutilation of the body, burning, covering or enclosing of the body, burial, movement, or dismemberment).

Written notes of the scene should be augmented with photographs and sketches. Close-up detailed photographs of different stages of insects (with a reference scale), may provide important clues useful in analyzing the entomological information from the scene. Some close-up photographs should be taken prior to collecting the insects. It may be possible for the forensic entomologist to have trained crime scene personnel take the needed photographs. Dual slide/print film can be used in recording entomological evidence. This film provides an uncut negative strip that demonstrates the filming sequence and facilitates duplication of photos. However, this film may not have as high a resolution as slide or print film. A record of the exact time when each photograph was taken also should be made.

The scene may be recorded with a video camera as well. While this is an excellent procedure for recording overall elements of the scene and observing entomological information from a gross perspective, the fine details and close-up imagery required in entomological investigations usually cannot be viewed on a video tape. Therefore, the video

camera should not be used as the only source for recording the details of the death scene. The 35-mm single lens reflex (SLR) camera, with a macro lens and flash, should be routinely employed to record the details of entomological evidence. If the scene processing team makes a video record, the entomologist should request a copy.

Once these observations and the areas of insect activity are noted and recorded, the forensic entomologist can begin the collection of insect and climatological data. Climatic data gathering can begin some distance from the remains and continue simultaneously with specimen collection. It is advisable to place the thermometer within a few feet of the remains (ca. 10 ft). A stabilization period should be allowed prior to reading and recording the temperatures. At the same time, collection of insect aerial samples can commence. This provides efficient use of time and also ensures that the aerial collections will be made prior to disturbance of the remains, thus increasing the recovery of the total numbers of specimens and species present. While many adult insects will return within a few minutes, some species may not be found after the initial disturbance of the remains.

The list of equipment and supplies outlined in the appendix of this chapter will facilitate proper collection and preservation of specimens from the death scene and during autopsy for delivery to forensic entomological laboratories in a physical condition necessary for evaluation by the entomologist. These items are adequate for collecting the insect specimens and other associated evidence, and for maintaining and preserving this evidence in good physical condition for subsequent study. Much of the listed equipment may be purchased from biological supply and scientific equipment companies.

Collecting Climatological and Temperature Data

Climatological information is critical when estimating the postmortem interval (PMI) by entomological means. The time required for insects to undergo their life-cycle development is determined largely by the temperatures and relative humidity in the particular environment to which they are exposed. Other climatological conditions (e.g., rainfall, full sun, snow cover, and fog) also may influence insect development rates, behavioral modes, and carrion-feeding habits. Therefore, the forensic entomologist should develop a working knowledge of climatology and its influence on carrion insect ecology. Proper collection and interpretation of climatological data is essential to the estimation of the PMI. Having the name, telephone number, and a working relationship established with a qualified climatologist can be of great value when you need specific data from a local region or interpretation of collected weather information. Meteorologists are usually more than willing to assist in an investigation and typically accommodate investigators quickly and precisely.

Several temperature readings that should be taken while processing the death scene are

1. Ambient air temperature recorded by readings taken at 1 ft and 4 ft (0.3 to 1.3 m) heights in close proximity to the body (Figures 3.4a,b).
2. Ground surface temperatures obtained by placing a thermometer on the ground on top of surface ground cover (e.g., leaves) (Figure 3.5).
3. Body surface temperatures obtained by placing a thermometer on the upper surface of the body (Figure 3.6).
4. Under-body interface temperatures obtained by sliding the thermometer between the body and the ground surface (Figure 3.7).

(a) (b)

Figure 3.4 (a) Ambient air temperature should be recorded at a height of approximately 4 ft (1.3 m) above (or in close proximity to) the remains. This temperature reading will be used to correlate scene temperatures with National Weather Service observations. (b) An ambient air temperature reading should also be recorded at a height of 1 ft (0.3 m) above (or in close proximity to) the remains. This temperature reading will allow the forensic entomologist to determine the occurrence and effect of microclimatic conditions at the scene. (Photos courtesy of Jason H. Byrd.)

(a) (b)

Figure 3.5 (a) Ground surface temperature should be recorded from the top surface of bare soil or any ground cover present (i.e., leaves). (b) Soil temperature also should be recorded at a depth of 10 and 20 cm, at a distance of 15 to 20 cm away from the remains. (Photos courtesy of James L. Castner.)

5. Maggot mass temperatures obtained by inserting the thermometer into the center of the maggot mass (Figure 3.8).
6. Soil temperatures taken immediately following body removal at a ground point that was under the remains prior to removal (Figure 3.9).

Soil temperatures also should be taken at a second point 3 to 6 ft (1 to 2 m) from where the body lay (Figure 3.10). These should be recorded from three levels: directly under any ground cover (grass, leaves, etc.), at a soil depth of 4 in (10 cm), and at a soil depth of 8 in (20 cm). (*Note:* the direct rays of the sun should not be allowed to shine on the thermometer-sensing element. Radiant heat from the sun will cause readings far in

Figure 3.6 Body surface temperature should be recorded directly from the upper surface of the body. In many cases, a temperature reading should be taken from both the skin surface (if exposed) and the upper surface of clothing or wrappings (if present). However, the remains should not be unwrapped at the scene. (Photo courtesy of Jason H. Byrd.)

Figure 3.7 A temperature reading should always be taken from the interface between the body and the substrate on which it rests (such as soil, vegetation, concrete, asphalt, or flooring materials). (Photo courtesy of Jason H. Byrd.)

Figure 3.8 If a centralized mass of larvae is present, the internal temperature of the mass should be recorded by simply inserting a thermometer probe into the center of the active mass. (Photo courtesy of Jason H. Byrd.)

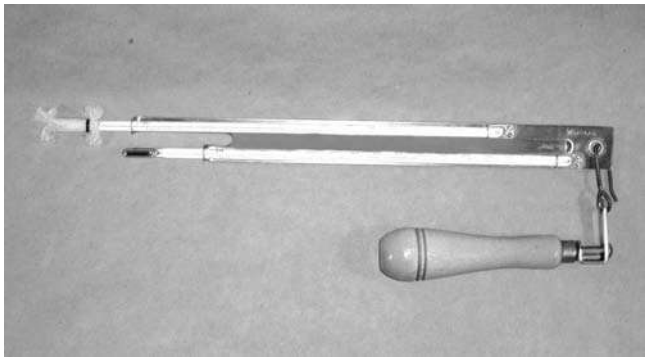
Figure 3.9 Immediately upon removal of the body, soil temperatures should be recorded from directly underneath the prior location of the remains. (Photo courtesy of Jason H. Byrd.)

excess of the true environmental temperatures. Always shade the thermometer from direct sunlight when taking temperature data.)

Estimate the duration of exposure of the remains to direct sunlight, broken sunlight, and shade for the total daylight hours. This can be accomplished by observing the surrounding and overhead vegetation and structures or the location of windows and noting their compass direction relative to the position of the remains. If there is any question as to this relationship, observation of the site periodically throughout a sunny day will provide additional information. When direct sunlight is shining on the body, external temperatures and some internal temperatures close to the surface may be higher than when the remains are shaded. These higher temperatures will be recorded even if the thermometer bulb is shaded and will reflect accurate temperature exposure for the body.

Relative humidity can be obtained at the scene through the use of a sling psychrometer (wet and dry bulb) or a battery powered psychrometer (Figures 3.11a,b). From these data,

Figure 3.10 It is important to record soil temperatures at a point 3 to 6 ft (1 to 3 m) from the body. These should be recorded from under any ground cover at a depth of 4 in. (10 cm) and 8 in. (20 cm). (Photo courtesy of James L. Castner.)



(a)

(b)

Figure 3.11 (a) A sling-type psychrometer (wet and dry bulb thermometer) used for determining relative humidity. (b) The sling psychrometer is spun rapidly on its handle for approximately 1 min before the reading is taken. Evaporative cooling of the moistened cotton wick of the wet bulb thermometer produces a lower than ambient temperature reading. The difference in temperatures is then utilized to determine the relative humidity. (Photos courtesy of Jason H. Byrd.)

dew points and dew wettings of the remains can be estimated. Under certain conditions, the moisture attributed to dewfall can be of greater cooling and wetting influence than those resulting from rain or snowfall. Even though there has been no precipitation recorded, a body may have been coated with surface moisture daily. Air movement and cooling from dew evaporation (evaporative cooling) can reduce the temperatures of the remains below ambient levels.

When insect collecting is completed at the scene, weather data for the time period should be obtained. This time period should extend from 1 to 2 weeks prior to a rough estimate of when death occurred, and to 3 to 5 days past the time the body was discovered. Weather data retrieval can be accomplished by contacting the nearest National Weather Service (NWS) station or other climatological data-gathering agency. Locating the closest National Weather Service recording station, or other climatological data gathering agency, to a death scene is necessary for the proper correlation of meteorological data collected directly from that death scene. These offices have records of other data collection sites and agencies in each state, and can indicate which station is in closest proximity to the death scene. Some NWS stations (including forest service fire towers and airport facilities) can give extensive weather data including hourly temperatures, humidity, extent of cloud cover, precipitation, and wind speed and direction. In addition, soil temperatures, water temperatures, river stages, tidal swings, soil moisture conditions, and evaporation rates also may be obtained from first order NWS stations. All climatic data are eventually sent to the National Oceanic and Atmospheric Administration (NOAA), where it is stored at the National Climatic Data Center (Asheville, NC). The needed weather data can be obtained in the form of recorded material officially certified for court documentation. Smaller stations may have only daily maximum and minimum temperatures, and total precipita-

tion. In many instances, such data may be all that are available for the entomologist to use in making the appropriate time interval evaluation.

Finally, it is important to conduct periodic temperature observations (3 to 4 readings over a 24-hour period for 3 to 4 days) at the death scene. In particular, these readings should be taken during the times of temperature maximums and minimums. These observations are valuable for the correlation of the site microtemperatures with those of the closest NWS station data. Great differences can exist even within short spatial distances, and the possibility for this type of error must be considered. For example, in a California case where a body was found next to a river, extensive periodic temperature data collection was conducted at the site. Temperature data were recovered over a period of days from the scene and compared to those documented at the airfield weather station. It was found that the site temperatures were approximately 11°F (6°C) lower than the same hourly temperatures recorded from the NWS station at a nearby airport. Once the calibration was made between these two sites, an additional 48 hours was added to the growth and development of the fly larvae due to the much cooler temperatures under the culvert at the water line. By making periodic visits (including times after dark) to the site over several days, accurate correlation could be made between the NWS station and the death site. When a NWS station is recording data on an hourly basis, an even greater degree of accuracy can be achieved by recording coincidental death scene readings.

It is recommended that these death scene temperature comparisons be taken at a time when weather conditions are similar to those noted during the time that body was at the recovery location. If a major frontal system has passed shortly following recovery of the remains, it may be advisable to wait until temperatures more closely approximate the levels that are representative of the time the body was *in situ*. Over a period of 4 to 6 days, if 4 or 5 temperatures can be taken at the scene per day, an adequate number of data points can be generated for a linear regression statistical analysis of the site vs. the NWS. To help accomplish this task, remote electronic temperature sensors that can be programmed may be calibrated and placed at the site for the required period of time. These sensors can record thousands of data points and recover the temperature every second if desired. Once the climatological data is collected, appropriate analysis of the pertinent climatic information can be made.

A recent case from New York demonstrated that solar radiation played a limited part in altering the decomposition of a body hidden in a car trunk for several days. As we all would expect, and as research from the Anthropological Research Facility in Knoxville, TN has demonstrated (W. Bass, personal communication), full sunlight on the car would likely have caused temperatures in the trunk of the car to be very high. However, upon examination of the cloud cover data for the region on the dates in questions, it was found that only 2 days showed full sun with 2 more showing scattered or broken clouds. The rest of the period was rainy and overcast, thus only limited influence was attributed to solar radiation on the heat loading of the car trunk where much more might have been expected.

Another evaluation rendered from climatological records involved the estimation of duration of the covering of a body with snow. In this case from northern Ohio, the remains of a young girl were found in late winter (second week of February) in a rural agricultural field. It was suggested by the landowner, who's house was approximately a half mile from the roadside ditch where the body was found amongst the tall weeds, that

the remains could not have been there since late October or early November. It was theorized that family members, field workers, and hunters would have seen the body either during hunting season or during the process of harvesting crops. Once weather records had been studied, it was found that there had been a 10-inch snowfall during the second week of November and temperatures were never above freezing until the later part of January. Another 5 inches of snow fell and covered the remains until the first week of February, when there was a considerable thaw. Thus, the girl's body had been covered with several inches of snow for over 2 months, conceivably concealing the remains from observation.

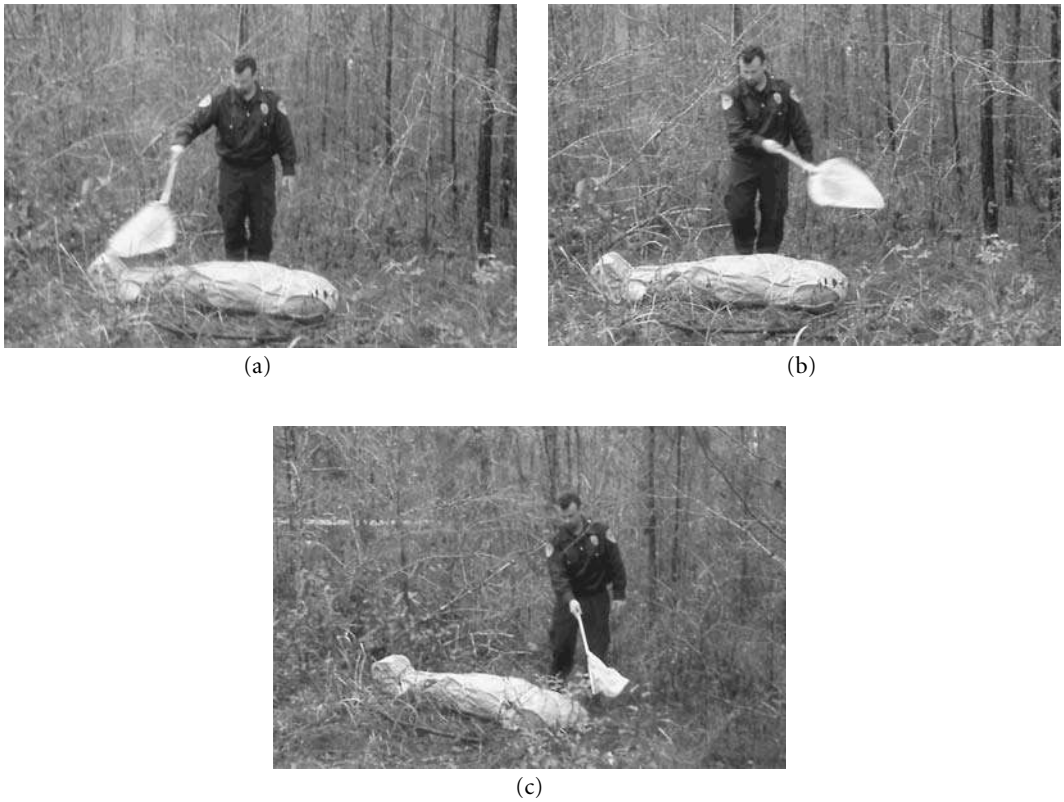
The primary purpose for obtaining climatological records is for the evaluation of temperatures over the period the deceased was missing. Temperature is the most important factor influencing insect growth and development if adequate food resources are available. Insects are cold-blooded (poikilotherms) and it is the external temperature influence that drives the rate of enzymatic action for growth within the insect's body. There are upper and lower temperature thresholds that go beyond enzyme capabilities to produce expected and desired reactions within the insect. Temperatures at these thresholds can create effects that are lethal to the developing organism.

Collection of Specimens Before Body Removal

Proper collection and preservation techniques, specimen labeling, and data recording are necessary for entomological data to be accurately evaluated and accepted by the criminal justice system. Accurate entomological data are essential for entry into the court record. On occasion, opposing attorneys have had opportunities to attack the expert entomologist when well-meaning evidence technicians, untrained in entomological recovery techniques, have omitted steps in the sequence of collection and documentation. To avoid such criticism, it is important to complete a thorough collection with supporting documentation regardless of the circumstances of the scene.

An aerial insect net is very useful during this stage of the entomological investigation for collecting fast flying and fast crawling adult insects. If flying insects are present over the body, the aerial sweeping technique for collecting can be employed. Flying insects associated with carrion are strong, fast fliers, thus netting of specimens requires some experience and practice. Appropriate netting techniques use several rapid, back and forth sweeping motions of the net (6 to 10 sweeps) with reversal of the opening of the net 180° on each pass (Figures 3.12a,b,c). On the last pass, the opened portion of the net is brought up to about chest level with rotation of the opening 180° (Figures 3.13a,b,c,d). This causes the netting material to be folded over the top edge of the large net ring opening, thus trapping the insects in the net bag (Figure 3.14). Another technique that can be employed to collect flying insects is to hold the tail of the net up and approach the insects from above with a swatting motion (Figures 3.15a,b). The natural escape behavior of the insect will cause them to fly up and into the net. With either technique, the insects can be easily confined in the end of the net (Figure 3.16).

The end of the net, with insects inside, then can be placed into a wide-mouth killing jar, which is then capped (Figure 3.17). The killing jar should contain either gypsum cement (plaster of Paris) or a few cottonballs soaked with fresh ethyl acetate (Figure 3.18). This will kill the insects after a few minutes (2 to 5 min is usually adequate). Following immobilization, the insects can be transferred into vials of 75% ethyl alcohol (ETOH) by placing



(a) (b)

(c)

Figure 3.12 Aerial netting of flying insects is one of the first entomological collection procedures to undertake. The insect net is swept rapidly back and forth above the body (a and b), with a rotation of the net opening 180° after each pass (c). (Photos courtesy of James L. Castner.)

a small funnel into the vial and carefully dumping the contents of the net into the funnel (Figures 3.19a,b). The insects also can be placed directly into alcohol by holding the end of the net up and reaching under the wire hoop and into the net with an alcohol vial and gently tapping the insect into the vial (Figures 3.20a,b).

Some of the specimens may be placed into a dry vial for direct pinning. If stored in this dry condition, however, the insects must be processed in a few hours because excessive moisture on the insects (condensation arising from within the closed vial) can promote mold growth that will quickly damage or destroy the specimens. Care should be taken that all insects are shaken out of the net and into the vial. Many insects have spurs or claws that can catch on the netting material and hinder their falling free. Aerial sweep-netting procedures should be repeated three to four times to ensure a representative sample of all of the flying insects present.

Many ground crawling adults can be collected with forceps or fingers. Preservation should be conducted in the same manner as with the adult flying insects. These insects may include some of the beetles (Coleoptera), ants, bees and wasps (Hymenoptera), true bugs (Hemiptera), springtails (Collembola), and newly emerged flies (Diptera). Some of these insects are quite fast, so it may be necessary to grab them with vigor and purpose as they are likely to scurry into the soil or under the remains or leaf litter.

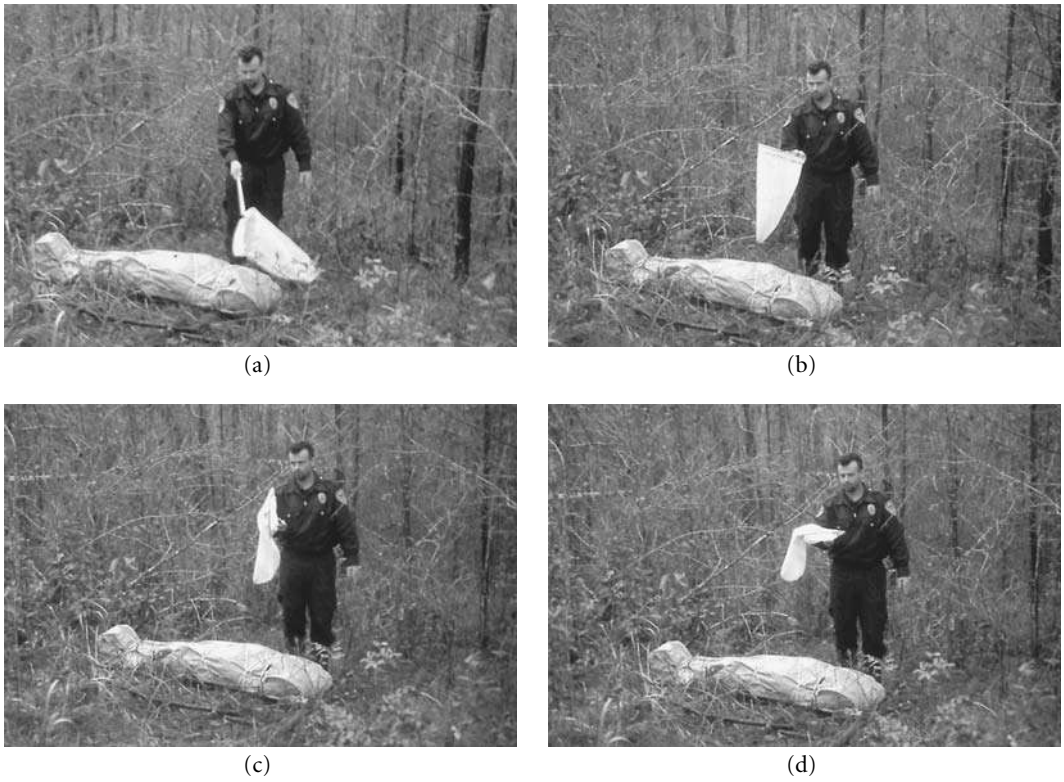


Figure 3.13 After the last pass (a), the net is quickly brought up to chest height (b) where the open hoop is rotated 90° (c). This rotation is continued for another 90° until the tail of the net lies across the wire hoop (d). (Photos courtesy of James L. Castner.)

Figure 3.14 In final position, the wire hoop has simply been brought to chest height and rotated 180°. This effectively traps the flying insects within the net and minimizes the chance for escape. (Photo courtesy of James L. Castner.)

(a) (b)

Figure 3.15 (a) An alternative technique to the sweep method involves approaching the insects from above while holding the tail of the net. (b) A swatting motion is then used to collect insects in the net. This is an effective method because insects will have a tendency to fly up in order to escape. (Photos courtesy of James L. Castner.)

Figure 3.16 Insects can be easily confined in the tail of the net with one hand for transfer into vials or kill jars. (Photo courtesy of James L. Castner.)

Figure 3.17 With the insects confined to the end of the net, it is then placed inside the kill jar and left 2 to 5 min. Other crime scene procedures can be conducted while the ethyl acetate takes effect. (Photo courtesy of James L. Castner.)

Figure 3.18 Commercially available kill jars are inexpensive and are made with plaster in the bottom to absorb the ethyl acetate. (Photo courtesy of Jason H. Byrd.)

The sweep-netting technique described previously also can be used to sample insects upon vegetation in the area surrounding the body. Many of the flies associated with carrion can be found resting on nearby plants or grasses. By sweeping the vegetation within 10 to 20 ft (4 to 6 m) from the remains, some of these adult insects may be collected (Figure 3.21). The aerial net can be used for this survey, but if the foliage is too thick or dense, or the plants are woody and stiff, the lightweight material of the aerial net will snag and tear. Instead, heavy-bagged sweep nets (such as those used by agricultural insect specialists for surveying insects on field crops) can be employed for collecting forensic insects resting on nearby foliage.

Once the surrounding area has been processed, collection of specimens from the body can begin. The person having overall authority of the scene must give permission to approach the body, although this should have been done prior to collecting around and over the corpse. An appropriate route to physically approach the corpse should be identified: many times a specific path is designated for movement into and out of the confines of a death scene to minimize destruction of evidence.

When the collection of insects is made before body bagging and removal, it is extremely important that nothing be moved or taken from the corpse except the insects which are on the surface and clearly visible. Do not disturb any portion of the clothing, the immediate area around the body, or the body itself. The entomologist should collect only those stages of insects which can be seen readily on the body, and use extreme caution when using forceps or other tools for collecting. Postmortem artifacts inflicted inadvertently while collecting specimens may be misleading and can cause needless questions and speculation. A thorough examination of the clothing and body bag can be conducted at the time of the autopsy for insects not collected at the scene. Some agencies require new body bags for each case to eliminate the question of residual evidence from previous cases. If not, the forensic entomologist must verify the absence of insects in the body bag before use.

(a)

(b)

Figure 3.19(a,b) Once the adult insects have been killed, they can be easily transferred to the alcohol vials by emptying the end of the net into a funnel. (Photos courtesy of James L. Castner.)

(a)

(b)

Figure 3.20 (a) Adult insects can be placed directly into alcohol, avoiding the use of kill jars and funnels. This can be accomplished by holding the end of the net up, and reaching under the wire hoop with a vial of alcohol and gently tapping the insect into the vial. (b) The insects trapped in the net will have a natural tendency to walk upwards. The open vial filled with alcohol can be brought up from underneath, and the adult insect can be tapped into the vial. (Photos courtesy of James L. Castner.)

Figure 3.21 The surrounding vegetation provides a resting place for insects that have been disturbed from the remains by the activity of the crime scene personnel. An effort should be made to recover these insects by the sweeping of nearby vegetation with the aerial net. (Photo courtesy of James L. Castner.)

Eggs and a mixed size sample of larval stages (several hundred) should be collected and preserved in one of the specified preservatives (Kahle's solution is preferred, see Chapter 4). The largest larvae observed should be collected and, if numbers permit, 30 to 60 of these should be placed into a preservative solution (Figures 3.22a,b). It is very important to put a label into each collection vial immediately (Figure 3.23). The sample number, hour, date, case number, and city/county should be included on each label as a minimum of data (Figure 3.24). A sample label format can be found in the Appendix of this chapter. It also is important that these labels be written in pencil, NOT INK! Labels made with a graphite pencil will not be affected by solutions used to preserve specimens. Duplicate labels should be made, with one being placed in the alcohol with the insect specimens, and the other printed on adhesive paper and affixed to the outside of the vial (Figure 3.25).

Double labeling procedures should be standard forensic entomological practice. This is done to ensure that data will not be lost due to external labels becoming loose and falling off. Also, when working with specimens, the internal label will be placed in the examination container that contains a portion of the specimens from the vial while the remaining specimens in the collection vial are still properly labeled, thus reducing the chances for mixing similar-appearing samples. The vial sample number and the data pertaining to where the sample was collected should then be recorded on the "Specimen Disposition and Identification Record" and also noted on the "Entomological Sample Log Sheet" (see Appendix).

Once a representative sample of the larvae has been properly preserved and labeled, a second equal-sized portion of these large larvae should be placed alive into maggot-rearing cups, either previously prepared or prepared while on scene (Figure 3.26). Companion sampling requires that identical samples be collected for both preservation and laboratory-rearing purposes. In order to keep the collected insects alive during shipment to the forensic entomologist, a specialized shipping container must be constructed so that the larvae can have constant access to a food source. Larval-rearing pouches may be constructed from aluminum foil by folding a 6 in. × 7 in. (15 cm × 18 cm) piece of foil into thirds horizontally, and then again into thirds vertically, ending with a rectangular piece approximately 2 in. × 2.5 in. (5 cm × 7 cm). This rectangle is then unfolded and the corners crimped together

(a)

(b)

Figure 3.22 (a) After completion of the aerial netting of the adult insects, the collection of the preserved larval samples can begin. This is best accomplished by having one member of the crime scene team collect the samples while the other completes the information required for the collection labels. (b) Larvae should be placed directly into a preservative solution upon collection. These samples will be used in conjunction with the living samples to help determine the postmortem interval. (Photo (a) courtesy of Jason H. Byrd. Photo (b) courtesy of James L. Castner.)

forming an open-topped, three-dimensional rectangular pouch. Plastic resealable sandwich bags, perforated with multiple pinholes also may be used as pouch-type rearing containers.

A palm-sized (approximately 3 to 5 oz or 90 to 150 g) piece of beef liver, or other rearing medium, is placed within the foil pouch. About 30 to 60 larvae (maggots) can then be placed into the foil pouch containing the food substrate (Figure 3.27). Once the larvae are placed on the food substrate, the top edges of the foil should then be tightly crimped

Figure 3.23 Data labels containing detailed collection information are immediately placed directly into the vial containing the preserved larvae and the preservative solution. These labels should always be completed in pencil. If ink is used, the alcohol will dissolve the print from the paper surface. (Photo courtesy of James L. Castner.)

Figure 3.24 The officer responsible for completion of the data labels should always include the case number, time of collection, date, geographic location, location of insect on the remains, and the name of the collector. Duplicate labels should be made on regular cotton bond paper (to be placed inside of the collection vial) and another printed on adhesive paper to be affixed to the outside of the collection container. (Photo courtesy of James L. Castner.)

Figure 3.25 A label printed on adhesive-backed paper, containing the same information as the label in the collection vial, should be affixed to the outside of all collection containers. (Photo courtesy of James L. Castner.)

Figure 3.26 Once the preserved collections have been made, a "companion" sample of living insects must be made. This should consist of insects from the same body areas as those preserved, and should reflect a representative sample of the larvae found on the body. The collection container for the living larvae is started by forming a pouch from aluminum foil. (Photo courtesy of James L. Castner.)

together to reduce desiccation and to help prevent the larvae from becoming dislodged from their food source during shipment (Figure 3.28). This is placed in a vented, pint-sized (16 oz) cardboard or plastic container with approximately 1 in. (2.5 cm) of medium size vermiculite or sand in the bottom (Figure 3.29). In especially dry climates, a wet piece of paper toweling can be placed into the pouch in order to prevent desiccation of the food substrate and the larvae. If plastic sandwich bags are used, the top should be sealed, providing that adequate ventilation has been provided by placing small holes in the plastic with a pin.

Eggs are treated in the same manner as the larvae. However, live puparia can be placed directly into the shipping containers that have vermiculite or sand in the bottom (Figure

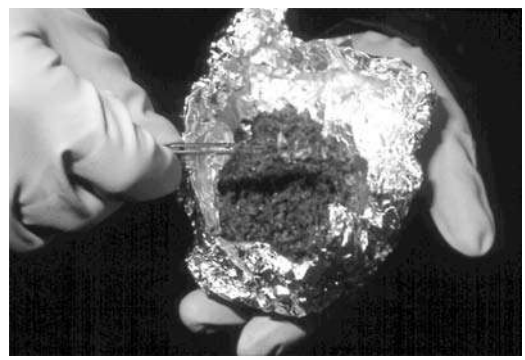


Figure 3.27 Once the foil pouch is created, a food substrate (typically ground beef or beef liver) is added to the pouch, and approximately 30 to 60 larvae are added. (Photo courtesy of James L. Castner.)

Figure 3.28 After the larvae are added, the top edges of the pouch should be tightly crimped. This prevents the larvae from being separated from the food source during shipment, and also helps prevent desiccation. The crimped pouch is then placed into a 16 to 32 oz plastic container for shipment. The plastic cup should have approximately 1 in. of soil or vermiculite in the bottom. This provides a burrowing substrate should the larvae reach the migratory stage during shipment, and also helps to absorb any fluids that may leak from the maggot pouch. (Photo courtesy of James L. Castner.)

3.30). It is not necessary to provide these containers with the pouch and food source as this stage does not feed. At the death scene, beetle larvae (generally recognized by having three pairs of legs) also may be collected along with the maggots. However, many beetle larvae are predacious on maggots. Therefore, the live beetle larvae should not be placed in the same shipping container with the live maggots. Extra maggots should be collected for use as a food source for them. These "feed maggots" may be held over an extended period under refrigeration (38°F or 4°C). Portions of beef liver or other substrates utilized as a maggot food source also may be used as an alternative food source for immature beetles.

Once samples have been collected from the body and its immediate area, the investigator should then focus on collecting the insects that have completed feeding and migrated away from the body. Generally, these insects will be older than those found on the remains and, thus, are extremely valuable in a death investigation. A search to recover these migrating larvae or puparia should be conducted. If the remains are recovered in an outdoor

Figure 3.29 The container holding the live insect samples should have duplicate labels as with all other collection vials. One should be affixed to the outside of the container and one placed inside with the collected insects. (Photo courtesy of James L. Castner.)

Figure 3.30 A representative sample of the puparia found at the scene should be collected and immediately placed into a preservative solution. A companion sample (shown here) should be placed into a collection cup containing approximately 1 in. of soil or vermiculite. Providing a food source is not necessary since the pupal stage does not feed. (Photo courtesy of James L. Castner.)

environment, the many larval species will burrow under surface debris or into the top couple inches (2.5 cm) of topsoil. Most of these migrating insects can be found within a radius of 20 ft (6 m), but this varies greatly depending on terrain. In general, migrating insects will aggregate in areas of soft soil, against vegetation and the trunks of trees, as well as under rocks and fallen limbs. These insects are easily recovered by gently removing the surface debris with a trowel or hand shovel (Figures 3.31a,b). The topsoil also should be sifted in order to recover other insect species that may have burrowed deeper than under the surface debris (Figure 3.32). The soil should be sifted over progressively smaller screen

(a)

(b)

Figure 3.31 (a) The surface debris in the immediate surrounding area should be removed to expose any migratory larvae or puparia. (b) These fully formed puparia are easily visible once the surface debris has been cleared away. (Photos courtesy of James L. Castner.)

Figure 3.32 The topsoil in the surrounding area should be sifted in order to collect any migratory larvae or puparia that burrowed into the soil, instead of simply resting under the surface debris. (Photo courtesy of James L. Castner.)

sizes so that insects, and insect fragments, that passed through the larger screen can still be recovered.

These living samples collected from the scene will be reared to adults in the forensic entomology laboratory. It is important that rearing the larvae to adults be undertaken to facilitate and confirm larval identifications. This procedure should be repeated for some smaller larvae or eggs, placing a portion of the specimens into preservative and also saving a portion alive for rearing. When collecting the pupal stages of flies, some should be preserved, while a companion sample can be placed into rearing cups without the foil pouch and food source. The companion sampling technique should be employed throughout the collection process.

Areas of the body where concentrated insect activity most likely will be encountered during the early stages of infestation are the nasal openings (Figure 3.33), ears, mouth, eyes, and sites of trauma (e.g., cuts, gunshot wounds, and blunt force injuries where the skin is broken). Skin creases of the neck also may contain egg masses, which may be found as well along the hairline and close to the natural body openings or matted in bloody hair (Figure 3.34). Wounds may have both egg masses and larvae associated with them and exposed genital and anal areas may contain egg masses and larvae, especially if these areas were traumatized prior to or after death.

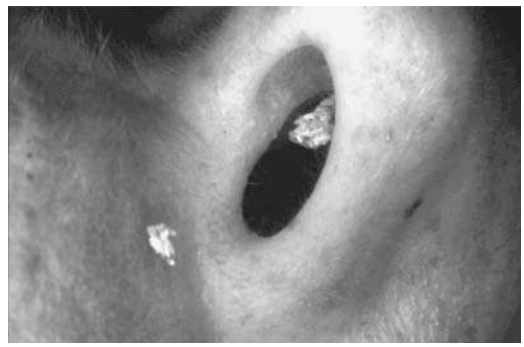


Figure 3.33 Blow flies colonize human remains in a very orderly array. The preferred sites for egg laying are the eyes, nose, mouth, ears, sites of soft tissue trauma, and the genital and/or anal area. In many cases the initial colonization is not readily observable as adult flies may deposit their first eggs deeply into the nasal cavity or the mouth. (Photo courtesy of James L. Castner.)

Figure 3.34 For some fly species, the hair is a frequent oviposition site. The hair should be examined thoroughly for the presence of fly eggs. In cases where flies have oviposited on individuals with long hair, the newly hatched larvae may desiccate before being able to reach the body. (Photo courtesy of James L. Castner.)

As stated earlier, thorough examination of the clothing and the body for entomological evidence should be conducted during the autopsy and not at the scene so that physical evidence relevant to the medical examiner's or forensic pathologist's portion of the investigation will not be disturbed or destroyed. At the death scene, the forensic entomologist should concentrate on collecting from around and under the remains, while collecting from the body should be superficial. The forensic entomologist should try to collect all apparent insect stages, but without causing postmortem artifacts on the body. Coordination of collection efforts with other investigators working the scene is critical.

Collection of Specimens After Body Removal

In cases where bodies are outdoors and heavily infested, many insect adults, larvae, and puparia (as well as other arthropods) will remain on the ground after the body is removed. The procedures described above should be followed for each of the different insect stages seen following removal of the body. A number of specimens of each immature stage should be collected and preserved, while a second sample should be collected alive for rearing.

Litter samples (e.g., leaves, grass, bark, and humus) or any material on the ground surface close to or under the remains should be collected and labeled. Many carrion feeding insects will shelter or hide in this material close to the body, and this should be thoroughly examined for additional faunal evidence. Collect handfuls of the litter down to the exposed soil, particularly litter in close proximity to the ground surface. This material can be placed into 2-qt cardboard or plastic containers for subsequent examination in the laboratory, or this can be examined at the scene if sifting and screening can be conducted properly on site.

Soil samples may yield insect specimens as well as provide samples for biochemical assay of decomposition fluids. Soil samples should be approximately 4-in. cubes or cylindrical cores (100 cc) of material from areas associated with different body regions (head, torso, and extremities). Soil samples of this size fit well into 2 pt (1 l) or slightly larger cardboard (cylindrical ice cream type) or plastic containers. Soil samples (approximately six total) should be taken from under, adjacent to, and up to 3 ft (l m) from the body, noting the origin of each sample in reference to the position of the body. These samples should be labeled according to the technique described for the insect vials.

Collection of Entomological Specimens During Autopsy

It is best for someone experienced in collecting insect evidence to be involved at each step of the investigation if the full potential of forensic entomology is to be realized. Ideally, the forensic entomologist should be present during each autopsy involving the collection of entomological evidence. Such collection is done in conjunction with the forensic pathologist performing the autopsy. The forensic entomologist should tell the pathologist the proper entomological evidence collection needs. This will preclude any later problems with the validity of evidence and prevent postmortem artifacts on the body. However, if circumstances prevent the presence of a forensic entomologist at autopsy, the medical examiner or coroner should be able to gather the necessary entomological evidence by following the appropriate collection procedures. If unable to attend autopsy proceedings, the forensic entomologist should provide telephone advice and direction to those tasked with specimen collection and preservation. If a certain insect life stage critical to a determination is not found during the autopsy, the investigator or entomologist should return to the scene in an effort to recover or confirm the absence of the missing insect life stage.

Most likely the remains will be enclosed in a zippered plastic "body bag" when it arrives from the death scene, particularly if the remains are in a state of advanced decomposition. In cases where the body is heavily infested with insects, the outer surface of the body bag may have larvae or adults. This evidence should not be overlooked; these insects should be collected and labeled using procedures described previously. Once the body bag is opened, the inner surfaces of the bag should be examined for insects that may have crawled from the body due to changes in temperatures or physical disturbance. The forensic entomologist should collect and label these insects with notations as to which area of the body (head, torso, and extremities) the insects were near.

Many times remains are stored for some time in coolers or refrigeration units prior to autopsy. This period may range from several hours to several days. Therefore, notation should be made of both the total time the body was cooling and the temperature of the chamber along with any other temperature information regarding the movement of the body to the morgue. Also, the temperature of the maggot mass should be recorded when the body is removed from the cooler. There may be little or no effect of the lower temperatures on insect development if the maggot mass was well established prior to the body being placed in the cooler. Maggot mass temperatures are commonly between 80 and 100°F (27 to 37°C) range even if the temperatures in the cooler are maintained between 30 to 40°F (–1 to 4°C). Bodies heavily infested with maggots should be autopsied as soon as possible. If even a weekend passes before the body is autopsied, the voraciously feeding larvae may consume valuable evidence.

After the remains have been taken from the body bag and placed on the autopsy table, an external examination is made. The entomologist and the pathologist can assist one another in the collection of evidence at this stage of the autopsy. If the body is clothed, a complete and detailed examination of the clothing is essential and may yield a variety of stages and kinds of insects. Folds in the clothing where eggs, larvae, puparia, or adults may be sheltering should be gently opened. Moist patches on clothing are excellent areas to search. Seeds and other plant materials should be collected at this time for analysis by a forensic botanist (Figure 3.35). In addition to the plant evidence, certain life stages of insects may be found in stems or seeds of plants that may give clues as to habitat, time, or geographic aspects in the case.

Figure 3.35 Investigators collecting entomological evidence commonly recover botanical evidence. Frequently small seeds or plant fragments adhere to the clothing of the victim or suspect. This can be crucial evidence of victim/suspect interactions. In this image, plant fragments are recovered on the clothing of the victim, which served to establish the postmortem interval. Plant material should always be recovered and placed in paper bags (or placed between sheets of newspaper) for shipment to a forensic botanist. In many cases botanical evidence can provide information about the scene unavailable by other means. (Photo courtesy of Jason H. Byrd.)

The bagging of the hands of the deceased in paper or sacks taped to the wrists is often a standard practice. This is done to preserve foreign trace evidence, such as skin fragments or hair, adhering to the hands. Inspection of these "hand bags" after their removal at autopsy is necessary as insects infesting hand wounds may crawl off the remains during transit and remain in such bags. After the clothing has been examined and removed, the areas of the body where concentrations of insect activity are found should be noted and photographed with a macro lens to show the extent of the infested area and insect composition. Collection of representative samples from each major infestation should be performed. If fly larvae are present, some should be kept alive and some preserved. On fresh bodies, the face is the most likely area to have insect activity. Flies, in particular, will seek the moisture and shelter of external openings (e.g., nostrils, mouth, and eyes) for deposition of their eggs. The genital or rectal areas, if exposed, will sometimes provide shelter and moisture attracting egg laying (ovipositing) female flies, especially if those areas have been traumatized or soiled with excretions. However, the urogenital–anal area is generally not a primary site for oviposition. It has been seen in research on human cadavers at the Anthropological Research Facility, Knoxville, TN and in case studies that there is a delay of several hours to a day or more in fly colonization of the pelvic area when these openings have not been traumatized. If trauma has occurred, there appears to be an increased fly attraction and these areas may be colonized as soon as, and in some cases exclusive of, the face even though the face was available for colonization. Traumatized areas of the limbs and torso, where breaks in the skin occur, also may contain insects (Figure 3.36). For example, insect infestations on the hands or forearms may suggest that the victim had sustained defensive wounds. This may not always be the case, however, and caution should be exercised when drawing this conclusion.

Very small arthropods such as fleas, ticks, mites, lice, or nits (lice eggs) may be present on both fresh remains and clothing. Many may be attempting to leave the cooling body and, therefore, can be found in the clothing or they may even be attracted to the investigator. Small ectoparasites also may be present on or within the tissues of the body itself. Thus, it is important to examine the hair close to the scalp for the presence of nits. The eye lashes or sebaceous gland areas of the face may harbor follicle mites, *Demodex*

Figure 3.36 Sites of soft tissue trauma are commonly infested with fly larvae. On fresh remains, insect activity in areas of normally unbroken human skin, not near a natural orifice, may be indicators of trauma. Although the insect activity alters and changes the appearance of any existing trauma, their presence in these areas on fresh remains should be an indicator that the underlying tissue and bone should be examined thoroughly by a forensic pathologist and a forensic anthropologist. (Photo courtesy of James L. Castner.)

folliculorum var. *hominis* (Simon) (Acari: Demodicidae). A few lashes are typically plucked and examined microscopically if the presence of this mite is suspected. Estimation of the postmortem interval may be determined based on whether or not these arthropods are still alive.

Once the internal portion (e.g., surgical entry into the torso and skull) of the autopsy has begun, major sites of insect activity may include the skull with natural body openings, hair and scalp, respiratory tract (including inner nasal passages), esophagus, genital and rectal areas, and antemortem wound sites. Also, the chest cavity and areas under desiccated skin, should be examined thoroughly. Insects collected from any of these body areas should be labeled, preserved, and their location noted.

Collection of Specimens from Buried Remains

Although burial slows the decomposition process, it does not necessarily exclude insect colonization. Both sarcophagid flies and muscid larvae (*Muscina stabulans*) (Fallén) (Haskell, personal communication) have been recovered from remains covered with several inches of soil. Blow fly puparia recovered from 100-year-old Indian skeletal remains from South Dakota suggested the season of the year in which the remains were buried (Gilbert and Bass, 1967). A giant bison skull exposed by erosion from a stream bank in Alaska was collected for study. The internal cavity of the skull contained two species of fly larvae and puparia (one species was identifiable) which were carbon dated back approximately 22,000 years (Catts, personal communication). Valuable entomological evidence can be gained from buried remains, often regardless of burial depth. It is essential to have a person experienced in the proper archaeological exhumation techniques and procedures present when unearthing remains. For a detailed discussion of this process, see Chapter 7.

Collection of entomological specimens from burial sites is very similar to procedures described in the section on collection of specimens at the scene. Collection of soil samples for examination for insect parts (or life stages) should be undertaken by the forensic entomologist as the excavation of the burial site proceeds. Soil from the ground surface down to the upper surface of the body should be sifted and examined as well as soil from the side and bottom of the burial pit after body removal. Insect larvae, puparia, adults, or

any insect fragments may be found in the surrounding grave. If life stages are seen on the remains, collection of samples for preserving and rearing from the exposed insect populations should be made, but with care not to cause postmortem artifacts.

Collection of Specimens from Enclosed Structures

Enclosed environments present several problems for the entomologist in the evaluation of insect colonization, development, and evidence collection. First, if the enclosed structure is very tightly sealed (e.g., newer automobiles with the windows and doors closed; tightly sealed rooms; metal containers with air seals; and newer, well insulated houses closed for air conditioner cooling), the chemical attractants used as cues by insects do not dissipate as rapidly as those from bodies that are left in the open. The question arises as to how much time has elapsed before the odors finally emanate from the restrictive confines. In some circumstances, the odors never permeate from the container. However, even in such cases, extensive visual searches should be conducted at the death scene to make certain no insect life stages are present.

Second, even if the attractant odor has emanated from the structure or container, insects may still be excluded from direct access to the decomposing remains. Thus, there may be considerable numbers of flies or other carrion insects found outside attempting to gain access to the remains. A concentration of blow flies outside a structure or container, likely indicates that something inside the enclosure is dead and beginning to decompose. Flying insects should be collected by aerial netting, and ground crawling insects should be hand collected. The duration of time that the remains have been in place may be indicated by certain assemblages of insects from a successional wave group (Megnin, 1894). This is due to the production of different odor attractants or other chemical cues associated with advancing decomposition.

Third, the enclosed structure may not have inside temperatures comparable to those reported from the local National Weather Service station. As explained in "Collecting Climatological and Temperature Data," temperatures in a particular outdoor habitat may be considerably different from those recorded at the NWS station, and independent temperature data should be collected from the scene to determine environmental conditions more accurately. This is even more crucial when dealing with enclosed structures. A car parked on a black asphalt surface with the windows closed, even on a mild sunny day (75°F or 24°C), may exhibit temperatures 30 to 40°F (20°C+) degrees higher inside the passenger compartment or trunk than the outside ambient temperature. This condition could accelerate larval development by a degree equivalent to several days, creating a considerable error in PMI estimation. By following the procedures described above or recreating the circumstances of the enclosed structure and recording temperature data, an accurate correlation can be obtained between the NWS data and the enclosed environment in question.

When investigating a death scene inside a building, always check the thermostat setting controlling the heating or cooling system. If activated, relatively constant temperatures may have existed for the period in question. This allows for a more accurate estimation of the rate of development of insects. Correlation of these data with onsite temperatures should be completed. Other problems with enclosed structures can occur. The first responder to a dwelling may open windows in the house in an attempt to dissipate the nauseous smell. In this event, the actual temperatures to which the insects were subjected

may be impossible to evaluate accurately until the building is closed again and the heating or cooling allowed to stabilize.

Collection of specimens from enclosed structures is conducted more efficiently with some knowledge of the likely places to which insects may migrate. In houses where the remains are in advanced stages of decomposition (skeletonization and mummification), it is possible that more than one generation of larvae have migrated off the remains, changed to pupae, and emerged as adults. Inspection of the edges of the room where the walls and floor make contact as well as under carpeting or carpet pads may reveal post-feeding (migrating) larvae and/or fly puparia. These maggots, that have reached the stage in which they no longer feed, may be found under carpeting, rugs, or any other covering that provides protection and seclusion for their transformation to the pupal stage. The fly puparia are small (approximately 9 mm), cylindrical objects that may be red, brown, or black in color. Crime scene investigators encountering them often ask why there were so many "rat droppings" in the room where the remains were found. These are most likely not rat droppings, but are fly puparia. Close examination will show segmental lines (sutures) on the fly puparia, which are not found on rat droppings (Figure 2.17b).

The larvae may migrate to pupate into other rooms as well. Occasionally, migrating larvae may travel up to 150 ft (50 m) in search of a suitable pupation site, so maggot migration in an enclosed environment can easily extend to the outer limits of most structures. A basement or crawl space under the structure will be an additional source of insects if the remains are in advanced decomposition and fluids have seeped through flooring and into the space below. Larvae, puparia, or adults may be found in such areas. When dealing with remains in automobiles, one should look under the floor mats and carpeting, down between the seats, and even under the upholstery which is in close proximity to the remains. Larvae also may migrate into the car trunk to pupate, so this area must be inspected thoroughly.

Because newly emerged adult flies and beetles will be seeking light and the outdoors, the backside of window blinds, shades, and curtains should be searched for adult flying insects. Window sills and ledges on the inside of the structure may hold high numbers of adult flies or other species which have completed development on the remains, have emerged from puparia, and sought to escape to their natural outdoor habitat. If the enclosed area is too hot, these insects may die from exposure to high temperatures.

Dark fly specks (fecal spots), and lighter colored food regurgitation spots, are frequently deposited by flies on the surrounding ceilings, floors, and walls (Figure 3.37) or even directly on the remains. Take note of the density of this spotting, as it may give some indication of the relative size of the fly population attracted to the remains. In advanced decomposition, insect feces (frass), can accumulate in conspicuous amounts around dried or mummified remains. Usually the cast skins of immature stages will be mixed in with the fecal material. In some cases, the dried feces still may be inside an extruded intestinal sheath called the peritrophic membrane. A mass of dermestid beetle fecal material (or frass) has the general appearance in color and form of pencil shavings or sawdust (see Figures 2.51a,b).

The same collection, preservation, and labeling techniques described earlier should be followed when collecting from these enclosed environments. Dead dried specimens must be handled very carefully because they are extremely fragile. They should be stored in fluid preservative to allow for rehydration or stored dry if toxicological analysis is anticipated.

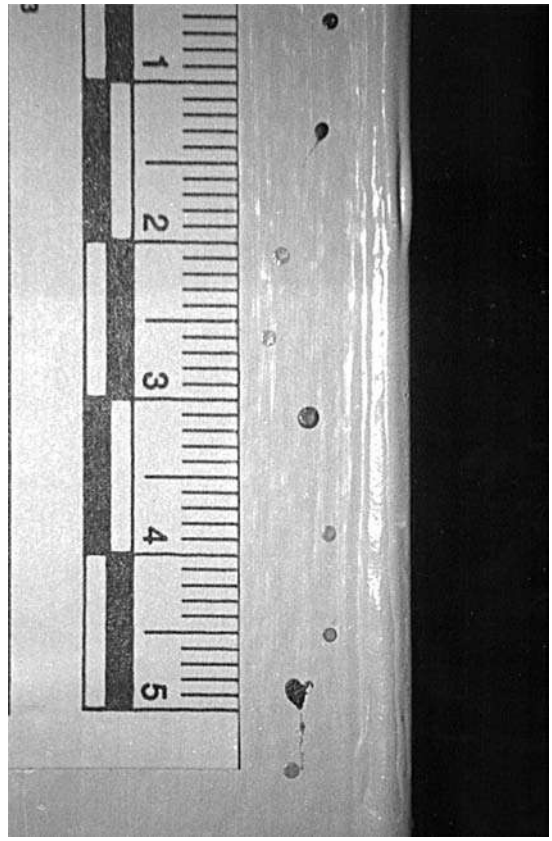


Figure 3.37 Fecal spots produced by flies may appear on any surface near the remains. These spots will test positive for human blood, and their occurrence should be consideration whenever an analysis of blood spatter is undertaken in an environment containing decomposed human remains. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

Collection of Specimens from Aquatic Habitats

At times it may be necessary to collect entomological evidence from bodies found in water. Sites may include shorelines, large bodies of water, rivers, ponds, irrigation or drainage ditches, sewage ponds, sewers, open wells, or rain barrels. In each of these habitats, specific insect species may have special survival adaptations unique to these environments. Specialized insects may help the investigator identify a particular geographic location or a specific time of year.

Generally, aquatic insect species are not known to be necrophagous (Haskell et al., 1989). However, some aquatic insects will use submerged or floating bodies for shelter or as a solid surface for attachment to facilitate feeding. Feeding usually will not include consumption of tissues from the body. Most often, aquatic insects utilize the body as an anchor for feeding on algal growth, or filter-feeding on small organisms, or for hiding from predators. A recent study of dead rats placed in an aquatic environment suggested that certain species of midges would colonize as time increased (Keiper et al., 1997). These aquatic insects may hold the answer to determining the length of submersion time in certain areas of the country. Aquatic arthropods (e.g., crawfish, crabs, or shrimp) will feed extensively on human tissue (Smith, 1986) causing postmortem artifacts.

Most aquatic insects spend a major portion of their life cycle in water, sometimes passing several years as a developing aquatic immature form before reaching the adult stage. In some cases, all life stages may be found in aquatic habitats, but in many species the growing and feeding stages are in water while the reproductive portion of the life cycle (winged flying adults) is of short duration and terrestrial. Therefore, death scene investigators usually will encounter only larvae, pupae, or other immature stages on submerged bodies. However, during warmer times of the year, newly emerged winged adults may be encountered on corpses found on the surface or along shorelines or riverbanks.

While common terrestrial insects (e.g., blow flies, flesh flies, clown beetles, carrion beetles) usually are not found on submerged bodies, floating bodies may contain many insects common to terrestrial scenes. These insects should be handled by using the same procedures described previously. If a corpse was colonized by terrestrial insect species prior to immersion, these insects may have drowned. This could indicate that the body was on the surface of the water for sometime before it sank to the bottom.

Some police agencies have procedures detailing recovery of bodies from water habitats. The protocol usually calls for some type of shroud or sheet to be fitted beneath the corpse before movement (if the corpse is floating) or for the body to be placed immediately upon a shroud if partially in the water. The shroud helps retain evidence on the body that may otherwise flush off when disturbed. This procedure is essential for proper recovery of aquatic insect specimens on the corpse. Most insects living on aquatic substrates will quickly detach at the slightest disturbance. Therefore, if a sheet or large, fine-weave mesh net can be slipped under and around the corpse before it is disturbed, most of the insects using the body as shelter or as a food source will be captured in this shroud.

Proper collection and preservation of the aquatic insects is essential for accurate identification of the organisms. The specimens may be collected as either preserved specimens or collected as live specimens for rearing to adults. There are several standard techniques available for rearing and preserving these fragile, soft-bodied insects (Merritt and Cummins, 1996).

Live immature specimens should be collected and transported in water taken from the environment in which they are found. During transportation, the collecting jars should be filled completely to reduce damage to the insects caused by excessive splashing. Also, the water must not be allowed to elevate in temperature because many of these immature insects are heat sensitive. Keeping the collecting jars shaded, covered with a wet cloth, or against chemical ice will help reduce excessive heating during transit. The collecting jars containing the specimens could be placed in a styrofoam or similar type ice chest with ice or icepacks inside. Portions of naturally occurring substrate found in close proximity to the corpse should be collected and placed into separate collecting jars.

Collecting procedures for preserving aquatic insects are very similar to those described under general collecting procedures. The processing and labeling techniques also are the same. Data labels should always be placed in the vials immediately after the collection is made using the same label format as discussed previously.

Collection of flying adult insects that are in close proximity to the body should be accomplished with an aerial net. The contents of the aerial insect net then can be funneled into a vial containing 70 to 80% alcohol (preferably ethyl alcohol) or a similar preservative. It may be possible to hand collect some adults from surrounding vegetation or directly from the body. In general, the wings are extremely important for identification of many of these fragile aquatic species. Eggs, larvae, and pupae also can be picked off the body

(substrate) by using forceps or fingers. If a net or shroud is not used in recovery, specimens should be collected at the body recovery site directly from the body before it is moved. If collection is delayed until the time of autopsy, many of these aquatic insects will have crawled off or dropped from the disturbed body and may not be found.

Often, aquatic immature stages are difficult to see due to their small size and their inconspicuous coloration. In one case, aquatic midge larvae (Chironomidae) also know as bloodworms, were collected from a corpse discovered in a river and were mistaken for red carpet fibers (Hawley et al., 1989). The investigator must look very carefully and closely at the outer portions of the skin and clothing. A hand lens may be necessary for these observations. Larvae also may be found under the slime and algae that coats the skin or clothing. These larvae may not be visible until this slime covering is scraped off. Caddisfly (Trichoptera) larvae or pupae may be found on and in clothing from bodies found in fast-moving streams. Once the specimens are collected, they should be preserved in one of the solutions listed in Chapter 4.

There has been very little forensic utilization of entomological evidence in aquatic environments (Holzer, 1936; Hawley et al., 1989; Haskell et al., 1990; Merritt, personal communication). Individuals lacking entomological training do not recognize the varieties of insects living in these environments as insects. Even with formal entomological training, many are difficult to identify to species, and there is little known of their behavior. This situation could be improved by involving entomologists knowledgeable of aquatic insects when bodies are recovered from aquatic habitats. The aquatic groups which may prove useful in yielding PMI and PMSI (Chapter 6) location information for death scene investigations include midges, caddisflies, mayflies, and some other small flies. If these insects can be identified and tabulated, data relating to their developmental time intervals might be used for making a PMI estimation.

Soft-bodied stages of aquatic insects should be preserved in fluids to prevent extreme distortion of the morphological characteristics resulting from drying. In contrast, several of the larger hard-bodied adult insects (dragonflies, dobsonflies, and damselflies) can be killed by using the killing jar method after which they may be pinned and labeled. Additional information on techniques of collecting and preserving the aquatic insects can be found in McCafferty (1981); Borror, Triplehorn, and Johnson (1989); Peterson (1967); Simpson and Bode (1980); and Merritt et al. (1984).

Processing Litter and Soil Samples

Insects and other arthropods that live in litter and humus can be collected by using a Berlese/Tullgren funnel. The funnel contains a wire screen platform to hold the litter sample in place but allows insect and mites to pass through the screen and down through the funnel. A small bottle or vial containing 75% ethanol at the bottom of the funnel cone captures the tiny animals. Insects and mites are driven to the bottom of the funnel by the heat from a low wattage (10 to 25 watt) light bulb or by fumes from a chemical repellant. Litter samples are placed in the funnel on top of the wire screen. The low wattage light or cloth bag containing the chemical repellant is suspended a few inches over the sample material and a vial or jar of alcohol is placed under the lower funnel opening. In 3 to 4 days the insects and mites will be driven from the litter material and into the alcohol bottle. These specimens then can be processed and examined.

Soil samples can be examined by using different size meshes of metal screens. First, a large 1/4-in. (6 mm) opening mesh screen should be used to separate large particles of soil and large insects. Sifting the soil onto a large, flat, white pan helps keep all of the evidence confined and allows the material to be spread out in a thin layer to expose the active insects. The white background makes the smaller, moving insects more visible. The insects should be preserved in the same manner as described earlier: adults in 75% ethanol and larvae in Kahle's or 75% ethanol. After these screenings are examined thoroughly, the finer particles are screened again using a 1.4 mm mesh screen. This will separate the large larvae, pupae, and the medium-size adults from the very tiny adults and larvae. A technique used for picking up very small insects and mites is to dip one point of a pair of forceps or a fine artist's paint brush into the collecting vial of preservative solution and touching the wet tool to the insect. The surface tension of the liquid will hold the insect as it is transferred to the collecting vial and the insect is "washed off" by the solution. Another method is to use a blow-type or electric aspirator to suck up the insects into a collection chamber for placement into ethyl alcohol. Mites may be collected from the soil samples by using the Berlese/Tullgren funnel technique for the soil sifted through the 1.4 mm screen.

The entomologist also should keep in mind that as the leaf litter or soil samples are being processed, other physical evidence may be discovered. Bone fragments, hair, teeth, projectiles, or other physical evidence essential to the case can be contained in samples. This evidence should be handled in the manner prescribed for collecting and labeling any other physical evidence and should be reported to the primary investigator and/or the forensic pathologist.

Conclusion

With the widespread acceptance of forensic entomology in the criminal justice system, it is hoped that the specialized training of crime scene technicians, investigators, medical examiners, and coroners will increase the frequency of recovery of entomological evidence. With the proper techniques and protocols established, it is possible for the personnel to conduct entomological evidence recovery without a forensic entomologist present at the scene. The collected evidence can then be shipped to the cooperating forensic entomologists at a distant location with no loss of evidentiary value. If training and cooperative efforts continue, the use of entomological evidence in legal investigations should further increase while valuable crime scene evidence will cease to be lost or ignored.

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APPENDIX

Collecting Materials and Equipment

1. Aerial and/or sweep insect nets (e.g., 15 or 18 in. diameter bags with 3 ft handles, 15 or 18 in. collapsible nets with variable length handles).
2. Collecting vials (1 to 2 dram size) with neoprene stoppers; screw cap collecting vials (4 dram size).
3. Wide-mouth (8 oz) pharmacy bottles with screw tops.
4. Light-tension larval forceps, needle point watchmakers forceps, medium or fine point dissecting curved forceps, dental picks.
5. Camel hair brush (No. 2).
6. Plastic or heavy cardboard containers (16 to 32 oz size).
7. Plastic specimen cups, screw cap lid type (4 oz size).
8. Paper labels, heavy quality paper (for placement inside of collection containers).
9. Paper labels, adhesive backed (for placement on the exterior of collection containers).
10. Dark graphite pencil (#2) (for marking paper labels). *Important:* Use only pencil for labeling since ink will run in alcohol-based fluids.
11. Hand trowel or 4 to 6 in. core sampling tool.
12. Thermometers, electronic and/or mercury.
13. Psychrometers, electronic and/or sling type (for measuring relative humidity).
14. Camera, 35 mm with macro lens and flash, dual slide/print film, video camera/recorder.
15. Paper towels and tissue paper. Can be used in rearing of live specimens (growing a particular insect stage to adult) or cleaning thermometer probes, forceps, or other equipment after collecting.
16. Solutions for preserving specimens (uses and formulations described in Chapter 4).
17. Disposable surgical or polyethylene gloves.
18. Eyedropper pipettes (for collecting very small insects in fluids).
19. Insect aspirator, battery powered or blow-type (for collecting small crawling and flying insects).
20. Flashlight or other portable light source.
21. Measuring devices, rulers, grids, tapes, etc. (for photographic purposes and/or for obtaining measurements and distances of other evidence).
22. Berlese funnel (for extracting fauna from leaf litter, soil samples, other material).
23. Shipping containers — styrofoam boxes with lids; small rectangular cardboard boxes (5 × 10 × 12 cm) (2 × 4 × 5 in.) or cardboard and metal screw cap cylinders (7 × 15 cm) (3 × 6 in.), shock absorbing-type packing.
24. Chemical ice (not dry ice) (for cooling and maintaining specimens collected alive).
25. Log book (for recording location, scene data, date, etc.).
26. Forensic Entomology Data Form.
27. Specimen Deposition and Identification Record.

Note: Not all of this equipment is essential for conducting an adequate or satisfactory entomological case evaluation. An insect net, forceps, and vials with the proper preserva-

tion solutions, labels, and maggot shipping containers have been used to facilitate many death-scene investigations. However, having the above listed material available when going to a scene or autopsy will enhance the success of the insect recovery. Much of the listed equipment may be purchased from biological supply and scientific equipment companies.

Insect Labels

Labels for the collected insect specimens must at least contain information as to date and time of collection, case number, location (state, county, and city), and sample number. Additional information can be included if space allows. Labels containing this information should be printed on both heavy bond paper and adhesive paper since one label will be affixed to the outside of the sample container and one label will be placed inside of the container with the insect samples. It is good practice to have many labels preprinted. Computers and widely available precut adhesive paper labels make this an easy task. However, it must be remembered that a laser printer should be used (ink jet and bubble jet printers will not suffice). If the labels are printed by hand, they must be printed with pencil, do not use ink! The alcohol used to preserve insect specimens will dissolve the ink and remove printed letters from the paper surface.

Sample Label

Date/Time:
Case #:
Location:
Sample #:

If space is a concern, the required information can be printed in an abbreviated format:

Date:
CS #:
Loc:
Sa #:

Entomological Sample Log Sheet

Case Number:	Agency:	Date:

NUMBER OF SAMPLES

Preserved:	Live:

WEATHER DATA

SUN	❑ Full		❑ Partly		❑ None
CLOUDS	❑ Completely	❑ Mostly	❑ Partly	❑ Scattered	❑ None
RAIN	Current Rainfall: ❑ Heavy ❑ Light		❑ None	Approx. 24 h total:	
WIND	Direction:		Approx. speed:	Gusts:	
SNOW	Current Snowfall: ❑ Heavy ❑ Light		❑ None	Approx. 24 h total:	

SAMPLE INFORMATION

SAMPLE 1:	Date:	Time:	METHOD: ❑ Aerial ❑ Hand
Location on Body:		Type: ❑ Maggots; ❑ Adult Flies; ❑ Puparia; ❑ Beetles.	
		❑ Preserved	❑ Live for rearing
SAMPLE 2:	Date:	Time:	METHOD: ❑ Aerial ❑ Hand
Location on Body:		Type: ❑ Maggots; ❑ Adult Flies; ❑ Puparia; ❑ Beetles.	
		❑ Preserved	❑ Live for rearing
SAMPLE 3:	Date:	Time:	METHOD: ❑ Aerial ❑ Hand
Location on Body:		Type: ❑ Maggots; ❑ Adult Flies; ❑ Puparia; ❑ Beetles.	
		❑ Preserved	❑ Live for rearing
SAMPLE 4:	Date:	Time:	METHOD: ❑ Aerial ❑ Hand
Location on Body:		Type: ❑ Maggots; ❑ Adult Flies; ❑ Puparia; ❑ Beetles.	
		❑ Preserved	❑ Live for rearing
SAMPLE 5:	Date:	Time:	METHOD: ❑ Aerial ❑ Hand
Location on Body:		Type: ❑ Maggots; ❑ Adult Flies; ❑ Puparia; ❑ Beetles.	
		❑ Preserved	❑ Live for rearing
SAMPLE 6:	Date:	Time:	METHOD: ❑ Aerial ❑ Hand
Location on Body:		Type: ❑ Maggots; ❑ Adult Flies; ❑ Puparia; ❑ Beetles.	
		❑ Preserved	❑ Live for rearing
SAMPLE 7:	Date:	Time:	METHOD: ❑ Aerial ❑ Hand
Location on Body:		Type: ❑ Maggots; ❑ Adult Flies; ❑ Puparia; ❑ Beetles.	
		❑ Preserved	❑ Live for rearing
SAMPLE 8:	Date:	Time:	METHOD: ❑ Aerial ❑ Hand
Location on Body:		Type: ❑ Maggots; ❑ Adult Flies; ❑ Puparia; ❑ Beetles.	
		❑ Preserved	❑ Live for rearing

FORENSIC ENTOMOLOGY DATA FORM

DATE:_____ **CASE NUMBER:**_____

COUNTY/STATE: _____ **AGENCY:**_____

DECEDENT: _____ **AGE:**_____ **SEX:** _____

Last Seen Alive: _____ **Date and Time Found:**_____

Date Reported Missing: _____ **Time Removed from Scene:** _____

Site Description:

_____

_____

_____

Death Scene Area:

Rural: forest_____ field_____ pasture_____ brush_____ roadside_____
barren area_____ closed building_____ open building_____
other_____

Urban/suburban: closed building_____ open building_____
vacant lot_____ pavement_____ trash container_____
other_____

Aquatic habitat: pond_____ lake_____ creek_____ small river_____
large river_____ irrigation canal_____ ditch_____ gulf_____
swampy area_____ drainage ditch _____ salt water_____
fresh water_____ brackish water_____
other_____

Exposure: Open air_____ burial/depth _____
clothing: entire_____ partial_____ nude_____
portion of body clothed_____
description of clothing _____
type of debris on body_____

Stage of decomposition: fresh_____ bloat_____ active decay_____
advanced decay_____ skeletonization _____ saponification_____
mummification_____ dismemberment_____
other: _____

Evidence of scavengers: _____

Possible traumatic injury sites: (Com ment below)

_____

Scene temperatures: ambient:_____ ambient (1ft) _____ body surface_____
ground surface_____ under-body interface_____ maggot mass_____
water temp, if aquatic_____ enclosed structure_____ AC/Heat: on/off_____
ceiling fan: on/off_____ soil temperature: 10 cm_____ 20 cm_____

Number of preserved samples _____ **Number of live samples** _____

NOTE: Record all temperatures periodically each day at the site for 3 to 5 days after body recovery.

SPECIMEN DISPOSITION AND IDENTIFICATION RECORD

Case # :	Date:
Agency:	

Date Obtained	Sample Number	Location on Body	Specimen Lengths (Range)	Number of Specimens	Stage/ Instar	Family/ Species

IDENTIFIER: ______________________________

Laboratory Rearing of Forensic Insects

4

JASON H. BYRD

Contents

Introduction

The laboratory rearing of insects collected from a death scene is an integral component of the analysis of entomological evidence, and it should not be overlooked. The purpose of laboratory rearing is threefold. First, rearing live insects collected from a death scene allows the entomologist to make a more positive species identification. In some cases, particularly with fly larvae, a definitive species determination cannot be made since distinct morphological differences may not exist. The adult stage of an insect species is usually more morphologically distinct than the larval forms and will permit identification with greater certainty. However, laboratory rearing of the larvae is not always essential when making a species identification as many distinct larval forms do exist, and definitive identification is possible by a qualified entomologist.

The second purpose for laboratory rearing is to more clearly define the postmortem interval, or PMI, since the rearing of subsequent life stages provides a better estimation of the amount of development the insects had undergone at the time of collection. Documentation of the time of onset and duration of the subsequent life stages allows the entomologist to accurately determine the time at which the eggs or larvae were deposited on the body. Lastly, the laboratory rearing of forensically important insects can provide

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the forensic entomologist with an invaluable database of insect development under controlled environmental conditions and such data can be accurately applied to future cases to assist in the further refinement of the PMI estimation.

The rearing of some insect species within a laboratory environment can prove to be an extremely difficult task, but fortunately the needs of most insects of forensic importance are easily met in captivity. The major component of a typical rearing setup in the forensic laboratory is the environmental chamber with programmable temperature, humidity, and lighting regimes. Such units are commercially available and relatively inexpensive when compared to the cost of other equipment commonly utilized within the forensic laboratory. However, if such environmental chambers are not available, effective laboratory rearing can still be conducted in a room in which the temperature is kept between 27 to 30°C and 80 to 90% relative humidity (Byrd, 1995). Such environmental conditions are acceptable and will work well for a variety of rearing situations conducted outside of an environmental chamber. A fume hood should serve as room exhaust, and if it is large enough to house several 10-gal (38 l) or 20-gal (76 l) "long" aquariums, rearing can be conducted within the fume hood to minimize odor. However, this practice is generally not recommended due to the high evaporation rates caused by the rapid airflow that occurs under a fume hood.

Aquariums such as those previously described are recommended for use in rearing insects since their size allows for the normal larval migration of most species to occur, and their lightweight construction allows them to be handled with little effort. Aquariums also are recommended because the vertical glass sides are easily cleaned and, when coated with a liquid or powder Teflon® such as Fluron® PTFE, the glass becomes an effective barrier to crawling insects that may attempt to escape. Aquariums also can be fitted with screen lids that will help to contain any flying insects that inadvertently hatch from laboratory rearing projects. These lids fit most aquariums tightly, are inexpensive, and found at most pet supply dealers. While aquariums are best suited for rearing the larval stages of flies, as well as both larval and adult beetles, they are not effective containers for adult flies. Adult flies should be housed within a screened enclosure, which is described in detail later in this chapter. Naturally, the number of aquariums and cages needed depends solely on the laboratory caseload and the number of species in each case that will need to be reared simultaneously.

In laboratory rearing projects of forensic importance, it is obvious that samples from differing cases should not be mixed, but different species from the same case also should not be reared within the same containers. Likewise, adult stages should generally not be mixed with larval stages. The larger adult silphid species such as *Necrodes surinamensis* (see Figures 2.36a,b,c) and *Nicrophorus orbicollis* (see Figures 2.42a,b,c,d), as well as larval and adult staphylinid beetles (see Figures 2.53 to 2.57), are best kept isolated or in low numbers in large containers that have plenty of hiding places. Many larval flies, and adult and larval beetles, are both predatory and cannibalistic. As a result of mixing species, or of overcrowding individuals, you could lose valuable and irreplaceable entomological evidence due to predation. Thus, it is best to keep like stages of the same species together and use as many rearing containers as necessary to accommodate the requirements of the caseload.

Insect Eggs

When collecting entomological evidence at a death scene, the forensic investigator is likely to encounter insect eggs. Their number will vary greatly, but those most commonly

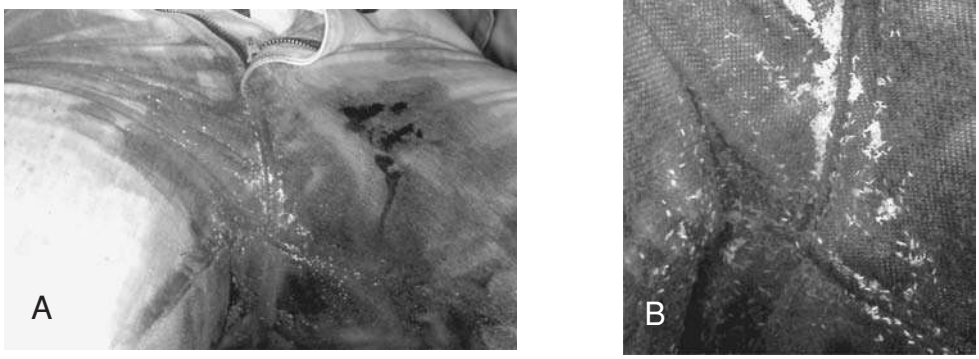


Figure 4.1 (a) Due to their small size, fly eggs often go unnoticed by the forensic investigator. In many cases, they are misidentified by the investigator as sawdust. The eggs are usually deposited around or in the natural orifices of the head, at sites of trauma, or in the genital/anal area. However, when excrement-soiled clothing is present, the adult female flies often deposit their eggs on the outer surface of clothing as seen here. (b) Detail of fly eggs and newly emerged larvae on the outer surface of the clothing seen in Figure 4.1a. Note the length of the eggs and larvae in comparison to the length of the stitch in the denim jeans. (Photos courtesy of James L. Castner.)

encountered are the masses of fly eggs that accumulate around the eyes, nose, mouth, genital/anal area, and around any sites of trauma. Unfortunately, most investigators discount them as "sawdust" or similar materials of inconsequence, and promptly brush them away in their search for "evidence." Fly eggs are often clustered together, and the individual eggs are difficult to detect unless the cluster is inspected closely (Figures 4.1a,b). Although individual fly eggs are visible to the unaided eye, a hand lens or a low-power dissection microscope will readily discern their distinct oblong shape. Collectively and singularly, the eggs have creamy-white to yellowish appearance, are dry to the touch, and will readily "flake" away from the substrate on which they were deposited by the female. Therefore, it is easy to understand why they are frequently overlooked or mistaken as sawdust. But, when any such substance is not readily identifiable at a death scene, it should always be collected for further analysis. Therefore, insect eggs should never go uncollected during the course of a proper death scene investigation.

When the eggs of flies are recovered at a death scene, a representative sample should be immediately placed into an 80% solution of either ethyl or isopropyl alcohol. Simply placing the collected eggs in alcohol is usually suitable for long-term archival storage of such specimens. It is not always a prerequisite that they should be first placed in a "fixative" or boiled as is done with the larvae of flies, but this is a good practice in order to ensure proper specimen preservation. This preservation process will be described in detail later in this chapter.

It is important for investigators to remember that the egg stage is short in duration and, if any unhatched eggs are not immediately preserved, hatch may occur only a short time later. If all of the collected eggs hatched, leaving none preserved, valuable time-interval

evidence becomes permanently altered. Also, if the time of onset and duration of egg hatch of eggs collected at the scene is not properly documented, it could result in a broader and less precise PMI being derived from the entomological evidence. The short duration of this stage makes it an extremely valuable life stage for the forensic entomologist to recover and analyze, as it allows for precise PMI estimation. It would be difficult for the forensic entomologist to obtain the same information from hatched eggs and their resulting larvae than could have been obtained from a proper collection of both living and preserved eggs. Such a collection would consist of a representative sample being immediately preserved, and another separate but equally representative sample being kept alive for rearing purposes. This concept of "companion sampling" should be followed for all insect life stages encountered at a death scene.

Equally as important as preserving a sample of the eggs at the scene, is the immediate shipping of live samples to a forensic entomologist for rearing purposes. In most cases, live eggs must be allowed to continue development in order to make a definitive species identification. With many fly species, it is not always possible to determine species identification utilizing only the egg stage. Even with the species that have a distinctive egg stage, it is a difficult, time consuming, and expensive task involving meticulous preparation of the eggs for scanning by electron microscopy. Therefore, the egg stage is probably the most important stage in which the practice of companion sampling should be employed. Fortunately, it is probably the easiest insect life stage to work with since unhatched eggs need little care, except to be protected from extreme temperatures. Thus, they are easily kept alive for rearing purposes and shipment to the forensic entomologist.

Larval Rearing

The larvae of many fly species of forensic importance can be easily reared on several different substrates. Various cuts of beef, chicken, and pork are commonly used as well as artificial diets (Byrd, 1998). Artificial diets, some of whose main component is dry cat food (Mandeville, 1988), also are popular in mass rearing operations because of their low odor and increased sanitation. However, they are not recommended for use when rearing insects within the family Calliphoridae for the purpose of PMI estimation in a forensic investigation. The larval rearing media is inherently important in forensic investigations since the type of larval food source can alter insect growth rates. For this reason, insects whose growth data will be utilized in legal investigations should be reared only on animal tissue.

For forensic laboratory rearing purposes, pork works extremely well because it does not produce excess liquid during the decomposition process and it does not undergo rapid desiccation. Utilizing ground pork, or beef, as a larval rearing medium is most popular because it is inexpensive and conveniently handled in the laboratory. Beef liver also works well, but it has the undesirable property of rapid desiccation in some situations. Successful development also will occur on chicken, but during decomposition it liquefies readily and the excess liquid may prevent some larval species from developing normally. This excess liquid also makes the laboratory cleanup process more difficult than necessary. For these reasons, pork or beef tissue should be the rearing medium of choice in the forensic laboratory.

Figure 4.2 Typical laboratory facility for the rearing of large numbers of individual case samples. Such a rearing facility can be established quickly and inexpensively for the processing of entomological evidence from multiple cases. (Photo courtesy of Montgomery Nelson, King County Medical Examiner's Office, Seattle, WA.)

Rearing Containers

Any fly larvae (particularly those in the families Calliphoridae or Sarcophagidae) that are part of the live insect collection made during the course of a death investigation, should be placed into a rearing environment as soon as possible once they are removed from the death scene. For proper collection procedures at the scene, please refer to Chapter 3. Once in the laboratory, the insects should be immediately placed into either small plastic (or foil-lined cardboard) containers that have been filled with approximately 150 g of beef or pork, using as many containers as necessary to rear all of the living samples collected (Figure 4.2). If needed, collected insects can be temporarily held (24 to 48 h) in a refrigerator with a temperature between 3 to 6°C (Haskell, 1990).

For laboratory rearing purposes, each larval container (Figure 4.3) should be placed in the center of an aquarium floor that has been covered with either sand or vermiculite to a depth of 1/2 in. (Figure 4.4). Each container will adequately rear approximately 75 to 150 larvae, and experience demonstrates that a 16 oz plastic container (a pint-sized yogurt cup) with short sides works well. The short-sided container serves to keep the larvae associated with their food source, but it will not restrict larval migration (Byrd, 1998). A lid is generally not necessary, or even recommended, as long as an adequate and continuous food supply is maintained. The larvae will always remain associated with their food source until the onset of migration.

If a lid is used, it should be well ventilated with small holes or slits and the lid must be removed at the onset of larval migration. Equipping the rearing container with a tight-fitting lid could alter their normal migration behavior and/or restrict the airflow too much, causing an increase in ammonia that is lethal to the developing larvae. High ammonia levels can accumulate to a toxic level in less than 6 h, killing all of the larvae within the rearing container. Interfering with the normal migration behavior of the insect larvae

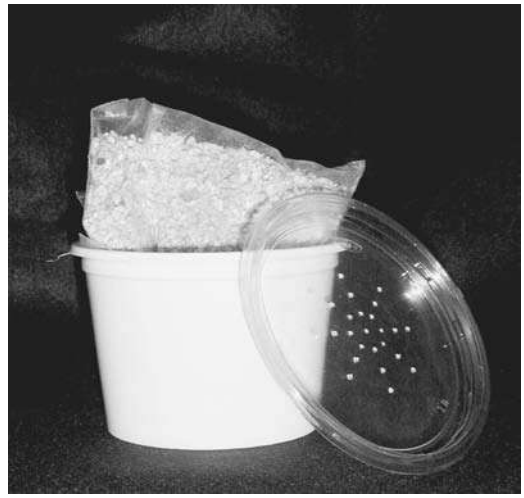


Figure 4.3 Typical larval rearing (or collection) container should be a short-sided plastic cup (16 to 32 oz size) with a tight fitting and ventilated lid. Vermiculite, kept in a resealable sandwich bag until needed, is a convenient way to provide a burrowing substrate for the larvae once they are collected. Containers such as this are inexpensive, can be assembled quickly, and kept in storage until needed. (Photo courtesy of Jason H. Byrd.)

reared as part of a PMI determination can be detrimental to a forensic investigation, as this may cause a delay in pupation and alter the time sequence of all subsequent life stages.

If the larvae are confined at the time of migration onset, pupation and emergence can be delayed or even prevented. It is important to remember that some insect species will die instead of undergoing pupation if they are confined in too small of an enclosure. This fact is particularly important in artificial rearing environments. However, some species

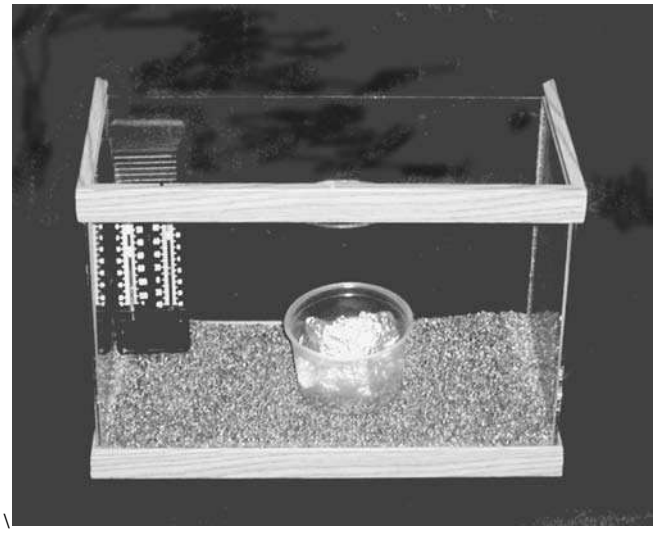


Figure 4.4 Larval rearing containers such as that shown in Figure 4.3 should be placed into a larger container, such as a small aquarium, to more adequately allow for the larvae to undergo their natural migratory stage. Providing this extra space is critical to the successful development of some species, or when large numbers of larvae are within the rearing container. (Photo courtesy of Jason H. Byrd.)

Figure 4.5 *Synthesiomyia nudiseta* pupae on the outer surface of clothing. Pupation in areas such as this is common with this species since the larvae typically do not migrate far away from the remains as in other species. (Photo courtesy of Jason H. Byrd.)

such as *Chrysomya rufifacies* (the hairy maggot blow fly) and *Synthesiomyia nudiseta* will still successfully pupate in a confined environment. Due to this behavior, it is always necessary to give any clothing found with the victim a thorough examination as the puparia of some species may be attached to the inner surfaces of the material, in folds of clothing (Figure 4.5), or along contact points between the victim and ground. Since other species such as *Cochliomyia macellaria* (the secondary screwworm fly) will typically die before pupation if trapped in a confined location, any large numbers of dead or inactive larvae should be noted and collected. A complete search of the surrounding area also should be made to recover any migrating larvae or to collect puparia that have formed after migration. Since there is typically more than one species inhabiting decomposing remains that prefer different pupation sites, it is always necessary to search all items in contact with the body as well as the surrounding area. A detailed discussion of such a search procedure is provided in Chapter 3.

As a result of the alteration in larval development due to improper rearing protocols, the PMI estimation derived may not be valid, and should be used in legal proceedings with great caution. To help eliminate this potential source of error, the use of an open container for larval rearing will allow for larval migration to occur naturally and without delay, thus reducing the possibility of improper rearing and alteration of larval development.

Monitoring Larval Growth and Archival Preservation

Once the larvae are placed on the food source in the plastic or foil-lined cardboard rearing containers, they should begin to feed immediately unless they have already entered the prepupal, or migratory, stage (Figure 4.6). Additional food substrate should be added as necessary (about twice daily), and a few larvae should be collected daily and immediately preserved in order to document the various stages of development. Detailed records should be kept as to stage of development, larval behavior, and time and manner of preservation. The number of larvae that should be preserved during rearing is strictly dependent on population size of the collected larvae. It is more important to have a few individuals reared to adulthood and less preserved as the immature stage, than to have several dozen preserved immature forms and no adults.

The proper preservation of maggots is an essential part of the collection and analysis process, and it should not be overlooked. Fly larvae can be preserved utilizing a variety

Figure 4.6 Migrating larvae of *Chrysomya rufifacies*. Larvae of this species may pupate directly on the remains or on clothing associated with the remains in indoor environments. However, in outdoor locations, the larvae usually migrate away from the remains before forming the pupal stage. (Photo courtesy of Jason H. Byrd.)

of methods. Many of these methods have equally good results, but it is imperative to inform the forensic entomologist of the method of preservation utilized in each case because each method produces various alterations of the larval body, which will affect the PMI estimation. Larval preservation is a two-step process. In order for the larvae to be properly preserved, they must first be "fixed" within 24 to 48 h after larval death. Maggots must be "fixed" because the alcohol will not fully penetrate the larval skin and the bacterial fauna in the insect gut will cause the larvae to decompose even though it is submerged in a solution of preservative alcohol. Decomposition of the larval body will alter its size, cause it to turn black, and make it unsuitable for use in PMI analysis. "Fixing" the larvae can be accomplished by either placing the larvae in a fixative solution such as Kahle's solution or KAA (see Appendix at end of chapter) or by boiling the larvae in water. If a fixative solution is used, the larvae should remain within that solution for not over 12 h and then be removed and placed into an 80% alcohol solution. Leaving the larvae in the fixative for too long will result in them becoming hard and brittle, making it difficult for the entomologist to work with. Due to this reason, and the difficulty sometimes encountered by some law enforcement agencies when attempting to purchase and keep chemical solutions on hand, the most recommended method for fixing larvae is by boiling in water. Since it is difficult to have boiling water at a crime scene, it is recommended that the larvae be placed into alcohol for on-scene preservation and then removed from the alcohol and boiled as soon as possible (or within 24 h after initial preservation), once back in the laboratory. Larvae should be removed from the initial alcohol solution and placed into boiling water and allowed to remain for about 30 sec. After this time, they should be promptly removed and placed into a fresh solution of 80% alcohol. The larvae will then be properly fixed and ready for long-term archival storage or for shipment to a forensic entomologist for analysis. However, the investigator should be advised that the act of boiling the larvae will fully extend the larval body, which could produce a specimen approximately 10% longer than it would be in life (Tantawi and Greenberg, 1993). This

Figure 4.7 When rearing large numbers of larvae in the laboratory, multiple rearing containers can be placed in a larger holding tank. This serves to keep larval aggregations small, reducing the effect of maggot mass heating, while still conserving laboratory space. The bottom of the larger holding container should be covered with at least 1 in. (2.5 cm) of burrowing substrate to allow for normal larval migration. (Photo courtesy of Jason H. Byrd.)

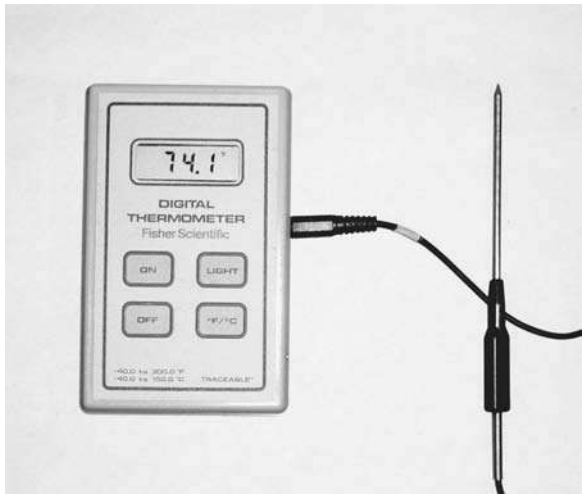
would result in an inaccurate PMI estimation unless the forensic entomologist was advised of the preservation method and compensated for such known changes. Therefore, the forensic entomologist must always be completely advised of the specimen preparation including preservation methods, and it is the duty of the collecting officer to keep detailed notes as to the time and method of preservation.

Problems with Mass Rearing

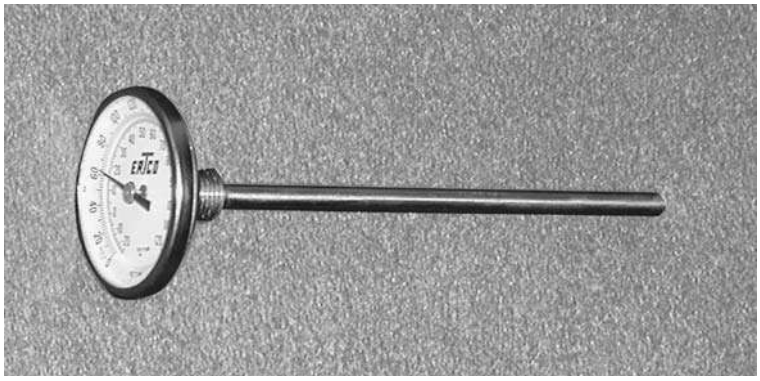
To rear larger numbers of larvae in situations where it is not necessary to keep samples separate (i.e., mass rearing operations), a setup similar to that shown in Figure 4.7 can be utilized. This process simply uses multiple food substrate containers as described earlier, which keeps the larvae/food substrate ratio low, and serves to keep larval masses small and manageable. It also helps reduce the odor produced when rearing large numbers of larvae in the laboratory. However, the most important aspect of utilizing multiple small food containers is the ability to keep the larvae/food ratio low. If there are a high number of larvae per gram of food substrate, the metabolic heat generated by the larvae will rise above the ambient temperature of the environmental chamber and unexpectedly shorten the developmental duration of the larvae (Goodbrod and Goff, 1990; Marchenko, 1988). Such an event is particularly undesirable since the PMI estimate derived from their development also will be shorter than it should be unless maggot mass heat is taken into account. Thus, it is a good laboratory practice to keep a temperature probe inserted into the larval food source to monitor temperatures and account for any unexpected temperature rises (Figures 4.8a,b,c,d). If any are noted to occur, they must be taken into account (or otherwise compensated for) when making the PMI estimation and included in the final report on the thermal history of the larvae.

Larval Migration

When the feeding stage is completed, the post-feeding larvae will attempt to leave their food source and exit their rearing container. This is known as larval migration. Migrating fly larvae will pose an escape problem since they can easily climb out of their rearing container, up the glass sides of the aquarium, and become widely dispersed amongst the



(a)



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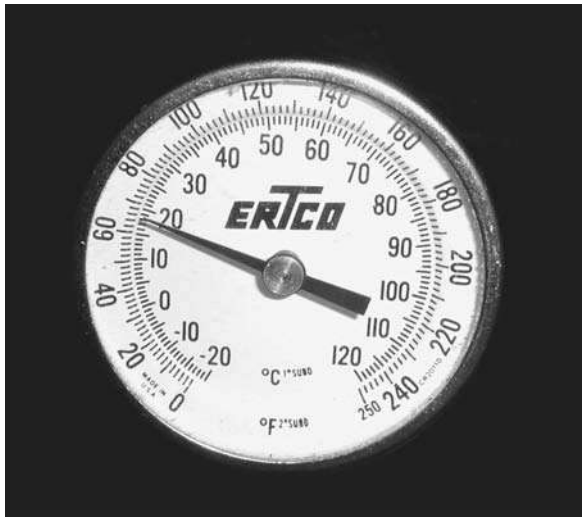
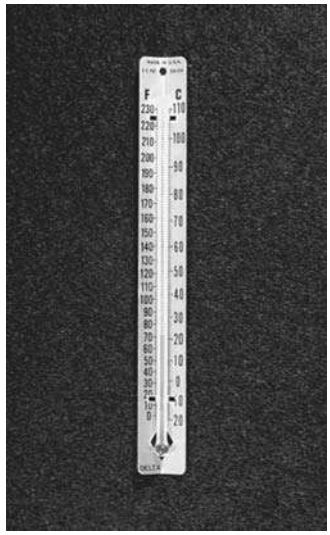


(c)

Figure 4.8 Continued on facing page.



(d)

Figure 4.8 (a) A digital thermometer, with a multipurpose temperature probe is ideal for recording the thermal history of entomological evidence. Such instruments are waterproof and have lighted LCD displays for easy reading in low-light conditions. The probe can be inserted into soil, fluids, and directly into larval masses for proper documentation of scene and laboratory rearing temperatures. (b) A "dial face" or "stem type" thermometer is inexpensive, very durable, and reasonably accurate. Thermometers such as this are ideal for rough service situations, and can be kept as equipment in crime scene units and field vehicles for long periods of time. (c) The face or "dial" of stem-type thermometers can be purchased in many sizes. Thermometers with a dial size of 1 to 1.5 in. (2.5 to 3.8 cm) are generally too small for the temperature to be read easily. However, many models of stem-type thermometers have a dial face of 2 in. (5 cm) making the dial more clearly visible under field conditions. These models are available with increments of either 1° or 2°F or C (shown here). (d) The least expensive and most widely available thermometer is the familiar mercury type; however, they no longer contain mercury. With reasonable care, these thermometers will provide adequate service. (Photos courtesy of Jason H. Byrd.)

equipment of a laboratory if not properly contained. One method commonly used to deter the larvae from crawling up the sides of the container is to cover the aquarium bottom with about 3/4 in. (2 cm) of builder's sand or vermiculite. They will readily crawl up the sides of their rearing container and drop onto the vermiculite or sand-covered aquarium floor. Most fly larvae have a natural tendency to bury themselves in the ground in order to complete pupation, and a substrate in the bottom of the rearing aquarium will allow the larvae to satisfy their natural burrowing tendencies. This substrate also will act to absorb the excess fluid that will accumulate upon migration. Once they find a suitable place for burrowing and concealment, they usually will not continue to wander unless they are too crowded and can not readily disperse. Allowing the insects to undergo their natural behavior will not create any unnatural delays in the time of pupation, which could alter PMI estimations calculated from their development.

Once on the aquarium floor, the larvae will begin to behave in a sluggish manner and their body length will start to decrease, becoming entirely immobile within 24 to 72 h after the start of migration. When fully shrunk and immobile, the larvae will begin to darken from the normal creamy-white color typical of the larval skin to a light red color, then

Figure 4.9 A commercially available rearing container that can be used for rearing terrestrial flying and some aquatic flying insects. The container is fitted with a cone that directs newly emerged adults into the vented top portion where they are held until the container is emptied. (Photo courtesy of Jason H. Byrd.)

changing to darker red, light brown, and finally to a dark brown that appears almost black (see Figure 1.35). Once migration has been completed, allow a couple of days for the larvae to complete pupation and for the pupa to harden before working with the insects. After this time period has elapsed, it is safe to gently sift the pupae from the sand or vermiculite substrate. Samples of the migrating larvae also should be preserved on a daily basis until pupation. A written record of the dates of preservation should be kept and recorded on a label contained with the preserved insects.

Once the pupae have been sifted from the burrowing substrate, they should be placed into an emergence container (Figure 4.9) and monitored closely for the emergence of any adults. The emergence container should be checked at frequent intervals and any emerged adults should be removed, counted, and placed into rearing cages and a small sample preserved. This procedure should be followed repeatedly until no more adults emerge. This practice will provide both the duration and rate of emergence, which is often essential and invaluable data in proper PMI estimation from entomological evidence.

Adult Emergence

At the onset of emergence, the adult inflates a balloon-like organ, called the ptilinum, on its head. The inflation of this fluid-filled organ causes the end of the puparium to split and the adult fly pulls itself out with its legs (Figure 4.10a). When newly emerged adults appear within the emergence container, they will look spider-like instead of fly-like (Figure 4.10b). The body will be gray in color and the small wings will not yet be fully extended. Shortly

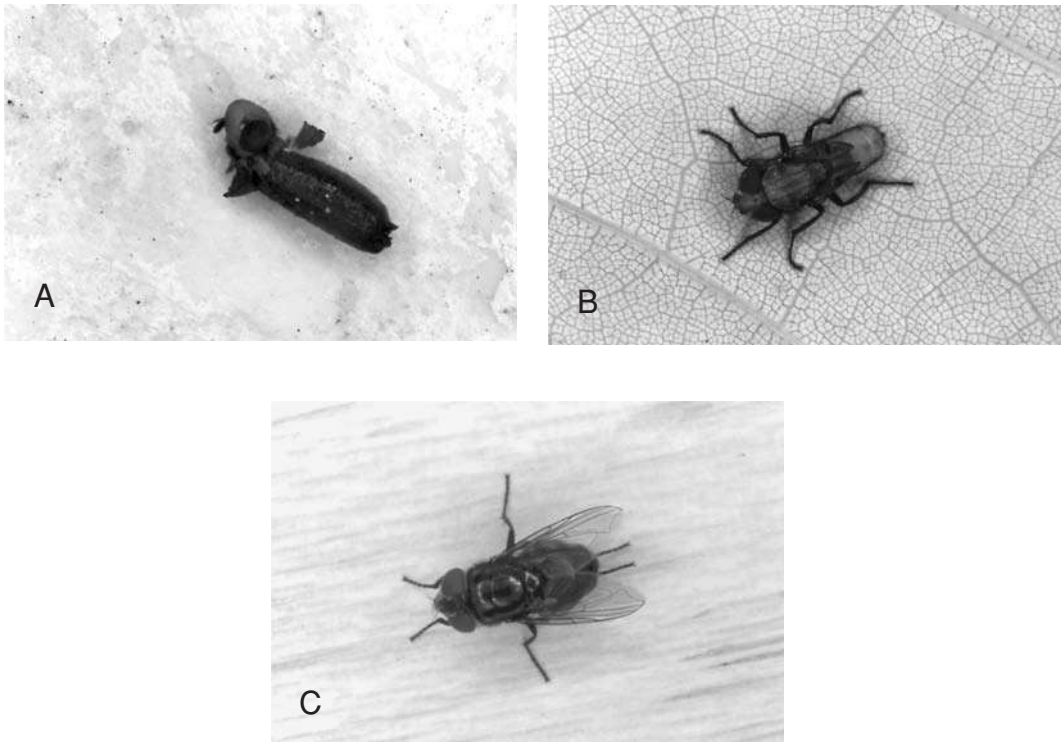


Figure 4.10 (a) An emerging *Cochliomyia macellaria* adult. Once the adult has split open the end of the puparium, it simply pulls itself out with its legs. (b) The newly emerged adult looks spider-like as it does not have its adult color or fully formed wings. They run about rapidly in search of shelter while they harden their exoskeleton, and as a result are often mistaken by forensic investigators as spiders. (c) The emerging adult seen in Figure 4.10A has finally attained its final adult color. This process usually requires several hours, but can vary widely depending on temperature. (Photos courtesy of James L. Castner.)

after emergence is complete, the adult will have expanded its wings, but not yet have attained the familiar shades of metallic green and blue commonly associated with blow flies. The adult fly usually gains the characteristic adult appearance within 8 to 24 h after emergence under typical laboratory conditions (Figure 4.10c). Once these characteristics appear, the adults can be moved from the emergence container and placed into the fly cages after a representative sample has been preserved. Until the time they attain their "normal" adult appearance, they are extremely soft-bodied and easily damaged. Care must be taken to minimize contact with them before they are able to "harden" their exoskeleton.

Some of the laboratory-reared adult insects should be put on pins or card points and properly labeled for further study (Figure 4.11). If the proper pins or points are not available, or the investigator is unfamiliar with pinning techniques, placement of the adult insects directly into an 80% solution of alcohol will adequately preserve adult insects. It is good practice to preserve the adult insects utilizing both methods. However, the investigator should first consult with the cooperating forensic entomologist, as some prefer that the adult insect specimens be simply frozen until analysis. Placing the adult insects in alcohol may alter their color or it may mat body hair so that taxonomic identification is difficult. Both methods will work well, and it is the sole discretion of forensic entomologists as to

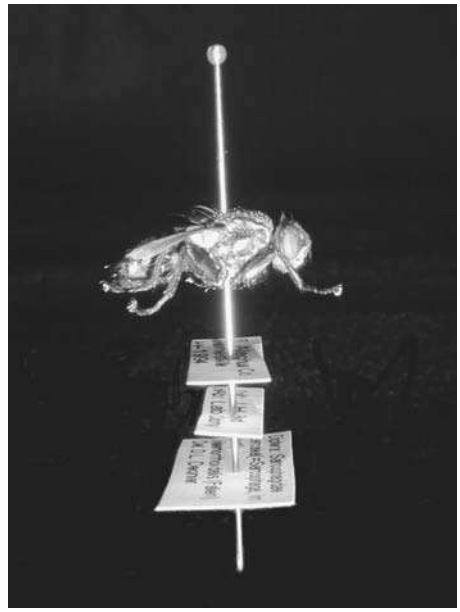


Figure 4.11 Properly pinned adult insect for archival preservation in a forensic entomology reference collection. Adult insects collected from forensic investigations can be pinned and placed into museum-type storage compartments. This properly preserves the adult insect and provides all necessary reference information on cotton bond paper affixed to the pin. However, if pinned improperly, critical identification characteristics can be damaged. (Photo courtesy of Jason H. Byrd.)

which method they prefer. In all cases, the date and time of pupation and adult emergence should be noted and entered into a written laboratory record, as well as a label placed in the alcohol vial or container with the preserved insects. Proper museum (or archival) preservation requires the adult insects to be "pinned" or "pointed" and such mounting should be left to individuals properly trained in this procedure. Improper mounting can permanently distort or destroy key identification features and possibly render the specimen useless for the species identification that is potentially necessary for legal investigations.

Adult Colonies

If necessary, adult flies can be maintained in a colony easily and a standard-size fly cage can adequately contain about 250 adult flies. Adult flies can be kept in a screened enclosure of any desired size, and commercially available fly cages that are approximately 14 in. (35 cm) square are a good choice. Most commercially manufactured fly cages are designed with a collapsible aluminum frame for storage and easy cleaning, and one side is fitted with an elastic "sock" so colony maintenance and the addition of food, water, and meat for egg laying can be accomplished with minimal fly escape. The flies contained within the colony should be provided with a 50:50 mixture of table sugar and powdered milk. Additionally, a supply of fresh water should be available at all times. A drinking container can be fashioned from a plastic specimen cup with a hole bored into the lid to allow for a cotton wick (Figure 4.12). A tray with a solid bottom should be placed under the fly cage to help contain the debris that will accumulate from the activity of the fly colony and to aid in maintaining laboratory sanitation.

Figure 4.12 In laboratory rearing colonies, an open cup of water can cause many flies to drown, eventually possibly decimating the colony population. A small specimen container, with a dental wick inserted through a hole in the lid works well as a constant source of fresh water for the fly colony. (Photo courtesy of Jason H. Byrd.)

Rearing Aquatic Insects

On occasion, it may be necessary to rear aquatic insects that have been collected as part of a legal investigation. Aquatic insect species are commonly found in manmade and natural freshwater and brackish habitats. These include lakes, rivers, sewers, ditches, and artificial containers. These insect species generally do not feed directly on decomposing remains as do terrestrial insects, but they will utilize the carcass as a substrate on which to attach themselves to filter the water for food, or simply use it for shelter. It is the insects and their protective cases attached to human remains and objects associated with crime scenes that allow investigators to determine both the geographic location of body deposition and the seasonality encompassing the PMI.

Unfortunately, a number of aquatic insects that are of forensic importance require an elaborate rearing system, as they need a habitat of flowing water. If they are deprived of these conditions, they can die in a period of minutes or hours. Also, many aquatic insect larvae will leave the body as soon as it is disturbed. For these reasons, it is usually a good practice to make detailed notes and preserve aquatic specimens while at the crime scene. However, for some species found in still water, laboratory rearing is relatively easy and a small aquarium or similar container will suffice. When possible, the collected insects should be kept in the water found at the scene. Tap water can be used, but only after it has been dechlorinated by allowing it to stand about 24 h. Although the collecting container should be completely filled to reduce damage to the insects from excessive jostling during transport, the rearing container utilized in the laboratory should be aerated by an air diffuser connected to a simple air pump such as those commonly used for freshwater fish aquariums. Sticks, rocks, or other vegetation protruding above water level should be provided

within the container so that emerging adult insects can have a refuge out of the water. Feeding aquatic insects can be best accomplished by submerging aquatic plant material found at the scene into the rearing container, and supplementing that with commercial flake fish food. As with terrestrial larvae, periodic collection and preservation should be conducted and a detailed account of the time, date, and method of preservation should be kept to provide the forensic entomologist with a detailed life history of the insects.

A conical screen "tent" apparatus with a removable clear plastic receptacle can be constructed over the top of the rearing container, so that emerging adults will become trapped after they leave the water and take flight. Emerged adult aquatic insects can be preserved by placing them directly into 80 to 90% alcohol (preferably ethyl alcohol). Aquatic insect rearing containers are commercially available and are the same as the containers earlier described as an adult emergence container for terrestrial flies. This container will work well for both terrestrial and aquatic insects and is an indispensable part of the rearing laboratory equipment.

Unique Species Requirements

The rearing of fly species such as *Musca domestica* (the house fly) (see Figures 2.25a,b,c) and *Hydrotaea anescens* (the bronze dump fly) (see Figures 2.23a,b,c) is conducted differently from the blow flies, as house flies and dump flies do not feed directly on the tissues of the carcass. They commonly feed on any fecal matter or clothing and material that have become fetid with urine or feces. In the past, laboratory rearing of species like these was conducted by using a mixture of horse and hog manure, despite its disagreeable nature. Fortunately, Richardson (1932) developed the first artificial rearing medium that consisted of 3.75 lbs (1.7 kg) of wheat bran mixed with 1.75 lbs (0.8 kg) of alfalfa meal. To this was added a mixture of water (5000 cc), yeast suspension (3000 cc) which consisted of 1lb (0.5 kg) bakers yeast and 2 l of water (kept refrigerated until used as needed), and malt sugar (25 cc) (Galtsoff et al., 1937). These ingredients are stirred thoroughly and added to rearing jars or tin cake pans. Larvae can be added to this mixture as soon as it is complete. There is no developmental difference between muscoid flies reared on natural vs. artificial substrates like the one described above, so it is a suitable alternative to the natural larval food source. Thus, artificial rearing medium is used extensively when dealing with muscoid flies due to decreased odor and increased sanitation.

Rearing Beetles in the Laboratory

Skin Beetles (Family: Dermestidae)

The necessary rearing of the various beetle species likely to be encountered during a forensic investigation will require a few modifications of the fly rearing process. For the various species of skin beetles that will be frequently collected in death investigations, beef, chicken, or pork will suffice. However, this food source must be relatively dry before being placed into the rearing chamber, and kept dry throughout the rearing process. In fact, species of *Dermestes* can be easily bred on almost any kind of dried animal matter (Abbott, 1937), and Trogid beetles (hide beetles) in the family Scarabaeidae have habits that allow rearing methods similar to those utilized for the various species of *Dermestes*. The larvae of the

dermestid beetles do not like unusually moist or wet conditions, as do many fly larvae. They also are negatively phototrophic (they move away from light) and are often concealed within or under decomposing remains. The beef or pork utilized for rearing dermestids should be whole and not ground, and it is usually not a problem to keep whole beef or pork dry during decomposition in the rearing environment. Chicken, if used, can be kept dry by cutting it in small strips. Although the food substrate and container should be kept dry at all times, a moist cotton pad covering a shallow petri dish of water should be available to both the adult and larval dermestids at all times.

As with the fly larva rearing protocol, a burrowing substrate should be provided to allow for migration and concealment of the prepupal larvae. If it is not provided, the rearing chamber should be checked daily and the newly formed pupae should be removed and placed into a separate container. If they are not removed, the feeding larvae may feed on the pupa of their own kind. Cannibalism is not uncommon, especially if the food source becomes depleted. Dermestids, unlike any other forensically important insect, can undergo a molting process to reduce their body size if food resources become scarce. Therefore, food must be available in excess, and any periods of food depletion must be accounted for when using the development of dermestids as a basis for PMI estimations.

Carrion Beetles (Family: Silphidae)

The insects of forensic importance in the family Silphidae are large beetles (typically 10 to 35 mm in length) and frequently observed associated with decomposing organic material (Anderson and Peck, 1985). Their most common habitat is that of the vertebrate carcass, and some beetles in this insect family even possess the uncanny behavior of interring small vertebrate carcasses. It is this behavior that has given some species the common name of carrion beetles, sexton beetles, or burying beetles. Most of the silphids are necrophagous as adults and, of the necrophagous species, the larvae feed exclusively on decaying animal flesh. However, the adults may feed on the carrion as well as the other insects present, especially the fly larvae that they readily find on carcasses.

The two subfamilies within the Silphidae, *Silphinae* and *Nicrophorinae*, have evolved radically different methods in which to utilize the carrion resource and avoid competition. Pukowski (1933) first described the life history of the beetles in the genus *Nicrophorus*, and numerous individuals have studied them since that time. A large amount of data have been collected about their development and behavior. When the *Nicrophorus* adults locate a carcass, they first crawl over the outer surface to assess its size and suitability. If the carcass size is small enough (i.e., a small rodent carcass), they will either bury it where it was found or attempt to move it to a different site. Milne and Milne (1976) describe in detail the complex behavior the beetle adults employ when moving a carcass. Once the carcass has been buried, the female then excavates a tunnel leading away from the carcass where she will lay as many as 30 eggs. When the eggs hatch, the larvae crawl toward the carcass and into a hole chewed by the female. Once inside of the carcass, the female regurgitates and feeds the larvae a liquid diet for at least 6 h before the larvae begin to feed on their own. This parental feeding behavior can last through the first couple of months, but it is not required as the larvae will continue to develop even if the female is removed.

The larvae undergo three stages, or instars, which are completed in about 22 to 26 days (Brewer and Bacon, 1975). Once the larval stages are complete, the larvae leave the carcass and crawl into the surrounding soil to pupate. Therefore, it is important to check

the surrounding soil at the crime scene for larvae and pupae of the Silphidae, as well as for the migrating fly larvae. The pupal stage typically lasts 15 days, depending on the species and ambient temperature. Usually, the beetles in the genus *Nicrophorus* do not compete with the Silphinae which commonly utilize larger carcasses (i.e., deer, bear, and human) as their food resource. However, large numbers of *Nicrophorus* adults may be seen on large carcasses, including human cadavers, where they consume both the carrion and the abundant fly larvae (Wilson and Knollenberg, 1984).

The life cycle of the Silphinae is not as complex, and unlike *Nicrophorus* that arrive early when the fly larvae are present, the Silphinae avoid competition for food with the fly larvae by waiting until maggot migration has occurred before their larvae colonize a carcass. There is no known parental–larval interactions within this genus, and development proceeds much more slowly than in *Nicrophorus*. The adults of the Silphinae mate after locating a suitable carcass and the females oviposit (lay eggs) in the surrounding soil. The larvae hatch from the eggs 2 to 7 days after being laid and move to the carcass to feed. On the carcass they undergo three larval instars and pupation takes place in the soil away from the carcass 10 to 30 days after hatch. The pupa has a duration of 14 to 21 days (Anderson and Peck, 1985). Again, sifting of the soil surrounding the body is a critical step in ensuring that all life stages have been recovered. This procedure is particularly important since the larvae and pupae found in the surrounding soil are typically older than those found directly associated with the carcass, which is critical information when determining the PMI based on entomological evidence.

Both the adult and larval forms of the Silphidae are quite common and easily collected. Laboratory rearing of the Silphidae also is relatively simple. Beetle larvae should be placed into an aquarium containing about 5 cm of moist soil and a few leaves added on the soil surface to serve as hiding places. A piece of carrion (beef, chicken, or pork) equivalent in size to a mouse carcass should be added as well as a shallow dish containing moist cotton to serve as a water supply. Once settled, the larvae will then move to the food substrate and burrow into the soil. They will remain in the soil directly underneath their food source and feed from below for about 2 weeks. If adults are present, they will bury the food source so that it is not visible from above. The successful rearing of the silphid beetles is largely dependent on seasonality and species, so expect to meet with varying degrees of success.

The practice of feeding fly larvae and dead adult flies to adult beetles in the family Silphidae, in addition to vegetative matter and decomposing meat, can improve rearing success in the laboratory. It also should be noted that with extremely young larvae of the genus *Nicrophorus* parental feeding might still be required. It is difficult to collect both the larvae and the parental adults unless the parental adult is observed in direct association with the larvae. However, the extremely young larvae that still require parental feeding are so small and inconspicuous that they are not likely to be recovered unless by a trained entomologist. Therefore, the parental feeding that occurs with the *Nicrophorus* is not a likely hindrance to laboratory rearing for forensic purposes. Beetles in the families Cleridae, Histeridae, and Nitidulidae can be reared in much the same manner as with the Silphidae.

Rove Beetles (Family: Staphylinidae)

The life histories and habits of beetles in the family Staphylinidae are little known, primarily because few are of economic importance. They also are not brightly colored enough to be of notice to the casual observer. However, some are large and conspicuous and a few species

are of economic importance as predators of insect pest species, and some life history information has been documented (Mank, 1923; Voris, 1939). As a result, only a select few species are useful in PMI estimations, and both adults and larvae should be collected whenever possible.

Staphylinid larvae can be expected to inhabit a decomposing substrate (animal, vegetable, or human) from about 9 days to over 2 months. While associated with carrion, they are mainly predators of other insects and their larvae that also are attracted to the decomposing remains. Unlike most insects of forensic importance, the amount of decomposition has little effect on their abundance. However, moisture is an extremely important factor, as they seem to prefer drier remains. The staphylinid larvae typically will not colonize a decomposing substrate that is extremely moist. Like many other beetle species, they prefer to feed on the fly larvae and, thus, should never be placed in the same container as fly larvae when collected. The collector should be advised that both adult and larval forms of these beetles remain well hidden and motionless within the cracks and crevices of a carcass and are sometimes difficult to find and collect. Adults seem to be found much more commonly than the larvae. When they are observed, they are usually walking, but are strong fliers and can take flight at any time. When the adults are threatened or disturbed they often turn their abdomen up and hold it over their head in a "stinging" posture. They have no stinging apparatus and this is merely a hollow threat. However, they are able to emit a very disagreeable odor when alarmed which cannot be easily washed away.

Both the staphylinid larvae and adults are hardy in captivity and are easily kept in small containers with about 1 in. (2.5 cm) of moist soil. They should be fed a diet of vegetable material, decomposing meat, and provided with a shallow dish of powdered milk. Live maggots and dead adult flies also can be added to their diet to improve rearing success. In their natural environment these beetles seem to prefer maggots and flies to decaying meat. Moistened cotton should be provided at all times as well as a source of water. The beetles should be kept in a dark rearing environment as they feed much more readily under cover of darkness. The egg-to-adult duration can be expected to be about 6 weeks, but will vary according to temperature (Mank, 1923). The larvae also are known to be cannibalistic and, thus, placing one larva per small container for rearing purposes may be the best method to utilize to prevent the loss and destruction of entomological evidence.

The rearing of entomological evidence is a task not often undertaken by the crime scene technician, law enforcement agency, or medical examiner's office during the course of a death investigation. Typically the laboratory rearing of forensic insects is left to the forensic entomologist. However, in an effort to best preserve forensic evidence, some law enforcement agencies and medical examiner offices are starting to rear collected specimens on their own in addition to the living sample collected for shipment to their cooperating forensic entomologist. Therefore, if the original shipment is lost or the larvae perish in transit, the investigating agency has living samples to serve as backup. Also, rearing operations at some agencies are undertaken simply due to the keen personal interest of some investigators in entomological evidence. These specimens are often incorporated into display or teaching collections at the respective offices. It is expected that more investigators will take it upon themselves to rear entomological evidence, at least temporarily until they can be forwarded to a professional forensic entomologist. This practice will help ensure the use of entomological evidence in criminal investigations, and help support the efforts of the forensic entomologist.

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Appendix

Preservation Solutions for Terrestrial Insects

A. Ethyl Alcohol (Ethanol)

This is commonly known as ethanol. The alcohol is best suited for entomological purposes at 75 to 80% and can be used to kill and preserve adult specimens and for preserving larvae after "fixing" in either Kahle's solution or KAA. Ethanol is usually purchased in "bulk" at 95% concentration. An 80% solution can be produced by adding 15 parts distilled water to 80 parts of the 95% ethanol.

B. K.A.A. (KAAD)

95% ethanol ..... 80 to 100 ml
Glacial acetic acid..... 20 ml
Kerosene..... 10 ml

This solution should be used only for killing larval specimens. The specimens should not remain in this solution for over 12 h as they become brittle and unsuitable for examination. Specimens in this solution should be transferred into a 75 to 80% solution of ethanol as soon as they have been killed.

C. Kahle's Solution

95% ethanol ..... 30 ml
Formaldehyde..... 12 ml
Glacial acetic acid..... 4 ml
Water..... 60 ml

This is another popular solution that should be kept available in the forensic laboratory. This solution can be used for killing and preserving adult insects, and for the preservation of larval specimens.

D. X.A.A.

This solution can be used in place of KAA (or KAAD), but it is not commonly used.

Isopropyl alcohol..... 60 ml
Xylene..... 40 ml
Glacial acetic acid..... 50 ml

Preservation Solutions for Aquatic Insects

A. Carnoy Fluid

Chloroform (30%) ..... 30 ml
Ethyl alcohol (95%) ..... 60 ml
Glacial acetic acid ..... 10 ml

Suitable as a killing agent and preservative for most soft-bodied aquatic insects. However, it is not commonly used due to the danger and restricted use of chloroform.

B. Ethyl Alcohol (90 to 95%)

Most popular solution for the preservation of most eggs, larvae, and pupae of aquatic insects. The adult form of most aquatic insects can be preserved in only 75 to 80% ethanol.

C. Kahle's Soultion

See comments for Kahle's solution above.

D. Pampel's Solution

Formalin ..... 10 ml
Ethyl alcohol (95%) ..... 30 ml
Glacial acetic acid ..... 7 ml
Water ..... 53 ml

Insect Succession on Carrion and Its Relationship to Determining Time of Death

5

GAIL S. ANDERSON

Contents

Introduction

Insects are usually the first organisms to arrive on a body after death, and they colonize in a predictable sequence. A corpse, whether human or animal, is a large food resource for a great many creatures and supports a large and rapidly changing fauna as it decomposes. The body progresses through a recognized sequence of decompositional stages, from fresh to skeletal, over time. During this decomposition, it goes through dramatic physical, biological and chemical changes (Coe and Curran, 1980; Henssge et al., 1995; Van den Oever, 1976). Each of these stages of decomposition is attractive to a different group of sarcosaprophagous arthropods, primarily insects. Some are attracted directly by the corpse, which is used as food or an oviposition medium, whereas other species are attracted by the large aggregation of other insects they use as a food resource.

0-8493-8120-7/01/$0.00+$.50

When the sequence of insects colonizing carrion is known for a given area and set of circumstances, an analysis of the arthropod fauna on a carcass can be used to determine the time of death. This procedure can provide accurate and precise methods for estimating elapsed time since death and is used in many homicide investigations worldwide.

When remains are found weeks, months, or more after death, insect evidence is often the only method available to determine reliably the time of death. Insects colonize in a predictable sequence, with some species being attracted to the remains very shortly after death; others are attracted during the active decay stage, and still others being attracted to the dry skin and bones. Insects continue to colonize a body until it is no longer attractive. When the insects migrate from the remains, they invariably leave evidence of their presence behind, such as cast larval skins, empty pupal cases, and even peritrophic membrane. Meanwhile, the remains themselves have changed and entered a stage of decomposition that is attractive to other, later colonizers. Therefore, when remains are found, the forensic entomologist will study not only the insects that are present on the remains at the time of discovery, but the evidence left behind by earlier colonizers. They also will note the species that are absent, but normally expected to be present, in the colonization sequence. From this information an accurate time of death can be established. However, insect succession on a corpse is impacted by many factors, including geographical region, exposure, season, habitat, etc.

Attraction to the Remains

Insects are attracted to the body immediately after death, frequently within minutes (Anderson and VanLaerhoven, 1996; Dillon, 1997; Dillon and Anderson, 1995; Erzinclioglu, 1983; Nuorteva, 1977; Smith, 1986). Blow flies are the first colonizers and can be attracted over great distances by odor. In South Africa, marked flies of the genus *Chrysomya* were caught in baited traps up to 63.5 km away from the point of release (Braack, 1981). Vision, color, and the presence of other conspecifics on the remains also play a role (Hall et al., 1995). Oviposition is elicited primarily by the presence of ammonia-rich compounds, as well as moisture, pheromones, and tactile stimuli (Ashworth and Wall, 1994). The remains also appear to be more attractive once one female has begun to lay eggs, with many females immediately laying large numbers of eggs in one area (Anderson, unpublished data; Barton Browne et al., 1969). This may be an evolutionary strategy to minimize desiccation and predation during the egg stage, which eventually results in large numbers of maggots on the body. Large maggot colonies (or maggot masses) can break down a body faster than individual maggots, and also can generate heat that can protect the insects against adverse temperature drops. This mass egg-laying behavior was found to be partially mediated by pheromones in Australian fly species (Barton Browne et al., 1969), although experiments on British species indicated that the odor produced by larval activity was neither attractive nor repellent to gravid females (Erzinclioglu, 1996). However, if this behavior is pheromone induced, it is possible that the pheromone is deposited on the egg chorion itself and dissipates after eclosion.

The odors emanating from a corpse change as the body decomposes, becoming more attractive to some species and less attractive to others as time progresses. Although blow flies arrive very shortly after death, they are no longer attracted when the remains have passed a certain stage of decomposition, or become mummified or dry (Nuorteva, 1977).

Some species, such as *Calliphora vicina* Robineau-Desvoidy, preferred decomposed remains to fresh when given a choice (Erzinclioglu, 1996).

In England, *C. vicina* and *Lucilia caesar* (L.) appeared on rodent remains within hours or minutes of death. *L. illustris* (Meigen) was not attracted to corpses in woodland until 76 h after death, and to corpses in open grassland until 48 h after death (Lane, 1975). This seems strange, as *L. illustris* is one of the main flies involved in blow fly strike in sheep in Britain, colonizing living animals (Wall et al., 1992), and is found to cause myiasis in man (Bauch et al., 1984; Greenberg, 1984; Khan and Khan, 1987). Since this species is clearly attracted to living animals, it would be expected that it also would be more attracted to a fresh carcass than one several days after death. However, this delay was not seen in British Columbia where it was found on pig carrion in open pasture within minutes of death, and with eggs being laid within an hour (Anderson and VanLaerhoven, 1996).

Irrespective of carcass size, *Phormia regina* (Meigen) is often reported to arrive later on remains than other blow flies, being attracted a day or two after death (Denno and Cothran, 1976; Goddard and Lago, 1985; Lord and Burger, 1984). In Missouri, experiments showed that although a few adult *P. regina* were collected in the first 24 h, many more were collected on carcasses 24 to 28 h old, and significantly more again on carcasses 48 to 72 h old (Hall and Doisy, 1993). In British Columbia, however, *P. regina* adults were collected from the remains immediately after death, although no eggs were laid until 2 days postmortem (Anderson and VanLaerhoven, 1996). This indicates that, in some cases, adults are attracted to the remains immediately, perhaps to obtain the protein meal required for ovary and testes development (Erzinclioglu, 1996). Apparently the remains are not considered an attractive oviposition media until a few days after death. *Cochliomyia macellaria* (F.) also is reported to be a later arrival and has been attracted to remains 18 to 48 h after death (Hall and Doisy, 1993).

The size of the carcass also seems to effect its attractiveness to blow flies (Erzinclioglu, 1996). For instance, *Calliphora vomitoria* (L.) has been reported to prefer larger carcasses (Davies, 1990; Nuorteva, 1959) while *Lucilia richardsi* Collin prefers to colonize small rodent carcasses (Nuorteva, 1959).

Blow flies are diurnal species and usually rest at night. Therefore, eggs are not usually laid at night, and a body deposited at night may not attract flies until the following day. One study in an urban area found that some blow fly species oviposited in low numbers on rat carcasses placed near sodium vapor lamps, but this is rare and nocturnal oviposition has not been observed in large-scale studies in other areas (Anderson, unpublished data; Haskell et al., 1997). Although blow flies rarely lay eggs at night, they will often lay eggs in dark areas during daytime. These areas include under wrappings, inside closets, in dark basements, in containers, and in chimneys (Anderson, unpublished data; Erzinclioglu, 1996). In fact, it has sometimes appeared that turning off the light in a lab situation can induce egg laying (Anderson, unpublished data). Therefore, it does not seem to be the darkness that inhibits oviposition, but rather the insect's diurnal rhythm. Although nocturnal oviposition is not normally observed in Hawaii, it has occurred in rare situations when a victim has been dumped close to resting blow flies (Goff, personal communication). In Canada, two young bear cubs were shot and disemboweled for their gall bladders late one evening at a large garbage dump. Despite the season (summer) and the fact that large numbers of blow flies were in the vicinity (due to the presence of garbage and other recent bear kills), no eggs were laid on the remains until the following morning (Anderson, 1999a). This difference may be geographical in nature or reflect differences in nocturnal

temperatures between Hawaii and Canada. Fly oviposition is strongly influenced by temperature (Erzinclioglu, 1996) and generally does not occur below 10°C, unless the substrate temperature has been influenced by solar radiation.

Geographical Differences in Succession

Insect colonization of carrion is dependent on many factors, but one of the most important is the geographic region or biogeoclimatic zone in which the remains are found. The biogeoclimatic zone defines the habitat, vegetation, soil type, and meteorological conditions of the area. This obviously has a major impact on the types and species of insects present, as well as their seasonal availability. It also affects the decomposition of the remains, which in turn impacts the insects that colonize them. Many families of carrion insects are relatively ubiquitous, but the individual species involved in decomposition vary from region to region. For instance, species, such as *Chrysomya* spp., found in the southern U.S. and other subtropical zones are not found in western Canada at all and are rare in the rest of Canada, with only one species found in southern Ontario (Cooper, personal communication). Decomposition itself also is quite different in various biogeoclimatic zones (MacGregor, 1999a; 1999b).

The species involved in the sequential colonization of the remains and their times of arrival will vary from region to region. Invariably, certain groups will colonize first, such as blow flies (family: Calliphoridae) and flesh flies (family: Sarcophagidae), but the species involved will vary. In tropical regions such as Hawaii, the first colonizers were *Phaenicia cuprina* (Wiedemann), *Chrysomya megacephala* (F.), and *Chrysomya rufifacies* (Macquart) in the family Calliphoridae, and *Bercaea (= Sarcophaga) haemorrhoidalis* (Fallén), *Parasarcophaga ruficornis* (F.), *Sarcophaga occidua* (F.), and *Helicoba morionella* (Aldrich) in the family Sarcophagidae, although individual species varied with region (Early and Goff, 1986). In contrast, the first colonizers in a Tennessee study were *P. coeruleiviridis* (Macquart) and *Phormia regina* (both calliphorids) (Reed, 1958), while in South Carolina the first colonizer was *Cochliomyia macellaria* (F.) (a calliphorid) (Payne, 1965). However, any given state or province may contain several biogeoclimatic zones, and colonization can vary between zones. In the coastal western hemlock region of British Columbia, frequent early colonizers include *L. illustris* and *P. regina* as well as *C. vomitoria* (Anderson and VanLaerhoven, 1996; Dillon, 1997; Dillon and Anderson, 1995). Farther north, *Protophormia terraenovae* Robineau-Desvoidy was the more common species in both casework (Anderson, 1995) and experimental studies (Dillon, 1997; Dillon and Anderson, 1996a).

Times of colonization of insect species and groups also vary greatly with geographical region. In many areas, dermestid beetles (Coleoptera: Dermestidae) are considered to be very late colonizers, frequently arriving when only skin and bone remains, sometimes months after death (Easton and Smith, 1970; Fuller, 1934; Payne and King, 1970; Reed, 1958; Rodriguez and Bass, 1983; Smith, 1986). In Hawaii, however, some adult dermestid beetles were collected as early as 3 to 10 days after death (Early and Goff, 1986; Hewadikaram and Goff, 1991), although larvae were not collected until later (Hewadikaram and Goff, 1991). In coastal British Columbia, dermestid larvae were first collected from pig carrion in exposed pasture 21 days after death, during the early stages of advanced decay. The majority was collected more than 43 days after death (Anderson and VanLaerhoven, 1996). In the northern region (sub-boreal spruce biogeoclimatic zone)

and the interior region of British Columbia (interior douglas fir biogeoclimatic zone), dermestid adults were first found on pig carcasses as early as the bloat stage, and larvae were found by the decay stage (Dillon, 1997; Dillon and Anderson, 1996a). During the summer in the interior region, dermestid larvae were found as early as the bloat stage, but this was rare (Dillon, 1997; Dillon and Anderson, 1996a).

Piophilidae (skipper flies) is another family with species often found at very different postmortem intervals, depending on the region. In early reports from Europe, piophilid larvae were considered to be late stage insects attracted to remains 3 to 6 months after death, arriving after Dermestidae (Leclercq, 1969; Smith, 1986). This was confirmed in Illinois when the majority of piophilid flies were collected during the decay stage, although no larvae were reported (Johnson, 1975). Isolated reports such as that by Smith (1975), indicated that piophilid larvae could be collected as early as 30 days after death, but these incidents were still considered anomalous. More recently, larvae have been collected from human cases in British Columbia as early as 26 days after death (Anderson, 1995) and from pig carcasses in open pastureland 29 days after death (Anderson and VanLaerhoven, 1996). Further large-scale studies confirmed these results (Dillon, 1997; Dillon and Anderson, 1996a; Dillon and Anderson, 1995). In Hawaii, piophilid flies have been collected from carcasses 33 to 36 days after death (Goff and Flynn, 1991).

Even when a species is found to be present in many different regions, it is possible that there may be within-species differences. However, Sperling et al. (1994) developed a DNA-based approach to blow fly identification and characterized three species, *P. regina*, *Phaenicia sericata* (Meigen), and *L. illustris* using specimens collected from British Columbia. It was then possible to develop a "DNA fingerprint" for these species which could be used forensically to identify a specimen to species, including a damaged specimen that otherwise could not be identified using conventional morphological characteristics. Recently, Gibo performed similar characterizations on specimens collected in Ontario from the same species and determined there were no genetic differences (Gibo, personal communication). However, Byrne et al. (1995) noted biochemical differences among geographic populations of *P. regina*. This could be of value in determining whether remains have been moved from the original death site to a secondary site after death (Byrne et al., 1995).

Geographical region obviously has a major effect on arrival times of different species of insects. This means that data generated in one region or biogeoclimatic zone should not be used to determine time of death in a different region. Databases should be developed for every biogeoclimatic zone in which insects are being used to determine time of death. Major databases are presently available for certain regions in North America including Hawaii (Goff et al., 1986; Goff and Flynn, 1991; Tullis and Goff, 1987), South Carolina (Payne, 1965; Payne and Crossley, 1966; Payne and King, 1972, 1970, 1969; Payne and Mason, 1971; Payne et al., 1968a; Payne et al., 1968b), and British Columbia. Other data also have been collected for specific local areas. In British Columbia, areas covered include the coastal western hemlock biogeoclimatic zone along the coast of British Columbia, including Vancouver Island (Anderson and VanLaerhoven, 1996; Dillon, 1997; Dillon and Anderson, 1995; Hobischak, 1997; MacDonell and Anderson, 1997; VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996), the sub-boreal spruce biogeoclimatic zone, the northern region of British Columbia (Dillon, 1997; Dillon and Anderson, 1996a; VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996), and the interior douglas fir biogeoclimatic zone in the interior of British Columbia (Dillon, 1997; Dillon and Anderson, 1996a). This author is currently working with colleagues to develop databases for Alberta, Manitoba, and

Saskatchewan. Preliminary studies also are being conducted in Nova Scotia (LeBlanc, 1998; Simpson, 1999). Once completed, these databases should cover most of the major biogeoclimatic zones in Canada. However, most biogeoclimatic zones cover vast areas, and there are many variations within these zones. Even over short distances, there can be a great deal of difference in microclimate that can have an effect on decomposition and colonization (Cornelison, 1999). This means that further studies within each biogeoclimatic zone also will be warranted.

Effects of Season

Season has a major impact on weather and the flora and fauna of a region. Thus, the faunal colonization of a body also is impacted. Many blow fly species vary in abundance depending on season. For instance, in Mississippi *Phaenicia (= Lucilia) coeruleiviridis* and *Cochliomyia macellaria* were dominant in the warmer summer months, from April to September, whereas *Calliphora livida* Hall and *Cynomyopsis cadaverina* (Robineau-Desvoidy) dominated in the winter months from October to March, with *P. regina* being found throughout the year (Goddard and Lago, 1985). In Maryland, *P. coeruleiviridis* and *P. regina* were found in both spring and summer, whereas *C. vicina*, *C. livida,* and *L. illustris* were found only in spring, and *P. sericata* was found only in summer (Introna et al., 1991). In a study in Finland, considerable seasonal fluctuation and regional differences were seen between blow fly species (Nuorteva, 1959). Recent and ongoing work in Australia also has shown a dramatic effect of season and sun exposure on pig carcass decomposition (MacGregor, 1999a; 1999b). *P. regina* is normally considered a cool weather species, but in British Columbia it was collected in spring, summer, and fall despite high summer temperatures (Anderson and VanLaerhoven, 1996; Dillon, 1997; Dillon and Anderson, 1996a; Dillon and Anderson, 1995). However, blow fly colonization also appears to be a function of altitude more than season in some regions (Dillon, 1997; Dillon and Anderson, 1995).

In addition to the blow flies, many other carrion insects are impacted by season and have specific peaks of activity. For instance, *Necrophilus hydrophiloides* Guéurin-Méneville (Coleoptera: Agyrtidae) was collected 128 days after death on pig carcasses placed out during the summer in British Columbia (Anderson and VanLaerhoven, 1996), but this elapsed time since death was reached in mid-October, which is the beginning of the species' period of activity, with peak activity reported between November and May (Anderson and Peck, 1985). Later work in this region showed that *N. hydrophiloides* was characteristic of temperatures below 10°C (Dillon, 1997). Therefore, time of colonization for some species may relate less to time since death and more to season. Colonization of remains in water by aquatic organisms also is influenced by season and corresponds to the life cycle of the organism (Hobischak, 1997).

The seasonality, or relative abundance, of certain insects and the potentially differing times of colonization of the remains in different seasons are important for several reasons. First, it means that carrion studies should be performed throughout the year in order to develop a valid database for an area. Second, it means that insects may be valuable in determining season of death. This may be useful when remains are discovered several years after death, although insects will probably be of little use in determining a precise time of death.

In one case involving skeletonized remains discovered in a shallow grave more than 10 years after the disappearance of the victim, the total lack of entomological evidence indicated to the investigators that death had occurred in winter (Rodriquez et al., 1993). This supported the police investigation that indicated a winter death and refuted the defendant's claim that an associate had killed the victim in summer (Rodriquez et al., 1993). However, care must be taken when interpreting such evidence as the time it takes for the natural decomposition of insect material (such as empty pupal cases and beetle exuviae) will be affected by many factors, such as amount of exposure, level of moisture, soil pH, etc. During the excavation of Arikara burials in South Dakota, empty pupal cases of blow flies and flesh flies were found in graves known to be 130 to 160 years old. This indicated that death had occurred between late March and mid-October when such flies are active in that area (Gilbert and Bass, 1967). The excellent preservation was attributed to the low annual rainfall in the region, and to generally dry conditions (Gilbert and Bass, 1967).

Conversely, in a case from the Lower Mainland region of British Columbia (a much wetter area), remains found after only 27 years had very few associated empty blow fly puparia. In this case, the decedent went for a walk in a park area of a temperate rain forest and disappeared during December. The decedent was found shallowly buried under the large root mass of a fallen tree. A large quantity of pill vials, previously containing therapeutic drugs, was found associated with the remains. It was believed that the decedent had wandered into the forest after taking the drugs, had become confused and had taken refuge under the upturned roots of a fallen tree. No foul play was detected. The decedent had died, and over many years the body had slowly become buried by the soil falling from the roots. The remains were skeletonized but had not been heavily scavenged, indicating that insects had probably removed the flesh. As the decedent went missing in December, and probably laid exposed for months or years before natural burial, the remains would probably have been colonized by blow flies in early spring and most of the flesh was probably removed before the body was gradually buried. Larvae would have entered the surrounding soil or the clothing, pupated and emerged, leaving behind the empty pupal cases. Therefore, it would be expected that large numbers of such puparia would have been found at the scene. However, despite the fact that the scene was very carefully searched and the soil in and around the natural grave was screened, only four to five such puparia were found, and these were highly deteriorated. It is probable that the high moisture level in the soil contributed to the destruction of the insect evidence. Therefore, a lack of insect evidence in an old grave can only be interpreted to indicate a winter death if the conditions would normally be considered suitable for the preservation of puparia, or other insect artifacts.

Effects of Sun Exposure

The placement of the corpse has an effect on the decomposition and faunal colonization of the remains. The most obvious effect is that of sunlight and heat. Bodies found in direct sunlight will be warmer, heating up more rapidly and decomposing faster. They will lose biomass more rapidly than bodies in shade and progress through the decompositional stages faster (Dillon, 1997; Dillon and Anderson, 1996a; Dillon and Anderson, 1995; Reed, 1958; Shean et al., 1993). Recent work in Australia has shown dramatic differences between decomposition rates of pig carcasses in sun vs. shade (MacGregor, 1999a; 1999b). Vertebrate scavengers also impact the decomposition and were found less frequently in sunny

habitats (Dillon, 1997; Dillon and Anderson, 1995), therefore having less effect on remains in such locations.

Blow flies exhibit habitat preferences within their regional distribution (Erzinclioglu, 1996) but these may vary by region. In Britain, *C. vicina* and *L. illustris* were found most commonly in open conditions, and *C. vomitoria* and *L. ampullacea* Villeneuve seemed to require dense cover. *L. caesar* appeared to be an intermediate species, preferentially being found in scrub areas with sparse trees (MacLeod and Donnelly, 1957). A later study in Britain revealed that *C. vicina* was found in both sun and shade (Lane, 1975), and in France, *L. caesar* was found to prefer shady habitats (Holdaway, 1930). In British Columbia, *L. illustris*, traditionally considered to be found only in direct sunlight (Smith, 1986), was found on pig carcasses in both open pasture (Anderson and VanLaerhoven, 1996) and in dense forest (Dillon, 1997; Dillon and Anderson, 1996a; Dillon and Anderson, 1995). However, most of the work that refers to *L. illustris* and other flies in the tribe Luciliini colonizing remains only in direct sunlight, originates from studies performed in Northern Europe (Smith, 1986). Therefore, it seems probable that habitat preferences may vary between Europe and Canada, explaining the behavioral differences observed. This is supported by observations in Germany (Haskell, personal communication) in which only flies in the tribe Calliphorini were attracted to bait when conditions were overcast, but flies in the tribe Luciliini were attracted as soon as the sun shone directly on the bait. This was in contrast to studies in the U.S. (Haskell et al., 1997).

Calliphora vomitoria is traditionally considered to be primarily a shade species (Smith, 1986). In British Columbia, it was usually found on pig carcasses in dense forest, although during the fall it was collected from carcasses in direct sunlight in open regions of the forest (Dillon, 1997; Dillon and Anderson, 1996a; Dillon and Anderson, 1995). It was not collected in open pasture (Anderson and VanLaerhoven, 1996). In the state of Washington, *L. illustris* and *C. vomitoria* were collected from a carcass in direct sun and from a carcass in shade. However, more *C. vomitoria* were collected in the shade, and more *L. illustris* were collected in the sun (Shean et al., 1993). *P. regina* was collected in both scenarios (Shean et al., 1993). The arrival time of several species of beetles in the families Carabidae, Staphylinidae, Silphidae, and Histeridae varied with exposure, and members of some families such as the Dermestidae, Cleridae, and Nitidulidae were only found on the exposed pig in Washington State (Shean et al., 1993). However, only one carcass was examined in each habitat, and it is difficult to determine whether these are actual trends for this area. In Canada, where a large number of carcasses were studied in a similar habitat, some beetle species were regularly found on both sun-exposed and shaded carcasses. Others varied in their preferences or times of arrival, although this also was impacted by season and geographic region (Dillon, 1997; Dillon and Anderson, 1996a; Dillon and Anderson, 1995).

In direct sunlight during summer in British Columbia, a study using clothed and unclothed pig carcasses show that the unclothed carcasses were heavily inundated by blow fly eggs, and they also mummified rapidly due to high temperatures. This resulted in the mass migration of undersized 2nd and 3rd instar calliphorid larvae in search of other food sources (Dillon, 1997; Dillon and Anderson, 1995). Such depletion of resources was not observed on shaded carcasses (Dillon, 1997; Dillon and Anderson, 1995), or on clothed carcasses in either sun or shade (Dillon and Anderson, 1996a). In Tennessee, Reed (1958) noted that insect populations were smaller at carcasses in pasture areas than in wooded, shaded areas, but this was not supported in other studies (Dillon, 1997; Dillon and Anderson, 1996a; Dillon and Anderson, 1995; Goddard and Lago, 1985).

Urban vs. Rural Scenarios

Some insect species are found in both urban and rural areas, yet others are very specific to one or the other, which indicates resource partitioning. The early colonizing blow flies include rural, urban, and ubiquitous species. This can be useful in forensic analyses, as certain species of blow flies found on remains may be used to indicate that the remains have been moved from an urban to a rural environment or vice versa (Catts and Haskell, 1990; Erzinclioglu, 1989). However, caution must be exercised since only some species are found exclusively in one or the other habitats, while many species can be collected in both. In an analysis of casework from British Columbia, *P. terraenovae* and *C. vomitoria* were found almost exclusively in rural areas, whereas species such as *P. sericata* were found exclusively in urban areas (Anderson, 1995). Others such as *P. regina*, *Eucalliphora latifrons* (Hough), and *C. terraenovae* Macquart were collected in both habitats (Anderson, 1995). Pig carrion studied in forested regions of British Columbia attracted only rural and ubiquitous species such as *P. regina*, *P. terraenovae, L. illustris,* and *C. vomitoria.* It did not attract more urban species such as *C. vicina* and *P. sericata* (Dillon and Anderson, 1997; Dillon and Anderson, 1996a; Dillon and Anderson, 1995). Pig carrion in open fields in rural southwestern British Columbia attracted only *P. regina* and *L. illustris* (Anderson and VanLaerhoven, 1996). Species such as *C. vicina* and *P. sericata* are commonly considered urban species (Reiter, 1984), but have been collected in rural regions (Anderson, 1995; Haskell et al., 1997). Therefore, caution must be used in determining whether remains have been moved based on insect evidence.

Rural blow flies survive on natural animal carrion, whereas urban blow flies are primarily associated with human refuse in the form of discarded food. Therefore, a further complication arises as many so-called rural areas are close to human habitation, with their associated human garbage, which encourages urban fly colonization and may increase the chances of accidental transport of urban species to rural environments by humans. Parks in urban areas also provide settings for rural insects to be found close to human habitation. At Simon Fraser University in British Columbia, both rural and urban species have been collected (Anderson, unpublished data). The university can be considered a large urban area due to its high human population and presence of human garbage, but is situated on a mountain surrounded by parkland, which in turn is surrounded by a large city. In New Zealand, *Phaenicia sericata* was commonly collected in urban parks and gardens, but was not collected in native bush remnants in urban regions (Dymock and Forgie, 1993). Similarly, *Lucilia cuprina* (Wiedemann) was only rarely collected in garbage despite being a common rural species (Dymock and Forgie, 1993). *Phaenicia eximia* Robineau-Desvoidy, *P. sericata, P. cuprina, Chrysomya putoria* (Wiedemann), *C. albiceps, C. megacephala, Musca domestica* L., *Ophyra* sp., *Fannia* sp., and several species of Sarcophagidae were associated with urban garbage in Goias, Brazil, although whether these species were considered to be solely urban was not reported (Ferreira and Lacerda, 1993).

Bodies Found Inside Buildings

The public and police alike often believe that insects will not colonize remains inside a building. However, insects will colonize remains indoors as easily as outdoors. The succession will be limited by the species that can and will enter a dwelling, and on how well the dwelling is sealed.

Blow flies are strong fliers that can follow an odor plume over a long distance (Erzinclioglu, 1996) and can easily enter buildings. In British Columbia, an analysis of cases over a 5-year period showed that *P. sericata* and *P. regina* were commonly collected from victims found inside houses, while *C. vicina* and *C. terraenovae* were sometimes collected indoors (Anderson, 1995). *P. terraenovae*, *E. latifrons,* and *C. vomitoria* were not collected indoors (Anderson, 1995). Other insect species such as *Piophila* spp., *Hydrotaea* spp. (Muscidae), *Thanatophilus lapponicus* (Herbst) (Coleoptera: Silphidae), and *Necrophilus hydrophiloides* (Coleoptera: Agyrtidae) were collected from human cases indoors, but not exclusively (Anderson, 1995).

Insects will be found even in high-rise apartments. In Germany, numerous carrion-frequenting flies were collected inside a multilevel apartment building, including *Fannia cannicularis* (L.) (the most numerous), *P. sericata, Sarcophaga carnaria* L., *C. vicina, Muscina stabulans* (L.), and *F. manicata* (Meigen) (Schumann, 1990). In Gdansk (Poland), *F. cannicularis, M. stabulans,* and *L. sericata* were found in an 11-story apartment building (Piatkowski, 1991). In Canada, *C. vicina* has been collected on the 18th floor of an apartment complex (Anderson, unpublished data). In all these cases, no remains were present to lure insects, so they were presumably attracted by the presence of normal household garbage. In Germany, the presence of *Parasarcophaga argyrostoma* (Robineau-Desvoidy) (Diptera: Sarcophagidae) is an indicator that remains have been outside at some time, as it is considered to be an exclusively outdoor species (Benecke, 1998).

In a comparison of insects collected from 35 cases of decomposing remains in Hawaii, in both indoor and outdoor situations, Goff (1991) found a greater variety of fly larvae associated with indoor deaths and a greater variety of beetles associated with outdoor deaths. Some species were restricted to remains discovered indoors, while some were restricted to outdoor deaths. Certain taxa were considered to be sufficiently restricted to one environment in this region (i.e., Hawaii) to serve as indicator species (Goff, 1991). Therefore, in some cases, it may be possible to determine whether remains have decomposed *in situ,* or have been moved either from an indoor to an outdoor scenario or vice versa. In a case in England, remains were concluded to have decomposed completely inside a house due to the presence of *Leptocera caenosa* Rondani (Diptera: Sphaeroceridae), a species that the author reported to be common in human habitations but rarely collected out of doors (Erzinclioglu, 1985). Care should be taken with such conclusions, however, as other members of this genus have been found in large numbers on remains buried outdoors in rural areas in both experimental studies (VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996; 1999) and in actual cases (Anderson, unpublished data). It should be noted that Smith (1975) collected this species from a dead fox in an outdoor environment. Moreover, species that may be considered to be indicators of an indoor scenario in one region may not necessarily be restricted to an indoor scenario in another region. For example, *Stomoxys calcitrans* (L.) (Diptera: Muscidae) was considered to be sufficiently restricted to indoor situations in the Hawaiian Islands to serve as an indicator species (Goff, 1991). However, this species is a major livestock pest frequently recovered in and around stables, and commonly found outdoors in other geographic regions (Kettle, 1990).

Effects of Burial

Disposal of the remains is often of paramount concern to a killer, but a human body is a surprisingly difficult object to dispose of and a commonly chosen method is burial. Bodies

buried feloniously, however, rarely are deeply buried as burying a full-sized human body at the traditional 6 ft depth requires a great deal of work and time. The more time a criminal spends with the victim, the greater the chance that evidence will be transferred, and also that the killer will be found with the remains. Therefore, a hasty, shallow grave is usually all that is dug.

Buried remains are still colonized by insects, but burial influences the time required for insects to reach the remains, the sequence of colonization, the species involved, and the rate of decomposition (Payne et al., 1968; Rodriguez, 1997; Rodriguez and Bass, 1985; Smith, 1986; VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996; 1999). The above also are affected by geographical region, soil type, whether the remains are disturbed after death, and the elapsed time since death before burial (Rodriguez, 1997; VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996; 1999), as well as the depth of burial (Mann et al., 1990; Rodriguez and Bass, 1985).

Many studies have looked at insect colonization of buried bodies, although much of the early work centered on human exhumations, rather than empirical studies (e.g., Gilbert and Bass, 1967; Motter, 1898; Schmitz, 1928; Stafford, 1971). Research on buried baby pig carcasses was performed in South Carolina (Payne et al., 1968a) and results indicated that several insect species were confined to buried pigs, and that decomposition was greatly slowed by burial. However, in this study, the carcasses were placed in small coffins, which may mimic a legal burial somewhat, but is unlikely to be representative of an illicit burial. In Tennessee, human burial experiments were performed using six cadavers buried at different times of year in an effort to study decomposition and insect colonization (Rodriguez and Bass, 1985). This work is noteworthy, as it was the only experimental work of its kind to utilize human cadavers. However, due to the limitations imposed by using human cadavers, relatively few were studied and no replication was possible. In addition, the cadavers were often received in varying conditions.

An extensive burial project using pig carcasses was conducted in British Columbia in 1995 and 1996 (VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996; 1999). In these studies, carcasses were buried during the summer in the forested areas of two biogeoclimatic zones. The coastal western hemlock zone, and the sub-boreal spruce zone (characteristic of the Vancouver and coastal region, and the cariboo regions, respectively) were selected. A large number of carcasses were buried with three carcasses exhumed at various time intervals over a 16-month period. Studies were also performed on carcasses buried 48 h after death, and on carcasses that had been disturbed after burial (VanLaerhoven, 1997). The results indicated that, in these areas, muscid flies were much more common on buried remains than on above ground carcasses. Although some calliphorid flies did colonize the remains, maggot masses did not form, and carcass temperatures remained very similar to ambient soil temperature (VanLaerhoven and Anderson, 1999). This is in contrast to results obtained from work in Tennessee on human cadavers in which temperature increases were recorded in the cadavers (Rodriguez and Bass, 1985). The difference may be due to geographic region and the resultant soil type, as soil type has a major influence on insect colonization (VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996).

Although a predictable sequence of insect colonization was clearly identifiable, it was quite different from that of exposed carcasses used in the control group of this and other studies performed in these regions (Anderson and VanLaerhoven, 1996; Dillon, 1997; Dillon and Anderson, 1995; 1996a). Some species were much more commonly found on buried bodies and others more commonly on exposed bodies. Also, species that colonized

both exposed and buried bodies frequently colonized them at different elapsed times since death (VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996; 1999). Most studies noted that decomposition was greatly slowed by even shallow burial (Payne et al., 1968a; Rodriguez, 1997; Rodriguez and Bass, 1985; VanLaerhoven and Anderson, 1999). Figures 5.1a,b,c,d,e,f represent a comparison of decomposition rates over time in pig carcasses above ground and those shallowly buried (≈30 cm of soil above carcass) for the coastal western hemlock biogeoclimatic zone of British Columbia. (VanLaerhoven and Anderson, 1999 provide a detailed description of the research.)

Insect colonization of remains also was delayed by burial by 2 or more weeks in some cases (VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996; 1999). However, when carcasses were left exposed for 48 h prior to burial (a common homicide scenario), the carcasses were colonized by calliphorid flies. These remains were presumably being colonized prior to burial as maggots were present on the carcasses exhumed 2 weeks postmortem after 48 h exposure, but not on carcasses which had been buried immediately after death (VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996).

Depth of burial also has an impact on decomposition and insect colonization (Mann et al., 1990; Rodriguez and Bass, 1985), but has not been extensively studied. Some reports have indicated that carrion beetles will not orient to carrion more than a few centimeters below the surface (Shubeck, 1985; Shubeck and Blank, 1982). However, both adult and larval carrion beetles were found on carcasses buried approximately 30 cm deep in soil in British Columbia (VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996; 1999). At present, work is being conducted in England on the ability of *C. vicina* to detect and exploit buried carrion at different depths (Fitzgerald, personal communication).

Geographic region, habitat, and season all play a major role in insect succession on exposed carrion. Further investigation is required to study the effects of these parameters on buried carrion. Preliminary burial studies are presently being conducted in Nova Scotia (Simpson, 1999).

Most cases of burial refer to illicit burial, in which the victim is usually buried hurriedly and shallowly in an effort to rapidly dispose of the corpse. However, even in traditional legal burials, certain species of insects have been reported to colonize a body buried deeply and housed in a coffin. Such species have undergone several generations on the remains (Colyer, 1954a; Stafford, 1971). *Conicera tibialis* Schmitz (Diptera: Phoridae) was the most common species reported to inhabit coffins, and this species is thought to burrow down to the corpse as an adult in order to lay eggs closer to the corpse (Colyer, 1954a). Adult flies have been observed to burrow upwards through the soil after adult eclosion (Colyer, 1954b). These reports are more than 40 years old, and it is doubtful whether such an insect could penetrate modern vaults and coffins.

Bodies in Water

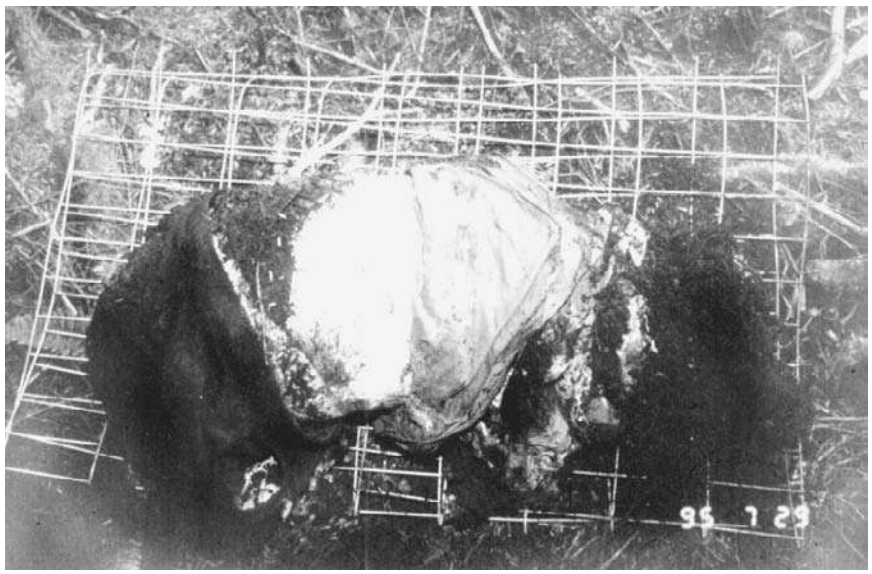
Bodies are frequently found in water, whether a result of a recreational death or an illegal disposal after homicide. When remains are found in water, faunal succession will be very different from that seen on land. This will be impacted by many factors including the body of water (e.g., lake, stream, ditch, ocean), temperature of water, season, presence/absence and type of clothing, scavenging, and biogeoclimatic zone (Hobischak, 1997; MacDonell and Anderson, 1997). In some cases, when the remains are only partially submerged, both

(a)

(b)

Figure 5.1 Clothed pig carcasses decomposing above ground compared with those shallowly buried. (a) Two weeks postmortem above ground. (b) Two weeks postmortem, shallow burial (~30 cm soil above body). (c) Six weeks postmortem above ground. (d) Six weeks postmortem, shallow burial. (e) Three months postmortem above ground. (f) Three months postmortem, shallow burial. (Photos courtesy of Sherah L. VanLaerhoven.)

(c)



(d)

Figure 5.1 *Continued.*

(e)

(f)

Figure 5.1 *Continued.*

terrestrial and aquatic fauna may colonize them. Research in British Columbia has examined pig carcasses in creeks and ponds in the lower mainland region (Hobischak, 1997; MacDonell and Anderson, 1997), and decompositional studies in the marine environment are presently being conducted by this author. However, a recent survey of coroners cases in British Columbia showed that entomological evidence was rarely noticed, and postmortem interval determination was left primarily to the time the decedent was last seen alive (Hobischak and Anderson, 1997). This determination may be valid in an accidental drowning but is unlikely to be accurate in a homicide (Hobischak and Anderson, 1999). Merritt and Wallace provide an extensive review of the colonization of a body in the aquatic environment in Chapter 6.

Bodies in Vehicles

Due to the nature of the crime, homicide victims are sometimes disposed of in somewhat unorthodox places. This can lead to a restricted or changed succession pattern. Cars and other vehicles are often used for suicide or for the disposal of a body. They provide an interesting environment for decomposition, as the vehicle itself may act as a barrier to some species, but will act as a protectant from rain and predators, and also have an effect on temperature and humidity.

A human body was found in a car trunk in the Lower Mainland of British Columbia in October. The victim was last seen alive the previous December, so had probably been in the car trunk through the winter, spring, summer, and into the fall. Winter temperatures in this region are mild, rarely going much below 0°C. The remains were mummified and relatively intact. It was apparent that a number of different species had colonized the remains over time. Dead adult blow flies were found in the car interior, along with a quantity of empty puparia in the car trunk and carpeting, as well as in the clothing of the cadaver. Calliphorid puparia were found along with the larvae of *P. sericata* and *P. regina*. When a body is exposed, the largest numbers of insects are the early colonizers, primarily the blow flies. The species that are considered later colonizers will also arrive, but usually in lower numbers than the blow flies. In this case, although the calliphorid were represented, the vast majority of insects and insect remains collected from the body were later colonizers. Larval sarcophagid (*Liopygia argyrostoma* Robineau-Desvoidy) flies were collected, together with puparia and larvae of two species of the skipper flies, *Stearibia nigriceps* (Meigen) and *Piophila casei* (L.). Also collected were species of Coleoptera, including *Dermestes* sp. (Dermestidae), *Necrobia ruficollis* (F.) (Cleridae), and *Nitidula carnaria* (Schaller) (Nitidulidae). These beetles were attracted both as scavengers and predators.

The calliphorid flies would normally have been attracted to the remains shortly after death. However, since the victim was last seen alive in the previous December and disappeared mysteriously at that time, it is probable that death took place in the winter. Therefore, it is probable that insects did not colonize the remains until the following spring (probably March) when temperatures in this region become consistently warm enough for insect colonization. If the body had been outside in this region from December until spring, it would not have mummified, but the dry interior of the car trunk allowed mummification to occur. This would have made the remains less attractive to blow flies, but still attractive to later colonizers.

The fact that a large range of species, including both Diptera and Coleoptera, were found on the remains indicates that the car itself did not provide much of an obstacle to the entrance of the insects. In fact, there are many entrances into most vehicles, including drainage holes in the trunk and rusted out areas in older vehicles. This vehicle remained parked on a city street for many months until the landlord insisted that it be moved in late summer. The landlord objected to the smell of the "stuff" that was appearing under the drainage holes in the trunk, and to the large numbers of flies that appeared to be attracted. The defendant, who had borrowed the vehicle, apparently sprayed it with a commercially available fly spray, and then drove the car around the corner parking it within meters of its original place. This apparently satisfied the landlord. Some time later, however, a police officer recognized the odor emanating from the vehicle and the killer was arrested (still living in the same apartment). He was convicted of murder.

It might be supposed that a vehicle would prove somewhat of a barrier to insects and that the reduced number of calliphorid flies collected in the above case may have been due to a reluctance to enter enclosed spaces. However, in a more recent case under very similar circumstances, it was shown that large numbers of blow flies could easily enter a vehicle. Human remains were discovered in the summer in the trunk of a small hatchback car parked on a city street in British Columbia (Figure 5.2). A blanket loosely covered the remains. The car was older with rusted areas, and one front window was open about 3 cm. Blow flies had colonized the remains, and larval and pupal *P. regina* and *P. terraenovae* were collected along with a few empty puparia of both species (Figure 5.3). In addition, a few larval *C. vomitoria* were collected inside the vehicle. This was a rare case in which *P. terraenovae* and *C. vomitoria* were found in an urban environment, possibly indicating that the vehicle had been moved. The numbers of blow flies on the remains were considerably less than those normally expected on remains exposed outside. This indicates that the vehicle did provide somewhat of a barrier, although a number of adults had obviously entered the vehicle for oviposition. Some pupae even showed evidence of parasitism, which indicated that pupal parasites also had been attracted into the vehicle.

Under the drainage holes in the trunk, a quantity of body fluids had leached out and a secondary colonization had occurred. Attracted by the fluids were *P. regina*, *P. terraenovae,* and *C. vomitoria.* Also collected at this secondary site were *E. latifrons* and *Hydrotaea* sp. (Muscidae). Beetles had not yet entered the vehicle but were also attracted to this drainage area. These included *Omosita colon* (L.) (Nitidulidae) and *Aleochara curtula* Goeze (Staphylinidae), as well as tenebrionid larvae.

The fact that the remains were inside an almost sealed vehicle in summer would have a strong impact on the temperatures to which the remains were exposed, which in turn would have an impact on the insect's development. Therefore, after the crime scene vehicle had been removed, a vehicle of similar design and color was placed in the same spot for 10 days. A SmartReader 1® datalogger was placed in the trunk under a blanket, and recorded the vehicle temperature every half hour. The temperature data showed that the daytime temperatures inside the car were much higher than the ambient temperatures; however, nighttime temperatures both inside the car and out were very similar (Figure 5.4). Certified weather records from the nearest weather station were collected from Environment Canada, and these data were compared using a regression analysis with those from the datalogger for the same time period (i.e., the 9 days after the discovery of the remains). A regression analysis is a mathematical way of using two known sets of data

Figure 5.2 Hatchback car parked into bushes on city street. Body was in trunk under a blanket. (Photo courtesy of Sgt. Allen Boyd, Vancouver City Police, Homicide Squad.)

(such as the known temperatures in the car recorded by the datalogger and the known temperatures for the same time period from a weather station), and determining the relationship between the two. If there is a good correlation between the two sets of data (traditionally R^2 above 0.5 or 50%), then the relationship can be used to predict the temperature at the death scene for any given day, using the known temperature at the weather station for that day. Utilization of the method allows the scene temperature to be

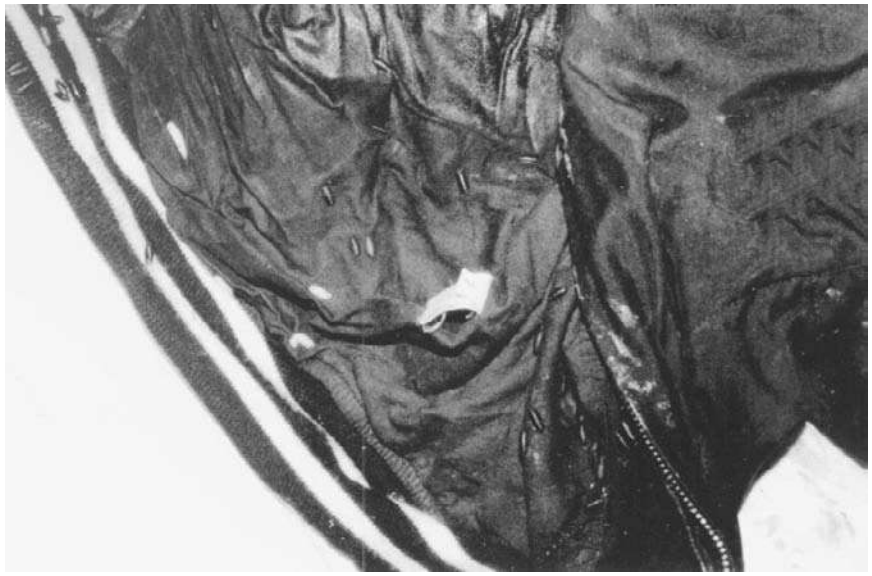


Figure 5.3 Calliphoridae pupae and puparia were found on the clothing and in the trunk of the car depicted in Figure 5.2. (Photo courtesy of Sgt. Allen Boyd, Vancouver City Police, Homicide Squad.)

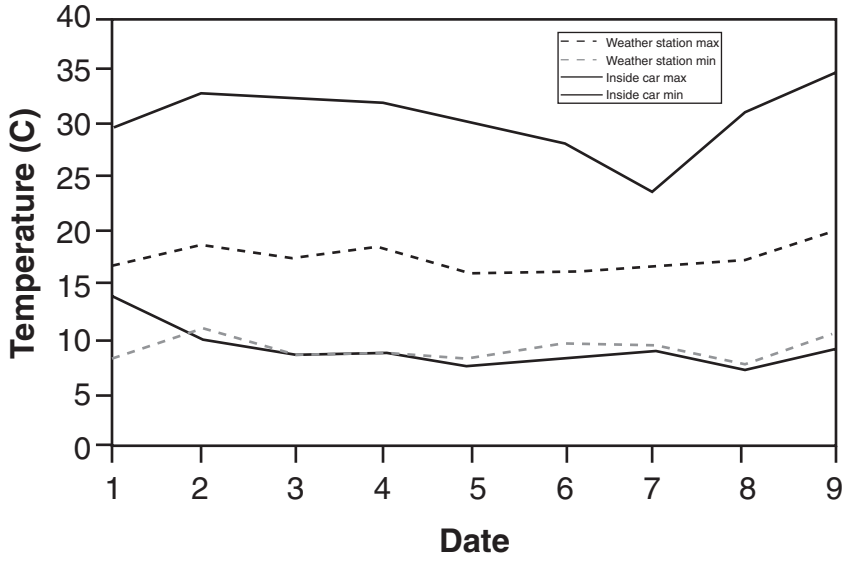


Figure 5.4 Minimum and maximum temperatures recorded inside vehicle over a 9-day period compared with temperatures recorded at Environment Canada weather station for same time period.

predicted. The regression analysis showed a good correlation between the two sets of data, with an R^2 of 0.74. The regression equation was then used to predict the temperature inside the vehicle (Figure 5.5). The entomological evidence indicated that oviposition had begun 15 to 17 days before discovery. However, since the victim was inside the car, it is probable that oviposition was delayed (possibly for a few days) until the odor from the remains attracted insects from outside. This case remains unsolved at this time and the period of delay involved cannot be verified as of yet.

Bodies in Enclosed Spaces

A human body was found inside a kitchen appliance that had been dumped down the side of a cliff in a rural area of British Columbia (Figure 5.6). The appliance had obviously been rolled over the edge and was found suspended in trees 60 to 100 cm above the ground. The remains were wrapped in a sleeping bag. A large number of maggots were present, together with several adult carrion beetles. Several maggot masses were present in the body, and the internal temperature of the remains was 34°C. The appliance was closed tightly, but the front handle was missing, resulting in a small hole approximately 1 cm in diameter, which had allowed entrance of the adult insects. This was the only entrance found. Large quantities of decompositional fluid were present in the bottom of the appliance, indicating that the base area was sealed. The blow flies all belonged to the same species, *P. regina,* and the oldest were in the third instar when discovered. Two species of beetles, *Creophilus maxillosus* (L.) (Staphylinidae) and *Nicrophorus defodiens* Mannerheim (Silphidae) also had located the remains and entered via the small hole. It was later determined that the victim had been killed inside a residence and placed into the appliance. The victim had remained in the appliance at this site for a few days and was then moved to a storage area.

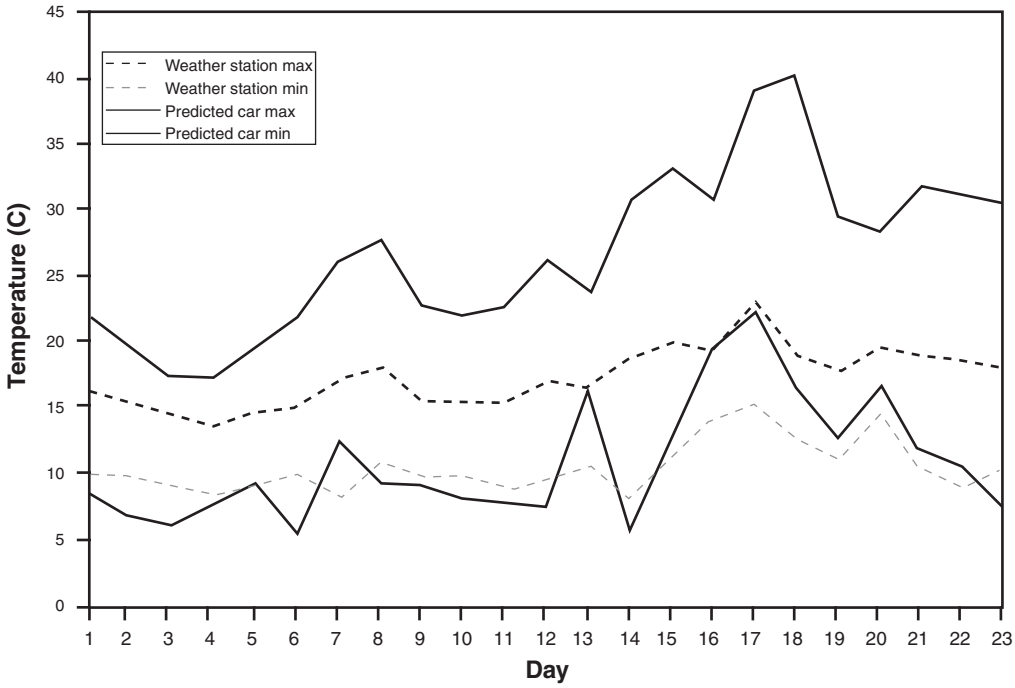


Figure 5.5 Actual temperatures recorded at Environment Canada weather station and car temperatures predicted with regression analysis.

After several days the remains, still inside the appliance, were moved to the rural site where they were dumped. Timing of colonization indicated that it was at this time that the insects entered the appliance.

Figure 5.6 Rural area in which remains were found inside a kitchen appliance. (Photo courtesy of Sgt. Allen Boyd, Vancouver City Police, Homicide Squad.)

Hanged Bodies

Hanging, either as a result of suicide or accident (or more rarely homicide), is a not uncommon form of death. If the body is suspended above the ground, it could present a unique environment for insect colonization. Although extensive research has not been published, some researchers have noted that hanging affects the insect colonization of the remains (Goff and Lord, 1994). They noted that hanging altered the insect colonization by excluding soil-dwelling taxa, thus changing the drying pattern of the body and, consequently, limited the activities of fly species. This reduced the numbers of insects collected and influenced which species colonized the remains as well as their times of colonization (Goff and Lord, 1994). Extensive work is presently being conducted on the taphonomy of hanging deaths in Alberta (Komar, personal communication).

Burnt Remains

Remains may often be burned either perimortem or postmortem. Little research has been published on the effects of burning on insect succession, but a recent study from Hawaii has shown that burned remains are colonized in a manner different from unburned remains (Avila and Goff, 1998). In this study, the remains were burned to give a CGS (Crow-Glassman Scale) level # 2 burn. The CGS scale is divided into five levels, depicting increasing destruction to the body relative to burn injury (Glassman and Crow, 1996). At level #2, the remains were charred with cracked skin (Avila and Goff, 1998). The arthropod fauna which colonized the burned and unburned carcasses were basically the same, but appeared slightly earlier on the burned carcasses (Avila and Goff, 1998) presumably due to the openings caused by the cracked skin. The burnt carcasses attracted much more fly oviposition than the unburnt carcasses, showing that burnt carcasses are still extremely attractive to calliphorid flies (Avila and Goff, 1998). Other work had previously suggested that oviposition was deterred by burning (Catts and Goff, 1992), but this no doubt depends on the level of burning and amount of incineration. In two cases in Italy, insect evidence (primarily larval calliphorid and sarcophagid flies) could still be used to determine time since death, despite the fact that both victims were burned (Introna et al., 1998). Killers often try to dispose of a victim by burning the body, but are unaware of the tremendously high temperatures and the time required to completely incinerate a human body. Even in the extreme heat of a professional crematorium, recognizable pieces of human remains are still present (Kennedy, 1996; Murray and Rose, 1993). The level and amount of colonization of burned remains by insects will no doubt be strongly influenced by the amount of flesh remaining, with more complete incineration reducing insect colonization. This author has participated in several cases of extreme burning in which no or very few insects colonized the remains.

Wrapped Remains

Remains, whether whole or dismembered, are frequently found wrapped in some material. This may be an effort to disguise the remains, to facilitate handling, or to prevent bleeding onto a carpet or vehicle. The type and extent of the wrapping may affect the insect colonization pattern of the remains.

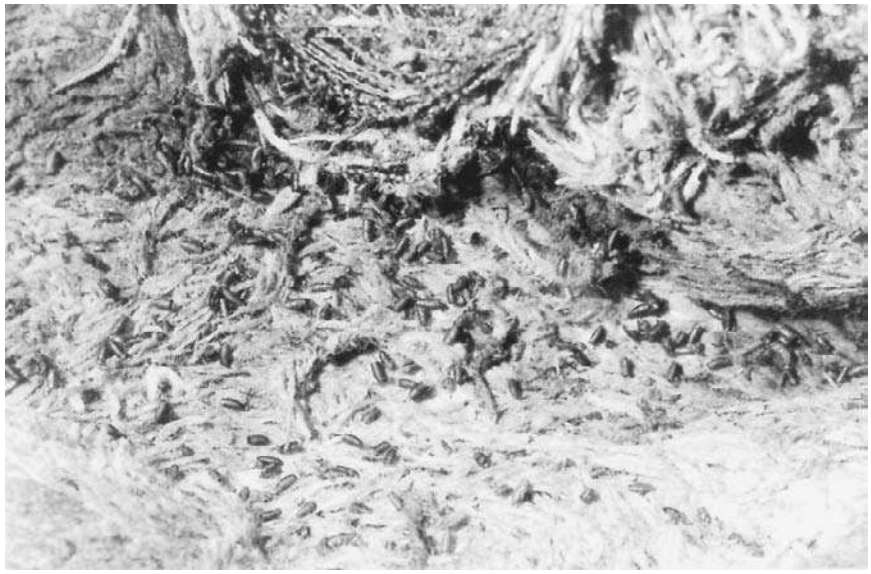


Figure 5.7 Large numbers of calliphorid puparia (empty pupal cases) on carpeting used to wrap body. (Photo courtesy of the Royal Canadian Mounted Police.)

A male victim was found halfway down a scree (loose rock) slope in British Columbia in spring. The remains were completely wrapped in an old carpet, but the carpeting was not sealed at the head or feet. The carpeting had not prevented insects from colonizing the remains, but resulted in the majority of prepupal insects remaining within the carpet to pupate. The remains were skeletonized, primarily due to insect activity, and large numbers of empty calliphorid puparia were collected (Figure 5.7). Later colonizing flies (such as Fanniidae) also had been present, as evidenced by their empty puparia. Therefore, these species were not dissuaded from the remains by the presence of the wrapping. This is probably to be expected as these species are collected in much larger numbers on buried remains than on exposed remains in B.C. (VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996; 1999), indicating a preference for protected, moister areas. Adult staphylinid beetles also were collected, but no other Coleoptera were found in this case. The entire remains were skeletonized, except for one foot located inside a boot, which had saponified to adipocere tissue. A few muscid flies (*Hydrotaea* sp.) were also collected from this area. In this case, it would appear that wrapping the body did not impede insect colonization, but possibly provided protection from predators and the elements. More secure wrapping may delay insect colonization, although this author has not yet seen a case where it was entirely prevented.

In Hawaii, a young female victim was found heavily wrapped in blankets in a rural, outdoor habitat. In order to determine the possible delay of insect colonization due to the wrapping, Goff simulated the case experimentally by wrapping a freshly killed pig carcass in a similar manner and observing how long it took for insects to begin to colonize the remains (Goff, 1992). Insects were first seen on the pig carcass 2.5 days after death, indicating a probable delay in colonization of 2.5 days in the human case (Goff, 1992). This later proved to be the case.

The previous case was outdoors, but a similar scenario was seen indoors in British Columbia, where a man was found in a basement completely wrapped in a large cloth bag

Figure 5.8 Garbage bag containing human thigh. (Photo courtesy of Sgt. Allen Boyd, Vancouver City Police, Homicide Squad.)

originally designed for sports equipment (Anderson, 1999b). The basement also was used as a hydroponics facility for marijuana and, thus, had controlled and known temperatures. *P. regina* had heavily colonized the body with all stages from early instars to empty puparia being collected from the remains and the surrounding area. The insects indicated that oviposition had begun between 13.5 to 16 days prior to discovery. The victim had last been seen alive 18.5 days prior to discovery, indicating a possible delay of 2.5 to 5 days before oviposition. Both the wrapping of the remains and the fact that the body was indoors would have compounded this delay. Although only blow flies were found, later succession insects might have eventually colonized had the remains been present for a longer period of time.

In the following case, the plastic wrapping was complete and secure. A human thigh was found tightly sealed in a plastic garbage bag in British Columbia during the late summer (Figure 5.8). The remains were suspended in a bush when found. The bag was a commercially available plastic garbage bag with drawstrings that had been pulled closed. No tears or cuts were visible on the bag. No adult flies were collected, and there appeared to be no possible entrance for adult insects. However, a number of third instar *P. regina* and *L. illustris* were collected from the distal portion of the leg, inside the bag (Figure 5.9). It is probable that the adult females detected the presence of the body part in the bag, and laid their eggs on the knot of the drawstrings. When the first instar larvae eclosed, they would have oriented to the carcass and would have been small enough to crawl around the knot and to the body part. Therefore, although it is probable that the presence of the knotted bag delayed insect colonization, it was not prevented.

In a case from northern France, plastic bags wrapping an entire corpse were interpreted as having prevented insects from colonizing remains (Bourel et al., 1995). Flies in the families Calliphoridae, Piophilidae, Fanniidae, Phoridae, and Sphaeroceridae had colonized the remains, among other insect species. This was interpreted to indicate that the remains had been exposed for a period of time (long enough for blow fly colonization) before being wrapped. It was thought that this wrapping excluded "second- and third-

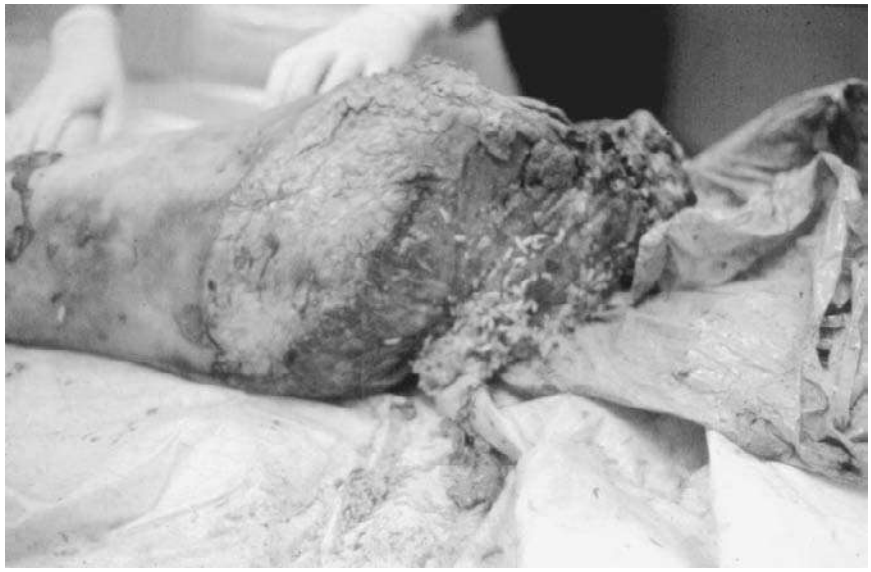


Figure 5.9 Human thigh, with third instar *Phormia regina* and *Lucilia illustris* (Diptera: Calliphoridae) colonizing the distal, cut end. (Photo courtesy of Sgt. Allen Boyd, Vancouver City Police, Homicide Squad.)

wave" colonizers, then months later, tears developed in the plastic and "fourth- and fifth-wave" colonizers arrived. An elapsed time since death of 11 to 12 months was predicted from this data (Bourel et al., 1995). However, recent work in British Columbia has indicated that many of these so-called fourth- and fifth-wave insects actually colonize much earlier than was previously thought (Anderson and VanLaerhoven, 1996; Dillon, 1997; Dillon and Anderson, 1995; 1996a; VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996; 1999). Thus, it is possible that the remains had been colonized earlier by the insects entering through knots or overlapping seams.

Other Factors which May Affect Succession

Scavenging

Scavengers other than insects also are attracted to remains, and can remove large quantities of flesh and even clothing (Dillon, 1997; Dillon and Anderson, 1996a; Hobischak, 1997). This can have a major effect on the decomposition rate and consequent insect colonization. In British Columbia, vertebrate scavenging (primarily from small rodents) occurred most commonly in shaded areas. Despite the cooler temperatures, pig carcasses situated at shaded sites were scavenged and lost mass at the same rate as those in direct sun. This equal mass loss rate was attributed to scavenging (Dillon, 1997; Dillon and Anderson, 1995; 1996a).

Carrion in aquatic habitats also was scavenged more often in shade than in direct sun (Hobischak, 1997; MacDonell and Anderson, 1997) (Figure 5.10). Scavenging was also more common in winter than summer (Dillon, 1997; Dillon and Anderson, 1995; 1996a). Vertebrate scavenging increased the rate of decomposition and, in some cases, eliminated

Figure 5.10 Mink scavenging pig carcasses in a creek in a forest in the coastal western hemlock biogeoclimatic zone. (Photo courtesy of Niki R. Hobischak.)

decompositional stages in pig, bear, and cougar carcasses. This resulted in less tissue available for later colonizers and, consequently, reduced the numbers of species and individual insects colonizing the remains over time (Dillon, 1997; Dillon and Anderson, 1995; 1996a; 1996b; 1997). Vertebrate scavenging appeared to be virtually eliminated in shaded areas in coastal British Columbia and greatly reduced in northern B.C. by shallow burial, although it did occur on rare occasions (VanLaerhoven, 1997; VanLaerhoven and Anderson, 1996; 1999).

Scavenging, in addition to affecting decomposition and insect colonization, may also produce postmortem artifacts that may be initially mistaken for wounds or mutilation (Anderson, unpublished data; Dillon, 1997; Dillon and Anderson, 1995; 1996a; Patel, 1994). Conversely, wounds originally mistaken as rodent damage may actually have other causes (Patel, 1995).

Scavengers, acting as opportunistic predators of insects, are common on remains. Although they may remove substantial numbers of colonizers (particularly blow fly larvae), they usually have little impact overall. However, some insect scavengers (due to their voraciousness and numbers) can have a substantial impact on arthropod colonization of remains. One such example is the fire ants (Hymenoptera: Formicidae: *Solenopsis* spp.) which may remove significant numbers of blow fly eggs and larvae (Early and Goff, 1986; Greenberg, 1991; Hayes, 1994; Stoker et al., 1995; Wells and Greenberg, 1994). Other species of ants also have been shown to have an impact (Cornaby, 1974; Lord and Burger, 1984), but most are present throughout decomposition as scavengers with little effect on overall decomposition rates (Anderson and VanLaerhoven, 1996; Payne et al., 1968a; Payne and Mason, 1971). In British Columbia, a pig carcass placed accidentally close to an ant nest was scavenged almost clean of blow fly eggs for 2 days until numbers of blow fly eggs were so large as to overwhelm the ant presence. After a few days, colonization proceeded as normal (Dillon and Anderson, 1995).

Presence or Absence of Clothing

Human victims are frequently clothed. The clothing may be complete or partial. In forensic cases in British Columbia, the majority of victims were completely or partially clothed. Alternatively, the victim may be naked but wrapped in a variety of materials such as carpet, blankets, sleeping bag, towels, etc., which act in a manner similar to clothing. Clothing can be expected to have an effect on insect succession on a corpse, as it affects the temperature and humidity of the remains, the amount of shade, and protection the body provides, etc. In British Columbia, clothed pig carcasses were found to have increased numbers and diversity of successional insects (Dillon, 1997; Dillon and Anderson, 1995). Most early instar larvae require liquid protein for survival (Smith, 1986). As the clothing becomes saturated with decompositional fluids, it provides more sites for oviposition than a naked corpse, resulting in larger larval masses and, hence, faster decomposition (Dillon, 1997; Dillon and Anderson, 1995; 1996a).

The clothing also can provide additional shelter for blow flies and their predators, increasing the number of Coleoptera on the remains and making the remains more attractive for species that prefer wetter environments (Dillon, 1997; Dillon and Anderson, 1995; 1996a). Conversely, clothing may make the remains less attractive to insects preferring dried remains. In aquatic environments, clothing provides shelter and extra attachment sites for aquatic fauna (Hobischak, 1997; MacDonell and Anderson, 1996). However, the effect of clothing depends on whether the body is completely or partially submerged. When the body is exposed above the waterline, clothing protected maggots from predation; whereas, below the waterline, organisms such as crayfish and caddisflies (Trichoptera) fed on unclothed regions preferentially (Hobischak, 1997; MacDonell and Anderson, 1996). Clothes permeated with lubricants, paint, or combustibles may double the time for initial colonization and have been shown to retard decomposition (Marchenko, 1980, cited in Greenberg, 1991).

Pig carcasses are frequently used to mimic human remains and are considered to be an excellent model for human decomposition (Catts and Goff, 1992). Most research has concentrated on naked pig carcasses (e.g., Anderson and VanLaerhoven, 1996; Hewadikaram and Goff, 1991; Shean et al., 1993; Payne, 1965; Payne and Crossley, 1966), although some work has included clothing (Komar and Beattie, 1998; Dillon, 1997; Dillon and Anderson, 1995; 1996a; Hobischak, 1997; MacDonell and Anderson, 1997; VanLaerhoven and Anderson, 1996; 1999; VanLaerhoven, 1997). Therefore, clothing can have a considerable impact on the decomposition, and it is important that future studies include this aspect of colonization as a consideration.

Insects also have been shown to move and tear clothing in a manner that may mislead investigators into assuming that a sexual assault has taken place (Komar and Beattie, 1998). Maggot masses have been able to move clothing from underneath a body, despite the overlying carcass weight. In carcasses clothed in skirts, the underwear and pantyhose were moved down to the distal hindlimbs while the skirt was pushed up (Komar and Beattie, 1998). Natural decompositional changes such as bloat tore clothing as well (Komar and Beattie, 1998). Heavy clothing may deter carnivore scavenging (Haglund, 1997), but carnivores also can cause clothing disarray. Such postmortem artifacts are usually easy to differentiate from that caused by insects (Komar and Beattie, 1998).

Pigs are chosen as good human models for many reasons, including the fact that they are relatively hairless. However, carcasses with a coat of fur also have been frequently used

to generate carrion data (Braack, 1981; Bornemissza, 1957; Denno and Cothran, 1976; Dillon, 1997; Dillon and Anderson, 1996b; 1997; Early and Goff, 1986; Easton, 1966; Jiron and Cartin, 1981; Putman, 1978; Reed, 1958; Smith, 1975). In British Columbia, the diversity of insects and times of colonization was found to be similar in bear and cougar carcasses to that seen in clothed pig carcasses, indicating that the fur acted in a similar manner to that of the clothing (Dillon, 1997; Dillon and Anderson, 1996a; 1997).

Conclusions

The predictable sequence of insect succession on a body has long been recognized as an excellent method to estimate the time since death. However, there are many diverse parameters that can affect the timing and species composition of the carrion fauna. It is vitally important to be aware of all the factors that can impact insect colonization of remains and to take them into account when analyzing a death. In particular, research is needed to develop a geographical database of insect succession on carrion in a variety of habitats and scenarios in North America.

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The Role of Aquatic Insects in Forensic Investigations

6

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Contents

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Introduction

Historically, different organisms such as plants, pollen, fungi, mammals, and insects have proven useful in forming evidentiary linkages among suspects, victims, or property with specific locations, particularly at outdoor crime scenes (Easton and Smith, 1970; Hall, 1990; Lane et al., 1990; Lord, 1990; Smith, 1986). In a court of law, medicocriminal forensic entomology is defined as the application of the study of insects and other arthropods to violent crimes such as murder, suicide, rape, as well as physical abuse. Lord (1990) stated that human corpses, whether they have been produced naturally or as the result of foul play, are processed by insect decomposers in the same manner as any other carrion. Although considerable amounts of important research and reviews in the field of forensic entomology were produced before 1990 (e.g., Keh, 1985; Meek et al., 1983; Nuorteva, 1977; Rodriquez and Bass, 1983; Smith, 1986), a large body of literature has emerged on arthropod colonization of terrestrial carrion (including humans) in the past 10 years. This has proved to be a great utility for forensic science (see reviews by Catts and Goff, 1992; Catts and Haskell, 1990; Haskell et al., 1997). In spite of this tremendous body of research, the role of freshwater and marine fauna in forensic investigations has received very little attention (Catts and Goff, 1992; Haskell et al., 1989; Hobischak, 1997; Nawrocki et al., 1997; Vance et al., 1995).

A review of the literature found that over 85% of studies pertained to terrestrial organisms, while approximately 15% pertained to aquatic organisms and their role, if any, in death scene investigations (Catts and Goff, 1992; Goff, 1993; Hobischak and Anderson, 1999; Smith, 1986). To date, most empirical evidence examining insect colonization in aquatic systems has concentrated on blow flies (Calliphoridae), and a few other terrestrial species that colonize a corpse after it bloats and rises to the surface. Those species that are restricted to aquatic ecosystems for survival in one or more life stages has been largely ignored (Goff and Odom, 1987; Hobischak and Anderson, 1999; Mann et al.,1990; Nuorteva et al., 1974; Payne and King, 1972; Smith, 1986; Tomberlin and Adler, 1998). In fact, submersion in freshwater environments can alter the terrestrial faunal succession on carrion or corpses and subsequently alter the process of decomposition (Haskell et al., 1989; Payne and King, 1972; Rodriquez, 1997; Smith, 1986).

Evolutionarily speaking, there are no truly sarcophagous aquatic insects that have evolved functionally to feed on carrion alone (Haskell et al., 1989). This is in contrast to the common terrestrial indicator species (e.g., blow flies and flesh flies) that can often provide a predictable time frame of succession, assisting in determining the postmortem interval. Frequently, dipteran larvae are the principal insect component within the terrestrial arthropod assemblage inhabiting vertebrate carrion and, therefore, forensic entomologists tend to focus on this group (Catts and Goff, 1992; Greenberg, 1991; Haskell et al., 1997). Because oviposition by terrestrial flies does not occur on a completely submerged carcass or corpse, it has been suggested by several investigators that the potential use of algae, silt and/or sediment deposition, or the presence of aquatic macroinvertebrates and the casings or structures housing them, may be of significance (Haskell et al., 1989; Keiper et al., 1997; Nawrocki et al., 1997; Siver et al., 1994).

As noted by Sorg et al. (1997) and modified here, human remains found in freshwater or marine ecosystems serve several ecological functions. Depending on the nature of the resting site (e.g., pool or riffle, depth and substrate), degree of decomposition, and the composition of the local fauna, human remains in water may: (1) become immediate or

eventual sources of food for a wide variety of invertebrates and fish, (2) provide a sheltered microhabitat for small non-scavenging species, (3) draw in a variety of secondary predatory species attracted to the original scavengers, (4) provide substrate upon which primary producers (e.g., algae and other periphyton) can colonize and grow, and (5) at advanced stages of decomposition, serve as substrata for invertebrate grazers attracted to bacterial or algal biofilms on bones, skin, or clothing, or to the bones themselves as a source of calcium and other minerals.

Since corpses are often found in aquatic environments (e.g., Haglund et al., 1990; Hobischak and Anderson, 1999), it is important that forensic scientists and police visiting a crime scene have an increased knowledge of the aquatic organisms that could potentially colonize humans and nonhuman models. They also should be aware of the environmental factors affecting their distributions. The objectives of this chapter are to first discuss the different stages of carrion decomposition in aquatic environments and then concentrate on the independent variables of importance (e.g., factors relative to the environment and/or to the corpse itself) that affect the postmortem submerged interval. The second part will characterize the aquatic insect community involved in decomposition and discuss their functional roles relevant to their importance in forensic science investigations. The third part will document and discuss several case histories involving aquatic insects. Throughout this review, we will concentrate on freshwater habitats and, to a lesser extent, marine environments.

Decomposition in Aquatic Ecosystems

Freshwater Ecosystems

The concept of phases or stages of decomposition in terrestrial habitats was developed in the 19th century with Megnin's (1894) description of eight series of changes and the arthropod assemblage associated with each stage. Presently, the five stages of decomposition recognized for corpses found in terrestrial settings include the following: fresh, bloat, active decay, advanced decay, and the dry/remains stage (see Anderson and VanLaerhoven, 1996; Payne, 1965; Smith, 1986; Tullis and Goff, 1987). Decomposition of a body submerged in an aquatic environment occurs at a rate roughly half that of decomposition in air, primarily due to cooler temperatures and the inhibition of insect activity (Knight, 1997; Rodriguez, 1997). Based on studies using immersed pigs (*Sus scrofa*) from June to November, Payne and King (1972) revised the stages of decomposition to accommodate corpses found in aquatic habitats and further divided the process into six stages: submerged fresh, early floating, floating decay, bloated deterioration, floating remains, and sunken remains (Figure 6.1). As with most other studies on carrion and corpses in aquatic and semiaquatic environments, these are primarily characterized or defined by the presence or absence of terrestrial insects, without any mention of aquatic insect colonization.

Since Payne and King's (1972) research on pig decomposition in water, more recent studies on the decompositional stages in aquatic habitats have been conducted. They have provided information which includes the duration of each decompositional stage, condition of the animal carcass, and the presence or absence of certain aquatic insects on various animals, including pig, salmon, and rat carrion in freshwater ecosystems of North America (Hobischak, 1997; Keiper et al., 1997; Minakawa, 1997; Schultenover and Wallace, unpublished data; Tomberlin and Adler, 1998; Vance et al., 1995).

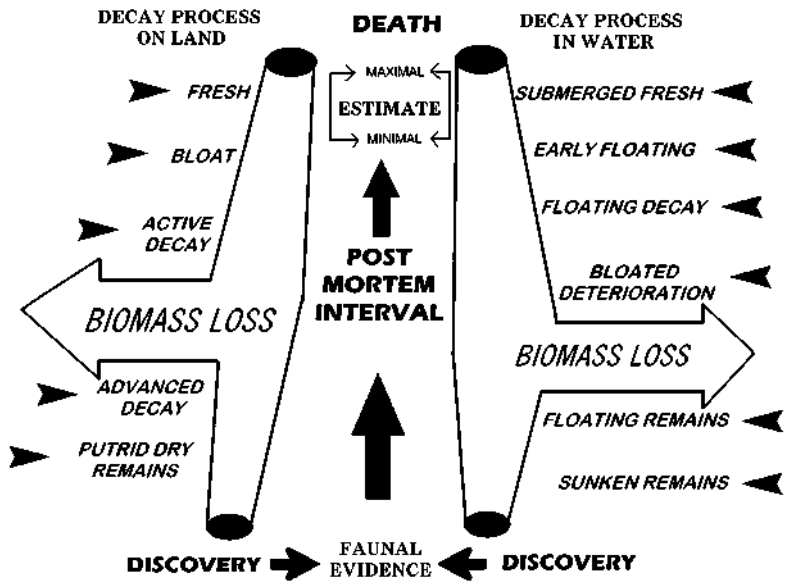


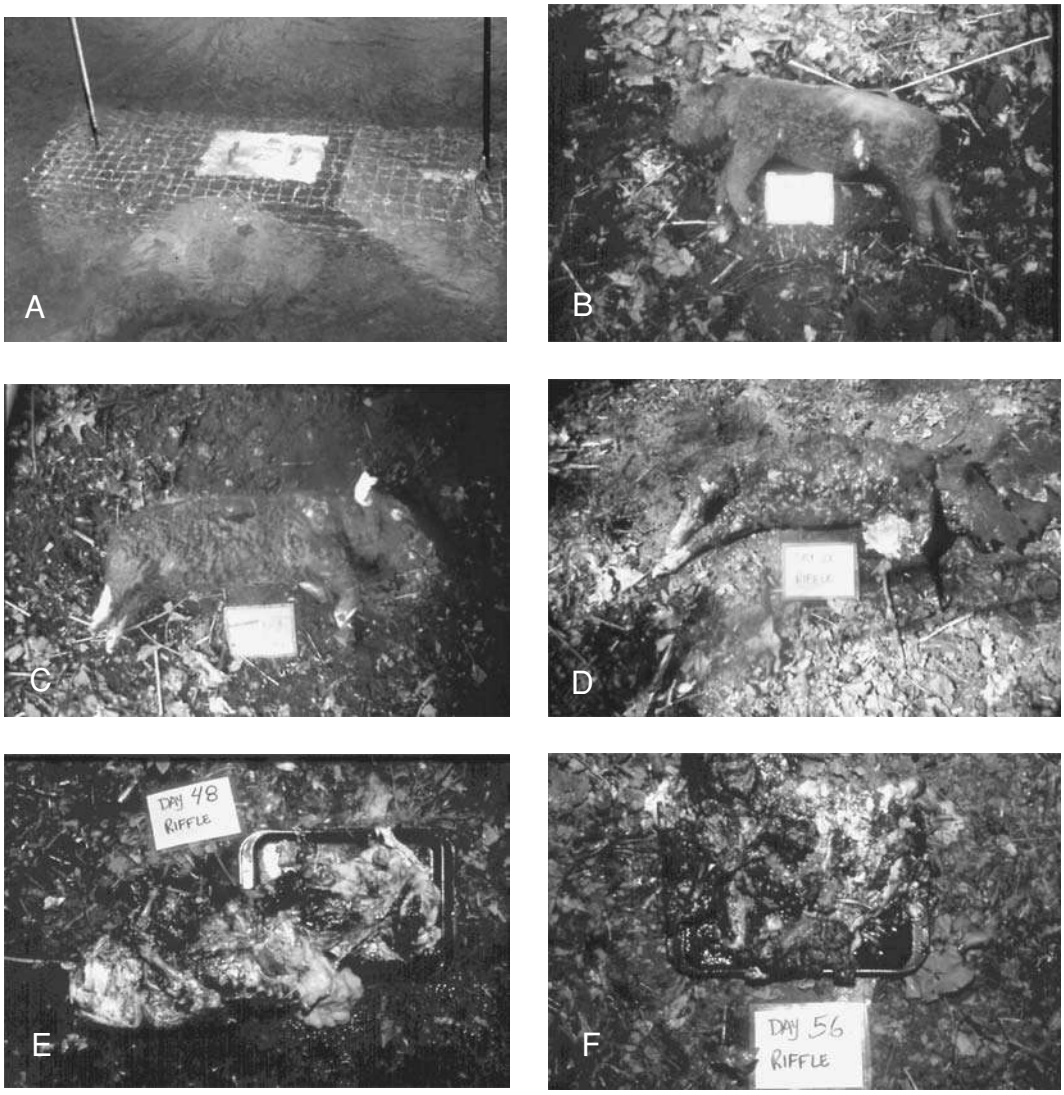
Figure 6.1 A comparison of the stages of decomposition on land and in the water. (Modified from Catts, E. P. and N. H. Haskell. 1990. *Entomology and Death: A Procedural Guide,* Joyce's Print Shop, Clemson, SC.)

First Stage: Submerged Fresh

The submerged fresh stage for pig carcasses in a stream is best characterized as the period of time between when the carcass is initially submersed and when it begins to bloat and rise to the surface (Figure 6.2a). Depending on the geographic location of a running or standing water habitat, the microhabitat within this water body, and the time of year, a pig carcass may not begin to bloat and rise to the water surface for 2 to 6 days in the spring in mid-latitudes and 11 to 13 days in more northern latitudes (Hobischak, 1997; Schultenover and Wallace, unpublished data). Truly aquatic insects in their immature stages, e.g., hydropsychid caddisflies (Trichoptera: Hydropsychidae), chironomid midges (Diptera: Chironomidae), and heptageniid mayflies (Ephemeroptera: Heptageniidae) were observed on carcasses by several investigators during this stage in running and standing water habitats (Hobischak, 1997; Keiper et al., 1997; Schultenover and Wallace, unpublished data; Vance et al., 1995), while adult hydrophilid beetles (Coleoptera: Hydrophilidae) also were collected on pigs in standing water (Vance et al., 1995).

Second Stage: Early Floating

As gases produced from anaerobic bacterial respiration in the abdomen increase, the pig carcass floats to the surface and either pushes the abdomen above the water surface, or in the case of a totally submerged pig in a cage, bloating will force the carcass against the roof of the cage and cause an indentation from the wire to form on the carcass (Schultenover and Wallace, unpublished data) (Figure 6.2b). Pig, rat, fish or human carcasses projecting above the water surface attract terrestrial insect species (e.g., blow flies (Calliphoridae) and related families (Muscidae, Sarcophagidae)) which lay eggs or larvae on exposed areas of the carcass. Carrion and rove beetles (Silphidae and Staphylinidae) may



Figures 6.2 (a,b,c,d,e,f) Different stages of pig decomposition (days after submergence) in a riffle area of a small southern Indiana stream. Pigs were removed from stream and placed on bank for photographs. (Photos courtesy of John R. Wallace.)

feed on larval blow flies or animal flesh. Yellowjacket and bald-faced hornets (Vespidae) generally prey on the adult and larval blow flies (Goff and Odom, 1987; Nuorteva et al., 1974; Payne and King, 1972; Tomberlin and Adler, 1998). However, for totally submerged carcasses, aquatic invertebrates such as hydropsychid caddisflies, chironomid midges, aquatic isopods, and heptageniid mayflies were found inhabiting the carcass during this stage (Haskell et al., 1989; Hawley et al., 1989; Hobischak, 1997; Schultenover and Wallace, unpublished data). It also was noted that the decay odor was quite evident and pronounced during this stage; tissues turned from pinkish hues to shades of blue-green, yellow fluids and gases oozed from the anus, and nails begin to slough off the hooves of pigs (Schultenover and Wallace, unpublished data). In addition, wire indentations remained on the dorsal side of each pig in both riffles and pools. Algal or periphyton growth increased significantly

more on pigs than on artificial substrates that were placed as controls in riffle and pool habitats (Figure 6.2b). This type of primary production may, in fact, provide food upon which mayfly larvae (Ephemeroptera) can graze.

The time of year when such studies are initiated plays an important role in determining the duration of each stage of decomposition. For example, Schultenover and Wallace (unpublished data) found that in the midwestern U.S. during spring, the early floating stage persisted for approximately 8 days in a riffle habitat and 6 days in a pool habitat. Conversely, in a cooler climate, Hobischak (1997) observed the bloat stage lasted from 23 to 37 days in pond and stream habitats in coastal British Columbia; however, this latter time period may have encompassed part of the "floating decay" stage described below.

Third Stage: Floating Decay

Intense feeding activity by calliphorid maggots has been observed on pig and rat carcasses floating above the water surface, creating many openings in the exposed skin (Tomberlin and Adler, 1998). Silphid, staphylinid, and histerid beetles were observed in high abundance during this time, searching for prey and copulating (Payne and King, 1972). Schultenover and Wallace (unpublished data) found that aquatic macroinvertebrate colonization of pig carcasses that were totally submerged varied both temporally and spatially between riffle and pool microhabitats. For example, they observed perlodid stonefly (Plecoptera: Perlodidae) larvae preying on both black fly larvae (Diptera: Simuliidae) attached to the carcass and chironomid midge larvae crawling on and in the carcass. Crayfish feeding activity during this stage increased on pigs in both riffle and pool habitats. The duration of this stage in their study was 8 days in the pool habitat and approximately 24 days in the riffle habitat. Although wire indentations were still present, skin sloughed off the hind limbs of the pig in a riffle habitat and both carcasses assumed a blackened color (Figure 6.2c). Gas released from the pig in a pool habitat was great enough to cause the abdomen to collapse even though it remained floating.

Fourth Stage: Bloated Deterioration

During this stage, most of the exposed tissues of pig or rat carcasses floating or projecting above the water surface have disappeared due to the continual feeding activities of blow fly maggots (Figure 6.2d). Conversely, pigs totally submerged were colonized mainly by chironomid and black fly larvae. Haglund and Reay (1993) and Schultenover and Wallace (unpublished data) found that hind limbs became disarticulated, blood and other fluids leaked from carcass orifices, large sections of flesh sloughed off, and severe ulcerations were observed on the abdominal wall (Figure 6.2d). The duration of the bloated deterioration stage varied between pool and riffle habitats by 12 and 8 days (respectively), and among seasons (Hobischak, 1997; Schultenover and Wallace, unpublished data; Tomberlin and Adler, 1998).

Fifth Stage: Floating Remains

Pig or rat carrion with limbs or parts of tissue projecting above the water surface show little maggot (Calliphoridae) activity, probably due to migration from the carcass, death by drowning, or predation from aquatic or other terrestrial insects. However, pig or rat carcasses floating at the tops of cages that were totally submerged showed significant sloughing of flesh and disarticulation of phalangial and limb bones (Keiper et al., 1997;

Schultenover and Wallace, unpublished data) (Figure 6.2e). Mostly chironomid midge larvae and some black fly larvae were recorded on pig or rat carcasses in both riffle and pool habitats during this stage (Keiper et al., 1997; Schultenover and Wallace, unpublished data). Some vertebrate predators (e.g., sunfish (Centrarchidae), dace (Cyprinidae), and sculpin (Cottidae)) were observed feeding on carcass flesh or on aquatic macroinvertebrates found on the carcass (J. Wallace, personal observation). In a case history reported later in this chapter, Merritt found that amphibians and fish were feeding on maggots that were exiting a victim's body in a pond during this stage. Hobischak (1997) found that carcasses also might experience scavenging from vertebrate species, such as mink (*Mustela vison*). The duration of this stage varied temporally and spatially from 4 to 14 days in the southeastern U.S. (Payne and King, 1972), 4 to 20 days in the Midwest (Schultenover and Wallace, unpublished data), up to 228 days in the Pacific Northwest (Hobischak, 1997), and ended when the remains sank. Singh and Greenberg (1994) suggested that the duration of submergence of corpses in forensic investigations may be estimated from the pupae of terrestrial sarcophagic insects that colonize, develop, and are later found adhering to or entangled in decaying flesh, hair, or clothes of a corpse even after complete submergence.

Sixth Stage: Sunken Remains

The duration of this stage is quite variable, but is primarily characterized with only bones and bits of skin remaining on the substrate. Payne and King (1972) noted that decomposition is completed by bacteria and fungi during this stage, while Schultenover and Wallace (unpublished data) noted that skull segments had become disarticulated, the remaining flesh resembled a soupy texture, and the decay odor was negligible (Figure 6.2f). In addition to fish scavengers, benthic aquatic fauna such as crayfish (*Orconectes propinqus*) may be found within the carcass remains as well as chironomid and mayfly (*Ephemerellidae*) larvae, annelids, snails, leeches, and amphipods (Hobischak, 1997; Nawrocki et al., 1997; Schultenover and Wallace, unpublished data; Vance et al., 1995).

Marine Ecosystems

Sorg et al. (1997) provided an excellent review of forensic taphonomy in marine contexts and noted that little research has been done on the process of decomposition of human remains, mainly because marine death assemblages including human remains are rare and the recovery of such is even more rare. In addition, human remains are often found long after the primary scavenging sequences have occurred and the participants departed, making it difficult to associate decomposition with a particular scavenger. Those studies that have focused on human remains in marine waters have done so as a by-product of forensic casework (e.g., Boyle et al., 1997; Donoghue and Minnigerode, 1977; Ebbesmeyer and Haglund, 1994; Giertsen and Morild, 1989; Haglund and Reay, 1993).

Whereas specific insects and their allies are generally the dominant scavengers of terrestrial and freshwater environments, crustaceans, fish, gastropod mullusks, and echinoderms are the dominate scavengers in marine environments (Sorg et al., 1997). Möttönen and Nuutila (1977) reported cases where crustaceans destroyed all the soft tissues of a body, including parenchymal organs, and inflicted crater-like lesions of varying size.

Lord and Burger (1984) observed that the process of decomposition and faunal succession by terrestrial arthropods of stranded marine Harbor seals on islands was generally similar and predictable to that reported for sheep carcasses in Australia (Fuller, 1934), dogs

in Tennessee (Reed, 1958), rodents in Great Britain (Putman, 1978), and pigs in South Carolina (Payne, 1965). They also noted that typical marine intertidal and wrack decomposers, such as crabs or amphipods, had a negligible impact on seal decay, as did the activities of scavenging seabirds. The absence of several beetle taxa (e.g., Silphidae) was of further interest in that carcass size and the harsh maritime conditions characteristic of rocky supratidal zones appeared to be important factors precluding these insects from visiting the carcasses. However, specimens of the beetle family Silphidae were encountered on gull and rodent carcasses located in more protected island habitats (Lord and Burger, 1984). Sorg et al. (1997) concluded in his review that only a few published studies (Skinner et al., 1988; Sorg et al., 1995) have actually described the decomposition of submerged remains in marine environments, and more documentation of the condition of human remains and the associated marine organisms is necessary to build sufficient case series from which marine models of postmortem change can be constructed.

Setting the Clock (PMI and PMSI)

The postmortem history and circumstances surrounding the discovery of the morbid remains of a corpse in a terrestrial setting is often provided by the arthropod community inhabiting the body (Catts and Haskell, 1990; Keiper et al., 1997). The postmortem interval (PMI), i.e., the time between death and corpse discovery for a body, can be estimated by an investigation of the composition and age of this invertebrate assemblage (Catts and Haskell, 1990; Smith, 1986). For corpses found totally submerged in aquatic environments, such as rivers, streams, ponds or lakes in which oviposition and larval development of terrestrial sarcophagous insects is prevented, determination of the postmortem submersion interval (PMSI) has proven more problematic. Ecological context is very important because even within one species, variations in behavior may occur in slightly different habitats or different seasons.

Although few indicators of time since death for corpses found in aquatic ecosystems are comparable in precision to the insect indicators used in terrestrial cases, there are observations which can be useful in suggesting or ruling out an approximate PMSI. In particular, the time intervals needed for certain growth phases of marine plants or animals that attach themselves to the remains can be used to estimate a minimum PMSI (Sorg et al., 1997). Sorg et al. recommended that research on marine cases be focused on sessile forms of fauna since with these organisms one can be sure that the animal/plant is truly associated with the remains. Further, in aquatic as well as in terrestrial habitats, the stages of decomposition (which embody the postmortem interval) can be affected by several environmental factors. These factors include, but are not limited to, temperature, water current regime, the aquatic organisms present, and other factors related to the corpse itself (e.g., presence or absence of clothing, and body habitus, i.e., submerged or floating).

Environmental Factors

The major variables that affect the rate of decomposition of human remains found in aquatic environments are summarized in Table 6.1. Physical-chemical parameters of water, such as temperature, current regime, oxygen content, and those associated with the corpse itself (e.g., clothing and trauma), not only play a part in decomposition of human corpses,

Table 6.1 Environmental and Corpse Factors that Affect the Postmortem Decomposition of Remains Found in Freshwater and Marine Ecosystems

Environment	Corpse
Water temperature	Presence of clothing
Current regime or wave action	Trauma
Siltation	Body weight
Salinity	Submerged or floating
Oxygen concentration	
Aquatic organisms	

Source: Modified from Haglund, W. D. and D. T. Reay. 1993. *J. Foren. Sci.,* 38:69–80.

but also influence the dominant pathway of decomposition. This decomposition is mediated through biological mechanisms, such as microbial and macroinvertebrate interactions.

Physical and Chemical Parameters

Temperature is probably the foremost environmental factor that influences the rate of corpse decomposition via the timing of insect oviposition or colonization, larval insect growth and survivorship, and hence is the most important factor in the estimation of the PMI in terrestrial ecosystems (Greenberg, 1991). Because temperature plays such an important role in forensic investigations in these ecosystems, it is reasonable to expect that temperature has the same effect in aquatic systems. As with terrestrial insects, aquatic insects respond to the summation of thermal units (i.e., degree days) as well as absolute ambient temperatures. Seasonal temperature fluctuations characterize most natural lotic (running water) and lentic (standing water) freshwater environments (Ward, 1992; Ward and Stanford, 1982). However, streams and rivers differ from lakes and ponds in that: (1) even though the annual temperature range is less in lotic waters, the seasonal rate of temperature change is often greater than in lakes and may affect surface and subsurface current patterns, particularly in large or deep rivers (Nawrocki et al., 1997); (2) typically diurnal temperature changes are often greater in streams than in the nonlittoral areas of lakes; and (3) thermal stratification is rare in natural streams and persists for only short periods relative to that in lakes (Brittain, 1976). In a specific case report of a corpse discovered in a swamp in Hawaii, Goff et al. (1988) found that oceanic island temperatures tended to remain relatively stable on both day/night and annual bases. This stability, unlike diurnal and seasonal fluctuations encountered in temperate continental areas, minimizes temperature-related variations in rates of arthropod development (Goff et al., 1988).

In general, cold water temperatures, whether in freshwater or marine ecosystems, retard the processes of decomposition, especially microbial breakdown. However, warm temperatures due to seasonal fluctuations in temperate climates or more stable environments as in tropical seas, accelerate decomposition (Sorg et al., 1997). This, in turn, influences trophic dynamics of aquatic invertebrates directly through its effects on feeding rates and indirectly through the food base available to these animals (Ward and Stanford, 1982). Higher temperatures increase larval growth rates of aquatic macroinvertebrates by altering the quantity (e.g., density and/or productivity of attached algae) and quality (microbial populations, such as bacteria and fungi) of organic matter associated with a corpse (Cummins and Klug, 1979). Direct effects on aquatic macroinvertebrate life histories can result

by temperature influencing the feeding rates, digestion, and respiration, as well as food conversion efficiencies, enzymatic kinetics, and endocrine processes (Sweeney and Vannote, 1981; Vannote and Sweeney, 1980). The dynamic nature of environmental temperatures and their effects on invertebrate life histories must be incorporated into forensic studies to further our knowledge of the role of aquatic organisms in the decomposition of human and other animal remains.

The physics of corpse movement in water, both horizontally (downstream) and vertically (to depths) have been summarized by Dilen (1984) and modeled by Ebbesmeyer and Haglund (1994). Dilen described four stages of motion of the body: sinking to the bottom, motion along the bottom, ascent to the surface, and drift at the surface. Dispersal of human remains in the sea is much more similar to that in lotic environments than in freshwater lentic or terrestrial sites. The principal agents of distribution in marine ecosystems are tidal, current, and wave action, as well as motile scavengers (Sorg et al., 1997).

Many factors can affect postmortem condition in aquatic environments and can interact in predictable and unpredictable ways. For example, soon after death a body may be clad in heavy clothing, but after deposition in a physically harsh aquatic habitat (e.g., riffles or rapids in streams and rivers, or intertidal zones of oceans), the force of the current or wave action could move the body between riffle and pools or remove the clothing in the immediate postmortem interval (Hobischak, 1997; Keiper et al., 1997; Sorg et al., 1997). Likewise, if a corpse is bleeding, large marine scavengers, such as sharks, may be attracted and cause severe alteration of the remains (Sorg et al., 1997). Small-scale variations in temperature may occur over short distances in streams and rivers and, therefore, the specific area where the body settles out within a stream reach may influence the rate of decomposition. For example, slow flowing pools may attain higher temperatures in summer than adjacent rapids and, therefore, any knowledge of body movement over time in an aquatic habitat may provide important information when determining the PMI or PMSI.

Oxygen level in the water also is an important factor to consider when determining the PMSI. Oxygen solubility in water is negatively correlated with water temperature, and oxygen levels vary with current speed and turbulence. That is, small fast-flowing, unpolluted streams are usually saturated with oxygen, whereas polluted streams, stream pools, small ponds, and stagnant bays (especially those with a high organic load of dead leaves or high sediment loads) can have relatively low levels of oxygen.

Certain groups of aquatic invertebrates have specific respiratory and behavioral adaptations to deal with low oxygen conditions in polluted streams or highly eutrophic lentic habitats, allowing them to survive in such habitats (Eriksen et al., 1996). However, many insect species are not able to deal with this respiratory demand. Many insects show a clear preference for cold waters (e.g., Plecoptera or stoneflies), possibly owing to the effects of temperature on oxygen availability as to the effects of temperature per se (Giller and Malmqvist, 1998; Ward, 1992). Therefore, depending on the oxygen concentration in a specific aquatic habitat where a corpse is found, the faunal community colonizing a body may be quite different (Hobischak, 1997). As a general rule, very low oxygen environments favor lower species diversity. However, higher numbers of individuals of those species that are able to tolerate low oxygen conditions and out-compete pollution intolerant groups are usually found (Hynes, 1960). In streams, ponds, or swamps that are highly polluted or anaerobic part of the time, there may be few, if any, organisms present that would be available to colonize a corpse, therefore resulting in a slower rate of decomposition. In contrast, an unpolluted stream generally has a higher diversity of organisms with fewer

numbers of individuals per species (Hynes, 1960). These water quality differences in oxygen concentrations therefore may affect the diversity and abundance of invertebrates colonizing a corpse in a specific habitat and the subsequent rate of decomposition over time.

Biological Mechanisms

The breakdown of dead organic matter, such as a corpse, is primarily a biological process involving three types of organisms: large and small particle detritivores (commonly referred to as macroinvertebrates), fungi, and bacteria. Although the critical role of microorganisms such as bacteria and fungi in stream ecosystems has been clearly established (Kaushik and Hynes, 1971; Suberkropp and Klug, 1976; 1980), little detailed work has been conducted on the specific microbial assemblages and the succession of different microorganisms associated with decomposing organic matter. Bärlocher (1982) reported that typically four to eight species of aquatic fungi dominate throughout the decomposition of leaves, but apparently no particular succession occurs on a single leaf. Fungi initially appear to dominate microbial assemblages in leaves as long as the tissue is more or less intact, while bacteria tend to increase when leaves become partially broken down and dominate the terminal processing stage (Baldy et al., 1995; Bengtsson, 1992; Suberkropp and Klug, 1976). If this is the case, a clear distinction between fungal and bacterial activity and diversity could be important in determining the postmortem submerged interval of a corpse colonized by these microorganisms in running waters. We are not aware of any published studies on the role of stream or pond microbes in the decomposition of carrion or human corpses. However, Siver et al. (1994) reported the successful employment of diatoms and planktonic algal communities in a pond to link three subjects to a freshwater crime scene in southern New England.

In aquatic systems, decomposition processes involve the dissipation of energy stored in organic matter (Allan, 1995). The major sources of energy in most stream ecosystems are (1) terrestrial inputs of organic matter in the form of leaf litter (commonly referred to as allocthonous material) (Cummins, 1974; Fisher and Likens, 1973), and (2) instream primary production (termed autochthonous material) brought about by photosynthesizing organisms such as algae (usually diatoms) and mosses (Lamberti and Moore, 1984). Nutrient recycling of this decomposed organic matter has long been recognized as an important function of aquatic ecosystems (Merritt et al., 1984).

Decomposing remains, such as salmon carcasses, may begin to influence nutrient dynamics as soon as they enter an aquatic ecosystem through the excretion of nitrogenous compounds resulting from protein catabolism (Mathisen et al., 1988; Schuldt and Hershey, 1995). Studies have shown that the decomposition of salmon carcasses in Alaskan streams increased algal biomass and primary production (Kline et al., 1990; 1997; Wipfli et al., 1998). Schultenover and Wallace (unpublished data) found that the introduction of pig carcasses to riffle and pool microhabitats in an Indiana stream also resulted in a significant increase of algal growth on these decomposing carcasses in both microhabitats over a 30-day period (Figure 6.2), as compared to artificial substrata (i.e., ceramic tiles) which showed no increase in algal growth. Keiper et al. (1997) also observed algal colonization of rat carcasses in riffle and pool areas of an Ohio woodland stream. Because algae is often a dominant component of primary production in streams, it is of major importance to organisms such as aquatic insects that utilize living plant material as a food source for growth and reproduction (Lamberti and Moore, 1984). Brusven and Scoggan (1969) observed algal-feeding caddisfly larvae feeding on dead squawfish after a fish kill, and

concluded that these larvae contributed directly and indirectly to the removal of the fish from the stream. Minakawa (1997) found 24 insect taxa associated with salmon carcasses in Pacific Northwest streams, with 10 caddisfly genera and 2 stonefly genera directly feeding on salmon flesh. He observed that one stonefly genus and three caddisfly genera associated with the carcasses had fungi in their mouths, suggesting that these insects might be feeding on the fungi and other microbes growing on the salmon carcasses. Kline et al. (1997) noted that macroinvertebrates invaded mouth and gill areas of salmon in fast currents and abraded body parts (tails, fins, and nose) in slower currents. They appeared to feed on gill membranes in the oral cavity but chose exposed muscle in the abraded areas, or fungal patches on the exposed parts of salmon. These findings may be important to forensic scientists in explaining how and why aquatic insects begin to colonize and utilize a human corpse in freshwater ecosystems and which groups one would expect to find on a corpse after a given time interval. Schultenover and Wallace (unpublished data) have shown that the algal species present and abundance of algal growth on a corpse (in this case, a pig) in a given stream system may be helpful in determining the PMSI in forensic investigations.

In addition to microbes (primarily bacteria, fungi, periphyton, or attached algae), the other basic food resource categories and a general classification system for aquatic insect trophic relations are presented in Table 6.2. They include:

1. CPOM, coarse particulate organic matter (particles greater than 1 mm in size), represented by litter accumulations consisting of leaves, needles, bark, twigs, and other plant parts, large woody debris, macrophytes (including macroalgae), and rooted and floating vascular plants. By definition, this also would include carrion of wild or domestic animals and human corpses.
2. FPOM, fine particulate organic matter (particles less than 1mm and greater than 0.5 μm in size) generally composed of unattached living or detrital material, including that created through the physical and biological reduction of CPOM.
3. Prey (all invertebrates captured by predators) (Merritt and Cummins, 1996c).

These nutritional resource categories form the basis of a functional feeding group classification system designed to identify macroinvertebrates involved in the processing of organic manner in aquatic ecosystems and assist with evaluating the role of these animals in death scene investigations.

Macroinvertebrate Functional Feeding Groups

Aquatic insects have been a major focus of many ecological studies in aquatic habitats. Due to their ubiquitous distribution, relative abundance, ease to collection, and large size (observable with the unaided eye) (Merritt and Cummins, 1996b), they lend themselves well to be the organism of choice in the study of death scene investigations. As pointed out earlier, unlike terrestrial habitats, the primary problem in aquatic environments is that there are no purely sarcophagous aquatic insects to compare with the common terrestrial indicator species, such as blowflies (Calliphoridae) (Haskell et al., 1989). There does not appear to be a clear predictable successional pattern on submerged carrion by different species of aquatic insects, yet Hobischak (1997) suggested a predictable succession of invertebrates colonizing pig carcasses (exposed and submerged) in the aquatic habitats she

Table 6.2 General Classification System for Aquatic Insect Trophic Relations

Functional Group (General Category Based on Feeding Mechanism)	Subdivision of Function Group		Examples of TAXA	General Particle Size Range of Food (in μm)
	Dominant Food	Feeding Mechanism		
Shredders	Living vascular hydrophyte plant tissue	Herbivores — chewers and miners of live macrophytes	Trichoptera: Phyrganeidae, Leptoceridae	$>10^3$
	Decomposing vascular plant tissue and wood — coarse particular organic matter (CPOM)		Trichoptera: Limnephilidae Plecoptera: Pteronarcyidae, Nemouridae Diptera: Tipulidae, Chironomidae	
Collectors		Detritivores — filterers or suspension feeders	Trichoptera: Hydropsychidae	
	Decomposing fine particular organic matter (FPOM)		Diptera: Simuliidae	$>10^3$
		Detritivores — gatherers or deposit (sediment) feeders (includes surface film feeders)	Ephemeroptera: Ephemeridae, Baetidae, Ephemerellidae Diptera: Chironomidae	
Scrapers	Periphyton — attached algae and associated material	Herbivores — grazing scrapers or mineral and organic surfaces	Trichoptera: Glossosomatidae Coleoptera: Psephenidae Ephemeroptera: Heptageniidae	$<10^3$
Predators (Engulfers)	Living animal tissue	Carnivores — attack prey, pierce tissues and cells, and suck fluids	Hemiptera: Belostomatidae,	$>10^3$
	Living animal tissue	Carnivores — ingest whole animals (or parts)	Odonata, Plecoptera: Perlidae, Perlodidae Coleoptera: Dytiscidae, Megaloptera Trichoptera: Rhyacophilidae	$>10^3$

Source: Modified from Merritt and Cummins, 1996a.

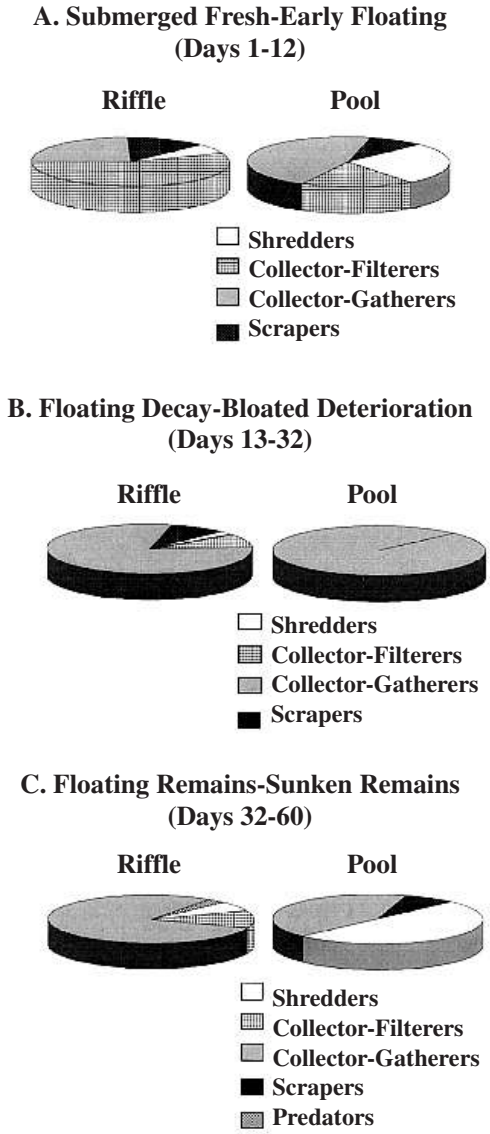
studied in British Columbia. However, she noted environmental conditions and organism habitat preferences influenced this pattern, and indicated that discretion should be used when evaluating succession for use of determining time of submergence or death.

Terrestrial indicator species of insects are ecologically characterized according to their trophic relationship with carrion, e.g., necrophages feed mainly on the corpse itself; omnivores feed on either the corpse or associated fauna, and parasites and predators parasitize or feed on the fauna associated with the corpse. The aquatic functional feeding group (FFG) method is based on the association between a limited set of feeding adaptations found in freshwater invertebrates and their basic nutritional resource categories discussed earlier (Table 6.2). The same morpho-behavioral mechanisms can result in the ingestion of a wide range of food items, the intake of which constitutes herbivory, detritivory, or carnivory (Merritt and Cummins, 1996c). Although food-type intake would be expected to change from season to season, habitat to habitat, and with growth stage, limitations in food acquisition mechanisms have been shaped over evolutionary time and these are relatively more fixed (Cummins and Merritt, 1996).

Corpses initially enter the aquatic system as coarse particulate organic matter (CPOM), much like that of leaf litter. Some of this material will enter the fine particulate organic matter (FPOM) and dissolved organic matter (DOM) pools following leaching, physical abrasion, and biotic processing, and some will enter the inorganic nutrient pool as a result of microbial activity (e.g., Cummins, 1974). Shredders, e.g., aquatic isopods and amphipods (Crustacea), some caddisflies (Trichoptera: Limnephilidae), and crane flies (Diptera: Tipulidae), are dependent on large pieces of organic matter (CPOM) such as leaves, needles, wood, and other plant parts derived primarily from the riparian zone. Collectors, e.g., net-spinning caddisflies (Hydropsychidae), black flies (Diptera: Simuliidae), and midges (Diptera: Chironomidae), utilize small particles of organic matter (FPOM) either by filtering from the passing water or by gathering from deposits in the sediments on the stream bottom. Scrapers, e.g., some caddisflies (Trichoptera: Glossosomatidae) and mayflies (Ephemeroptera: Heptageniidae), are adapted to remove algae or periphyton attached to rock or log surfaces. Predators, e.g., caddisflies (Rhyacophilidae), stoneflies (Plecoptera), and beetle larvae (Coleoptera), are adapted to capture prey organisms through behavioral mechanisms or specialized body parts (Table 6.2). The development of an aquatic insect community on an introduced substrate, such as a corpse, is highly dependent on available colonizers in addition to other factors such as seasonality, temperature, current speed, and depth as discussed previously (Haskell et al., 1989).

The FFG approach can be used to track the decomposition of carrion or a human corpse over time and it can document the changes in functional feeding groups based on changes in the nutritional resource categories available to the different taxa. The presence and abundance of the various FFGs and their percent composition is a direct reflection on the availability of the required food resources and the condition of the related environmental parameters. Recent empirical evidence by Schultenover and Wallace (unpublished data) presented below demonstrates the utility of this approach to track some type of predictable successional change in stream macroinvertebrate FFGs on, in, or around a corpse as decomposition proceeds over time.

Figure 6.3a shows that greatest percentage of FFGs on pigs in riffle and pool habitats were collector-gatherers (col.-gath.) and collector-filterers (col.-filt.) during the freshly submerged and early floating stages of decomposition. The percentage of the FFG collector-gatherers was higher in the pool (46%) vs. riffle (25%) habitat where chironomid midge



Figures 6.3 Percent composition and comparative changes in macroinvertebrate functional feeding groups (FFGs) colonizing pigs at different stages of decomposition in riffle and pool habitats in a southern Indiana stream.

larvae made up the major portion of the col.-gath., and net-spinning caddisflies (Hydropsychidae) made up the col.-filt. Although present, shredders such as crayfish and crane fly (Tipulidae) larvae, as well as scrapers (Ephemeroptera: Heptageniidae) and predators, were not very abundant in either habitat. The diversity of aquatic macroinvertebrates was greatest in these early stages of decomposition as compared to the remaining stages.

During the floating decay and bloated deterioration stages, the col.-gath. FFG was the most abundant on pigs in both habitats compared to all other feeding groups (Figure 6.3b). In fact, midge larvae were the only macroinvertebrates found on the pig carcass in the pool

habitat. This may have been due, in part, to the sediment accumulation on the pig providing an ideal habitat in which these insects could construct feeding burrows or tubes. The pigs in the riffle habitat were colonized by a more diverse assemblage of insects, including some scraper mayflies and net-spinning caddisflies (Figure 6.3b).

As pigs in both stream habitats reached the floating remains–sunken remains stages of decomposition (Figure 6.3c), shredders represented by amphipods and limnephilid caddisflies, and col.-gath. midges became the dominant groups in the pool habitat. However, in the riffle habitat, col.-gath. chironomid midges were the most abundant FFG group represented (79%). During these two stages, predator species such as hellgrammites (Megaloptera: Corydalidae) and fish were observed feeding on or around the pig in the riffle habitat.

Aquatic Insects of Importance to Forensic Science

Although only 3% of all species of insects have aquatic or semiaquatic stages (Daly, 1996), in some freshwater biotopes insects may comprise over 95% of the total individuals or species of macroinvertebrates (Ward, 1992). Merritt and Cummins (1996b) trace the interest in aquatic insects from its earliest limnological roots (e.g., Forbes, 1887), to sport fishery-related investigations of the 1930s and 1940s (Needham, 1934), and to the use of aquatic insects as indicators of water quality during the 1950s and 1960s. More recent work on aquatic insects includes most major areas of ecological inquiry, including biomonitoring as a stream management application (Cairns and Pratt, 1993; Rosenberg and Resh, 1996). Forensic science is the latest field of interest to embrace the use of aquatic insects (Haskell et al., 1989).

Identification of aquatic macroinvertebrates is the first step toward a basic understanding of the role these organisms play in death scene investigations. It is not the purpose of this chapter to provide an all-inclusive identification key to aquatic insects; this can be found in other major reference works (e.g., Merritt and Cummins, 1996a; Thorp and Covich, 1991). Rather, we have chosen to include a simplified, illustrated, dichotomous key to functional feeding groups of lotic macroinvertebrates modified from Cummins and Wilzbach (1985) and Merritt and Cummins (1996c) (See Appendix). The key emphasizes higher level taxonomic separations that permit reliable categorization of functional feeding groups. It is organized in two levels of resolution. The first level can be done in the field with a minimum of taxonomic skill and the second level should be done in the laboratory. The amount of taxonomic effort and skill required and the need for the use of a microscope increases with the second level of resolution. While this key is primarily aimed at insects found in streams and rivers, most of these groups also are found in standing water habitats. Although it is possible to key out aquatic insects to major families using this key, we recommend that if lower resolution is required or if the specimens are to be used as evidence in court, they be taken to an aquatic entomologist for verification. For a discussion of marine invertebrates associated with human decomposition, see Sorg et al. (1997) and references contained therein.

The evolution of a vast array of morphological, physiological, and behavioral adaptations in aquatic insects enables these organisms to inhabit virtually all bodies of water. Truly aquatic or semiaquatic insects occur in every conceivable aquatic habitat that a human body could be found. This includes such specialized habitats as hot and cold springs, intertidal pools, temporary and aestival ponds, intermittent streams, saline lakes, marine intertidal zones, as well as in less harsh running and standing water habitats (Ward,

Table 6.3 Occurrence of Life Stages in Major Habitat Types for Aquatic and Semiaquatic Representatives of Insect Orders Most Likely Associated with Carcasses or Corpses[a]

Order	Terrestrial	Freshwater	Estuarine	Marine
Ephemeroptera	A	L	L	—
Odonata	A	L	L	—
Hemiptera	A	A, L	A, L	A, L
Plecoptera	A	L	L	—
Coleoptera	A, L, P	A, L	A, L	A, L
Diptera	A, P	L, P	L, P	L, P
Megaloptera	A, P	L	—	—
Trichoptera	A	L, P	L, P	L, P

[a] Key: A = adult, L = larvae, P = pupae.

Source: Modified from Ward, J. V. 1992. *Aquatic Insect Ecology, I. Biology and Habitat,* John Wiley & Sons, New York.

1992). In fact, rarely are conditions in natural or even polluted waters so extreme as to totally exclude insects. The virtual absence of insects in the open sea suggests that other marine organisms are of more importance to forensic investigators in this habitat.

Approximately 8 of the 13 orders of insects containing species with aquatic or semiaquatic stages are likely to be associated with carrion or corpses in aquatic habitats (Table 6.3). The following brief synopsis describes those orders of aquatic and semiaquatic macroinvertebrates, their functional role in aquatic ecosystems, and relative importance in previous or ongoing forensic case histories.

Mayflies (Order: Ephemeroptera)

Except for a very few species that venture into brackish areas, mayflies occur exclusively in freshwater. The ephemeral nature of the adult stage, generally 2 to 3 days duration or less, accounts for the Latin name of the order Ephemeroptera or "short-lived" (Ward, 1992). Mayflies are morphologically and behaviorally diverse and the larvae have been grouped into four life forms: (1) swimming, (2) creeping and climbing, (3) flattened and streamlined, and (4) burrowing (Ward, 1992) (see Appendix, Keys 5 and 6). Both swimming (Baetidae) and flattened and streamlined (Heptageniidae) mayflies have been observed feeding on or near pig carcasses (Hobischak, 1997; Schultenover and Wallace, unpublished data). Heptageniid larvae and the larvae of other families (e.g., Ameletidae) remove attached algae and periphyton from substrata such as rocks, logs, or in some instances corpses. Collector-gatherer mayfly larvae, such as in the Baetidae or Ephemerellidae, obtain their food (largely FPOM) by simply gathering it from wherever they can find it, such as under rocks, in deposition zones, or on the surface of stones or other substrates. FPOM accumulates in many places on the streambed wherever the current slackens enough to permit it to settle from the water column and accumulate in these deposition zones. Minakawa (1997) and Wipfli et al. (1998) found the scraper mayfly families Ameletidae and Heptageniidae, and collector-gatherer families Baetidae and Leptophlebiidae, associated with salmon carcasses in Pacific Northwest streams. Vance et al. (1995) collected baetid and caenid (Caenidae) mayflies off pig carcasses submerged for only 2 days in a small lake. Interestingly, Schultenover and Wallace (unpublished data) found heptageniid mayflies grazing on periphyton or algae that had accumulated over

time on pigs in riffle and pool habitats. They provided baseline data that indicated periphyton growth increases significantly over time on pig carcasses compared to growth on control tiles in the same aquatic habitat. This enriched feeding substrate may attract increased numbers of scraper insect species, such as heptageniid mayflies, as has been demonstrated in salmon carcass-enriched streams elsewhere (e.g., Wipfli et al., 1998). These trends are preliminary and require further testing; however, such a strong correlation between periphyton growth on corpses and increased abundance of a specific species utilizing this resource may provide some important entomological evidence useful in future death scene investigations.

Stoneflies (Order: Plecoptera)

Stoneflies are associated primarily with clean, cool running waters, although a number of species are adapted to life in large oligotrophic, alpine lakes. Stonefly larvae have specific water temperature (cold stenotherms), substrate type (usually cobbles, boulder surfaces), and stream size (small to medium) requirements in their distribution and succession along the course of streams and rivers (Stewart and Harper, 1996). While eggs may hatch soon after deposition, the larvae grow slowly and reach maturity nearly a year later. Major growth occurs during the winter and most species reach the adult stage early in the year (Wallace and Anderson, 1996). Some adult stoneflies are the earliest species of aquatic insects to emerge during the spring when snow still covers the ground.

Most species of stoneflies are clingers or sprawlers and can be divided into two functional feeding groups; shredders or predators (see Appendix, Key 5). Families of both shredders (Taeniopterygidae, Nemouridae, Pteronarcyidae) and predators (Perlodidae, Perlidae) have been observed colonizing or feeding on submerged salmon carcasses or pig carrion in streams, with larvae of Pteronarcyidae feeding on salmon flesh in the laboratory (Hobischak, 1997; Minakawa, 1997; Schuldt and Hershey, 1995; Schultenover and Wallace, unpublished data). Based on the restricted habitat requirements of stoneflies and the predatory nature of many species, the presence of larvae on a body may indicate that it was probably deposited and remained in a riffle zone of a stream or river. Thus, it may have been in the stream for some time to allow for the colonization of other insects, which may serve as prey for the stoneflies associated with the corpse at that time.

Caddisflies (Order: Trichoptera)

Caddisflies occur on all continents except Antarctica, and are found in freshwaters (e.g., streams, rivers, ponds, lakes), brackish waters, and occasionally in marine intertidal areas. The high ecological diversity of caddisflies has been attributed to their ability to produce silk. The larvae use silk to construct fixed retreats or nets that trap and collect food particles in the current, or to build their portable cases (Wiggins, 1996) (see Appendix, Keys 2 and 3).

Different families of caddisflies use different organic materials (e.g., twigs, leaves, and other plant parts) or inorganic matter (sand grains, pebbles, stones) and sometimes a combination of each to construct their cases (see Appendix, Key 2). As a caddisfly larva grows, it either adds on to its present case or changes to another type of case to accommodate its increase in size, depending on the availability of case-building materials. Often different stages of the same species use different materials to make their cases. For instance, some early larval stages of two caddisfly species belonging to the family Limnephilidae, *Pycnopsyche lepida* (Hagen) and *Pycnopsyche luculenta* (Betten), construct cases composed

Figure 6.4 Larvae and cases of first through fifth instar of *Pycnopsyche lepida* showing changes in the type of case building materials with increasing size and age of larva. Instar T-5 = 1st instar, instar T-4 = 2nd instar, and so forth to Terminal instar = 5th instar, Terminal instar (burrowed) = prepupa. (Photo courtesy of Richard W. Merritt and K. W. Cummins.)

of leaf disks surgically cut from tree leaves that have accumulated in the streams (Figure 6.4). As these larvae grow and mature and as the available leaf material significantly declines over time in these streams, *P. lepida* changes its case type to include more mineral deposits and sand grains glued together with silk, and also moves to faster moving water (Cummins, 1964) (Figure 6.4). Conversely, *P. luculenta* switches from the same type of leaf disk case to a case lined with twig portions as ballast, and moves to slower water such as that found along stream margins (Wallace et al., 1992). Therefore, knowing the case material used by the larva in a particular stage of its development, one could identify the season of the year, or even a specific month, that a body may have entered the water if a caddisfly larva and its associated case were found on the victim's remains.

Caddisfly cases found on a corpse also may indicate whether the corpse had been moved from a specific habitat within a lotic environment (e.g., riffle or pool), or transported by the current to a different location downstream. Many caddisflies also have known geographic ranges and phenologies (i.e., the timing of specific biological events within their life histories). Based on the species collected on the corpse, one could determine both spatial and temporal relationships (such as the likelihood of the corpse being transported long distances, or the time of year a corpse may have been placed in a given aquatic system).

Shredder species of caddisflies (Limnephilidae: *Pycnopsyche* spp.) and filtering-collector species (Hydropsychidae: *Hydropsyche* spp.) have been observed on pig carcasses in stream habitats in Indiana (Schultenover and Wallace, unpublished data) (Figure 6.5c). Brusven and Scoggan (1969) observed caddisfly larvae of the family Limnephilidae feeding on dead squawfish in a river in northern Idaho, and Hobischak (1997) found the same family associated with submerged pig carrion in ponds in British Columbia. Minakawa (1997) and Kline et al. (1997) observed larvae of several genera of Limnephilidae feeding on dead salmon flesh in the field and in the laboratory (Figure 6.5a), with some individuals actually penetrating the salmon skin (Figure 6.5b). The latter authors found over 1000 caddisfly larvae (Linnephilidae: *Ecclisomyia*) on one fish head in an Alaskan stream. Limnephilid caddisfly larvae were observed moving to salmon carcasses and feeding for up to 15 min. before leaving, whereas chironomid midges colonized the carcass fungal mat for

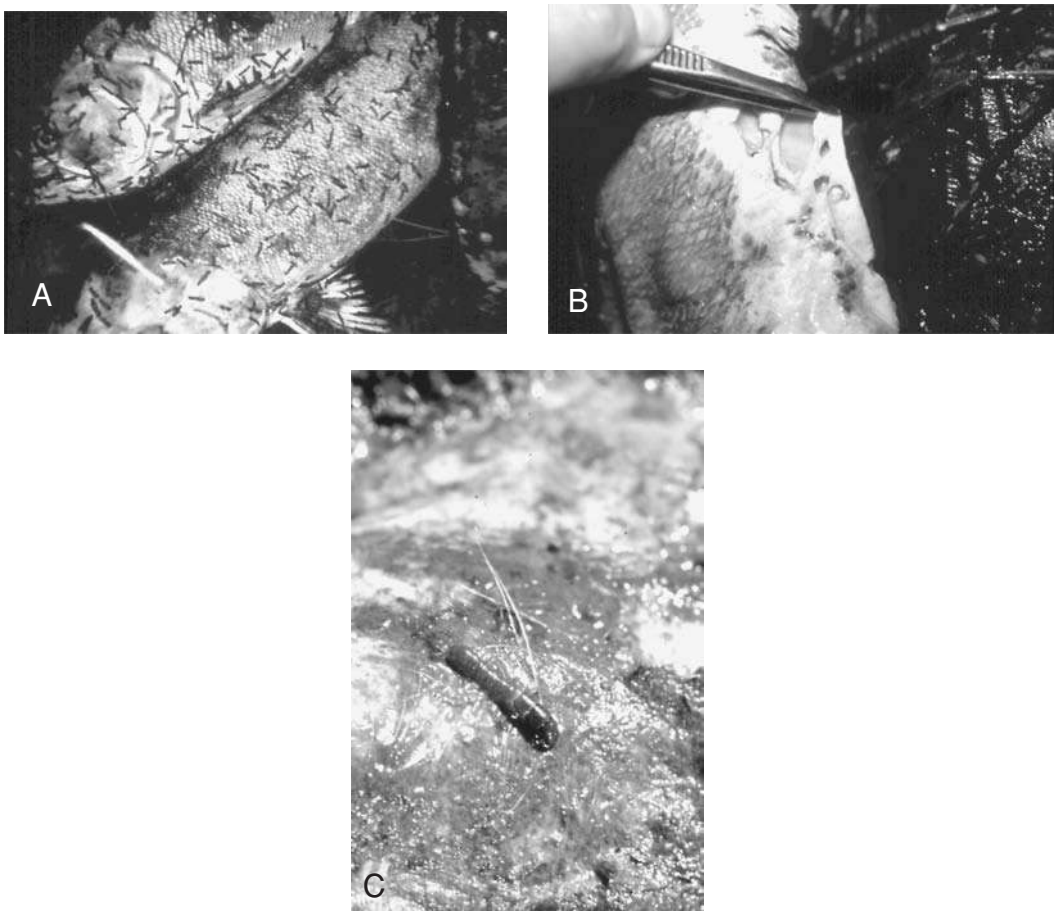


Figure 6.5 (a) Coho salmon carcass in stream with caddisfly larvae (Limnephilidae: *Ecclisomyia* sp.) attached to skin. (b) Coho salmon carcass showing feeding marks in skin made by caddisfly larvae (Limnephilidae: *Ecclisomyia* sp.). (Photos (a) and (b) courtesy of Jason Walter and Brian Fransen.) (c) Larva of net-spinning caddisfly (Trichoptera: Hydropsychidae) on skin of submerged pig in southern Indiana stream. (Photo courtesy of John R. Wallace.)

longer periods (Kline et al., 1997). Haskell et al. (1989) discussed the utility of caddisfly casings as forensic entomological evidence, and Nawrocki et al. (1997) described a case where several caddisfly cases made of tiny stones (possibly Limnephilidae) were cemented to the floor of the nasal cavity of a corpse found in the water.

True Flies (Order: Diptera)

Over half of all known species of aquatic insects are dipterans, with midges (Family: Chironomidae) constituting the largest family of freshwater insects (Ward, 1992). Many dipteran families that have aquatic representatives are largely composed of species that inhabit water as larvae (Ward, 1992). Dipterans are found in virtually every conceivable aquatic environment and, in fact, may be the only insects in freshwater habitats with extreme environmental conditions. These include hot springs, petroleum pools, and saline lakes. This order contains the most successful insect colonizers of the marine intertidal environment.

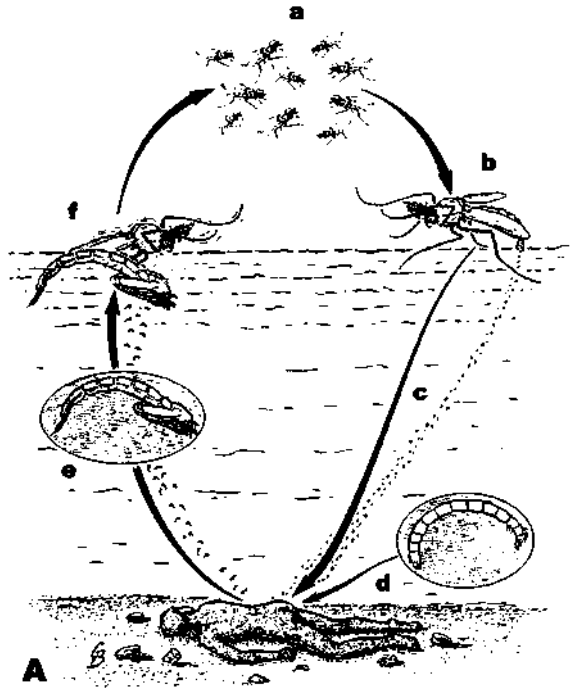


Figure 6.6a Generalized diagrammatic life cycle of a chironomid midge (Diptera: Chironomidae) colonizing a human corpse. For explanation, see text. (From Haskell, N. H. et al., 1989. *J. Foren. Sci.,* 34:622–632. With permission.)

The chironomid midges are of particular interest to forensic investigators because of their overall diversity and presence in nearly every aquatic habitat (Figure 6.6a). They often account for over 50% of the total macroinvertebrate species diversity, occurring at densities in excess of 50,000/m^2 (Armitage et al., 1995; Berg and Hellenthal, 1991; Coffman and Ferrington, 1996). As an example, in a small woodland stream in eastern North America, 143 different chironomid species were recognized (Coffman, 1973). The midges also are the most diverse family of Diptera in their selection of aquatic habitats, occupying every conceivable water body from the smallest streams to the largest rivers and from large lakes to very small pools. They occupy both fresh and saltwater environments as well as clean and polluted waters of varying depths (Pinder, 1995). The red color of some midges (Figure 6.6b*), leading to the name "bloodworms," is caused by the respiratory pigment hemoglobin which enables the larvae to recover rapidly from anaerobic periods in low oxygen environments (i.e., sediments) (Eriksen et al., 1996).

Chironomid larvae are known to feed on a great variety of organic materials representing all functional feeding groups. These include:

1. Coarse detrital particles (leaf shredders).
2. Medium detrital particles deposited in or on sediments (gatherers and scrapers).
3. Fine detrital particles in suspension, transport, or deposited (collector-filterers and gatherers, and scrapers).

* See color section insert following page 78.

4. Algae (benthic, planktonic, or in transport) (scrapers, collector-gatherers, and some filterers).
5. Vascular plants (miners).
6. Fungal spores and hyphae (gatherers).
7. Other animals (predators) (Berg, 1995; Coffman and Ferrington, 1996) (Table 6.2).

The ability of chironomid larvae to spin silk has great adaptive importance and plays a major role in filtering collectors and gatherers (Wallace and Merritt, 1980). It allows them to construct a silken tube that houses the larva, and spin a conical catchnet across the lumen of the tube (Appendix, Key 3). For those inhabiting soft substrates in lotic and lentic habitats, a U- or J-shaped burrow is constructed in which they live. Because of their diverse food habits and mode of existence, it is not surprising that most studies dealing with submerged carrion of any type have noted the presence of chironomid midges (Haskell et al., 1989; Hawley et al., 1989; Hobischak, 1997; Keiper et al., 1997; Minakawa, 1997; Vance et al., 1995; Wipfli et al., 1998). They often are found to be the most abundant insect on corpses and are usually represented by several different genera and species. Also, because egg masses and young larvae of chironomids are known to drift in streams and rivers and disperse in ponds and lakes (Pinder, 1995), they are one of the first colonizers to arrive on a corpse.

Midges have four distinct life stages: egg, larva, pupa, and adult (Figure 6.6a, a–f). The duration of the larval stage, with four instars, may last from less than 2 weeks to several years, depending on species and environmental conditions. However, chironomids in temperate climates usually have from one to two generations per year and, in general, exhibit shorter life cycles in warm, nutrient-enriched waters (Coffman and Ferrington, 1996). As noted above, early instars may be planktonic and in the water column, while later instars are usually benthic and on substrates or in the sediments (Figure 6.6, d). Most identification keys to the Chironomidae are written for mature larvae. The pupal stage (Figure 6.6, e) begins with the separation of the larva from the underlying pupal integument. After ecdysis, or shedding the old skin, the pupa usually remains hidden in debris until it swims to the surface where adult emergence takes place (Figure 6.6, f). Chironomid adults usually live a few days, although some species survive for several weeks. Adults generally do not feed and this stage performs the functions of reproduction and dispersal. Mating either takes place in aerial swarms (Figure 6.6, a), on the water surface in skating swarms, or on solid substates. Females may broadcast the eggs at the water surface where they sink to the bottom sediments (Figure 6.6, c) or, more frequently, deposit gelatinous egg masses on the open water or on emergent vegetation (Coffman and Ferrington, 1996).

Family-level identification of these insects may not be that helpful in determining the PMSI in an investigation because of the high species diversity and numbers of midges one could find in any aquatic environment. However, it may help to associate a corpse with a specific type of aquatic habitat, or to determine whether or not a body was totally submerged for a period of time, or even determining the time of year when the body was deposited. In most cases it will be necessary to have the specimens sent to an aquatic entomologist or chironomid specialist to determine which species were found on the remains. Once the species and stage of the larva are determined, existing information on the life cycle and duration of each stage can be used to help determine the PMSI.

In studying the colonization of rat carcasses by aquatic insects in riffles and pool areas of a small woodland stream, Keiper et al. (1997) observed that midge larvae were the

dominant insects colonizing the corpses, although no patterns in numbers of larvae over time were evident. However, the diversity of chironomid genera increased after 29 days in the riffle area and larvae of the chironomid genus *Orthocladius* did not begin to colonize the carcasses until 13 days after submersion in the riffle and after 20 days in the pool. They concluded from this study that some patterns in midge colonization were detectable over time, but suggested that different indices for pool and riffle habitats need to be developed when determining the PMSI on corpses based on midge colonization rates. Schultenover and Wallace (unpublished data) found that aquatic insect diversity on submerged pigs generally decreased over time; however, midges were the dominant organism found in or on pig carcasses. Wipfli et al. (1998) observed Chironomidae burrowing into salmon carcasses in the field and was abundant within the salmon tissue after 30 days. The presence of midges in all of these studies suggests that the carcasses were submerged for some time.

Studies of midges occurring in some Iceland and Canadian lakes (Harper and Cloutier, 1986; Lindegaard, 1992) have shown that specific species complexes are associated with different depth zones of the lake, such as the surf zone (lake margin), littoral (0 to 10 m), sublittoral (10 to 20 m), and profundal (> 20 m). The highest number of individuals and species were found in the shallowest sites and the lowest number of both occurred in the deepest sites. If this information was known about a lake in which a corpse containing chironomids was found, then it might be possible to determine where in the lake a body was originally deposited before it floated to the surface and if it was possibly transported by wind and/or wave action. Also, there may be published life history information on some of the species present that could assist with determining a PMSI.

Midges and black flies (Simuliidae) have both been evaluated in death scene investigations during the past decade and are discussed in more detail in the Case Histories section of this chapter.

Collecting and Rearing Aquatic Insects from Corpses

Collecting and preserving insects from aquatic environments is somewhat different than from terrestrial sites because one is mostly dealing with larvae rather than adults. All larvae and adults collected from aquatic habitats should be placed in vials, labeled and preserved in 70 to 80% ethanol (ethyl or isopropyl) as soon as they are carefully removed from substrates, clothing, or the corpse with forceps. It is helpful to have waders or hip boots and shoulder-length rubber gloves (trapper gloves) to gain access to the body in cold water and remove insects before moving the body to land. A large magnifying glass or hand lens in the field is helpful to spot insects on the habitus. Labeling instructions for insect specimens are detailed in Catts and Haskell (1990) and in Chapter 3 of this book. It also is important to keep larval caddisflies with different cases in separate vials, as they tend to abandon their case when placed in alcohol and sometimes the case is a key character in identifying the specific taxa of Trichoptera. Although there are other solutions that can be used to preserve aquatic insects, e.g., Carnoys, Hood's, Kahle's (see Catts and Haskell, 1990), ethanol is the preferred medium (Merritt and Cummins, 1996a). If one is collecting stream-bottom samples containing detritus and insects, it is preferable to use 95% ethanol to account for dilution from water in the samples (Merritt et al., 1996). A white enamel pan is helpful in providing contrast when sorting through silt and detritus for insect specimens. It is very important that specimens are *never* placed in water as a preservative

because they will decay rapidly (within 1 to 2 h) and not be identifiable. In most instances, one will not see many adult insects flying around the habitat or corpse that are associated with the larvae in aquatic environments.

In some instances, it may be necessary to transport live larval insects from the field to the laboratory so that they can be reared to adults for specific identification. One of the most common problems encountered in rearing aquatic insects involves mortality during transport due to inadequate oxygen supply and/or temperature control. Agitation during transport will maintain oxygen levels, but may damage delicate specimens. Alternative methods include transporting the animals in damp moss, burlap, or paper towels with a small amount of cold water and using small "bait bucket" aerators that can be purchased at most pet stores. To maintain cool temperatures, thermal containers or ice coolers should be used (Merritt et al., 1996).

Laboratory rearings can be maintained at field temperatures using an immersible refrigeration unit or by recirculating water through a cooling reservoir. If laboratory temperatures do not match those in the field, mortality can be reduced by allowing temperatures to equilibrate slowly. To maintain water quality in the laboratory, tap water should be dechlorinated and distilled, or spring water added to replace evaporation loss. Algal and detrital food supplies are often best maintained by periodic replenishment from the field (Merritt et al., 1996). For a further discussion of rearing techniques, see Merritt and Cummins (1996a) and Chapter 4 in this volume.

Case Histories Involving Aquatic Insects

Case No. 1

In late June 1989, a pair of recreational scuba divers were exploring the waters of the Muskegon River in western Michigan when they discovered a car lying upside down on the bottom of the river, with the dead body of a woman inside (Figure 6.7a). The car was found in a 15-ft deep hole so it was not observable from the riverbank or the bridge above. Police hauled the car from the river and traced it to the woman's husband. Medical examiners found contusions on the dead women's skull which did not appear to be caused by the accident. Based on other evidence, the husband became the prime suspect. He initially claimed he had argued with his wife in late September of the previous year, and that she had driven away upset into a foggy night. He further stated that he had not seen her since that night. However, the car in which his wife was found was in relatively good condition, suggesting that it had been pushed into the river and had not gone into the water as the result of a crash.

Even though the police thought the husband was lying about the circumstances of the case, the cold water had preserved the woman's body, making it difficult to determine the time of death. Interestingly, when the car was brought up from the river, the detectives noticed that there were aquatic insects attached to the windshield, fenders, and door panels (Figure 6.7a,b). Specimens were collected from these substrates and sent to the author (Merritt) in the Department of Entomology at Michigan State University for identification. Three different insect taxa were identified from the car: caddisfly cases, chironomid midges, and black fly pupal cocoons and some larvae. It was difficult to establish a time that the car had gone in the river using the caddisflies or midges; however, the black fly cocoons provided evidence that was significant to establishing the PMSI.

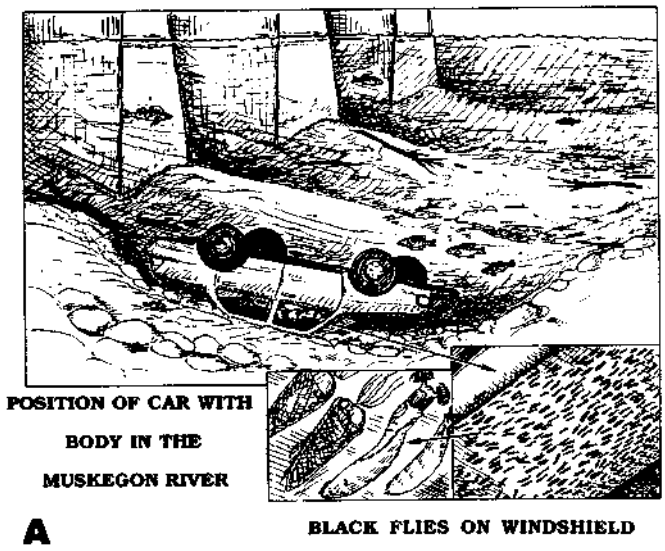


Figure 6.7 (a) Drawing showing position of car with body inside found by recreational divers near bridge abutments in the Muskegon River, MI. Inset shows larvae and pupal cocoons of black flies on windshield. (b) Close-up photograph of car door panel with arrow pointing to attached black fly pupae. (Photo (b) courtesy of Det. Sgt. Richard Miller, Michigan State Police.)

Adult female black flies, in this case belonging to the genus *Prosimulium*, lay eggs in the late spring or early summer. These settle into the sediment and undergo obligate diapause (an arrested state of development) until the following fall or early winter (late November to January) (Figure 6.8, eggs). The eggs hatch into larvae and these attach to a specific substrate in the stream (e.g., rocks or vegetation, in this case a car) where they filter very small particles from the current with fan-like structures on their head (Figure 6.8, larva). Larval growth is slow at low water temperatures prevailing in the river during January and February. Following snowmelt, growth increases and pupation on the substrate occurs in late March or April (Figure 6.8, pupa).

Emergence follows shortly after (early to mid-May) and adults are present for 1 to 2 months during which time they mate, take a suitable blood meal (if required), lay eggs, die, and the life cycle begins again (Figure 6.8, adult). In this particular case, numerous pupal cocoons (consisting of "silken" threads in a dense sleevelike structure of specific

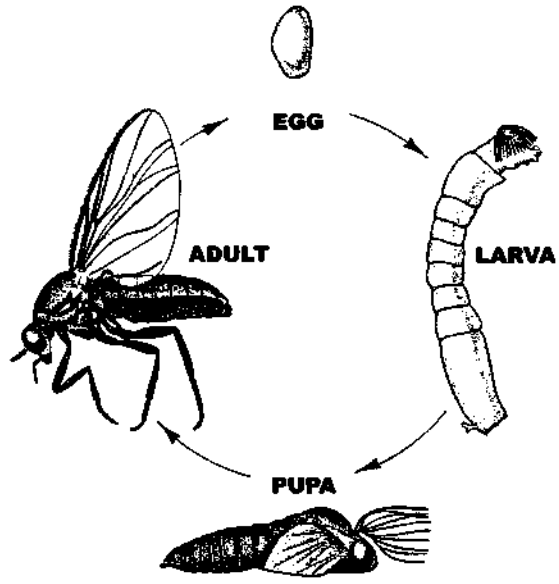


Figure 6.8 Generalized life cycle of a black fly (Diptera: Simuliidae) showing all stages.

shape and texture) (Figures 6.7a, 6.8, pupa) remained attached to the car after the adults emerged. Pupal cocoons of black flies are sometimes species-specific and used in keys to identify specimens found in lotic habitats (Merritt et al., 1996). Based on the specific identification of the cocoon and known life cycle of the black fly species present on the windshield of the car in late June, it was determined that the car had to have gone into the river long before June 1989 and most likely the previous fall. The suspect later claimed that he had spoken with friends who had been in contact with his wife during late winter and spring of 1989. This would not have been possible based on the species of black fly found on the car. In April 1990, based on autopsy, insect, and other evidence presented by the prosecutor and expert witnesses, it was proven that the husband was lying and his wife had disappeared the previous fall and the car had been in the river for approximately 9 months. He was found guilty of second degree murder in the death of his wife and sentenced to 20 years in prison. The man was convicted of murder, based in part on the life cycle of an aquatic insect (Wolkomir and Wolkomir, 1992).

Case No. 2

On July 5, 1997, the dead body of a partially submerged 19-year-old female was discovered by turtle hunters in a small lake in western Michigan (Figure 6.9) 3 days before she was supposed to testify against a man who had sexually assaulted her. Unconfirmed reports placed her alive on June 5 or 6, 1997. The woman's eyes and mouth were duct-taped shut and the body had been weighted down with two cinder blocks and bound with chains before she was thrown into the lake alive. The body was submerged within the lake prior to rising to the surface due to gaseous build-up of putrefaction. Insect larvae (fly maggots belonging to the family Calliphoridae) were collected by the police on July 7 from the head and face exposed to the air when the body was pulled from the water (Figure 6.10). No puparia were observed on the body by the detective in charge. Specimens were sent to the author (Merritt) at Michigan State University for identification.

Figure 6.9 Photo of Oxford Lake, MI with arrow pointing to human corpse floating on the surface. (Photo courtesy of Det. Sgt. Richard Miller, Michigan State Police.)

An analysis of the fly larvae showed that they were mature third larval instars of *Phormia regina* (Meigen), the black blow fly, a common blow fly in the Midwest during the summer months. This species is a late arriver at carrion, and at 80 and 90°F temperatures, *P. regina* will generally have a 24 to 48 h delay in oviposition (Hall and Doisy, 1993). However, the situation here was somewhat different and more difficult to interpret because the body had been under water for some time and surfaced in a different physiological state than would have occurred if the victim had just been killed minutes earlier and never exposed to water. Based on the degree-days required for larval development of the oldest

Figure 6.10 Close-up of lake victim's head during autopsy showing larvae of blow flies (arrow) crawling on face. Adult flies colonized the head region after body floated to the surface. (Photo courtesy of Det. Sg. Richard Miller, Michigan State Police.)

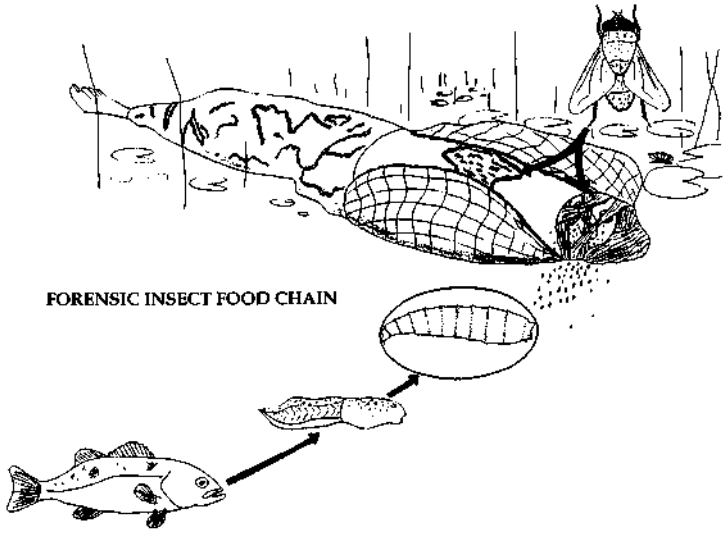


Figure 6.11 Drawing of forensic insect food chain in a lake environment showing a case where blow fly maggots colonizing a body exited the body into the water. Tadpoles were observed feeding on maggots and then being eaten by fish. Fish also were observed feeding on maggots. (Drawing courtesy of Ethan Nedeau.)

and largest specimens, the floating remains of the body would most likely have been exposed to colonization by this species between July 1 and 2. This PMSI date turned out to be extremely close to when police suspected the victim had surfaced, and was in line with the estimation of how long the body had been submerged in the lake.

The time required for the body to resurface from being submerged is primarily dependent on water temperature and typically decreases with depth (Rodriquez, 1997). With fairly warm water temperatures, as was the case for this shallow lake in July, and the observed presence of algal blooms (Figure 6.9), a body would be expected to surface within a few days to a week. Other factors which affect the rate of decomposition in water include bacterial content and salinity (Rodriquez, 1997). A corpse submerged in a highly eutrophic shallow lake such as the one in this case will decompose much more rapidly than a corpse in a relatively cooler, deeper lake with a lower bacterial count. Of course, the weight of the body, as well as the cinder blocks and chains used to keep it submerged, also were factors to consider.

An interesting aspect to this case was the observation by the detective at the crime scene that tadpoles and small fish, probably bass and blue gills, were feasting upon the decomposing tissue of the body and that dipteran larvae were exiting the body and falling into the water. From an ecological standpoint, this could be the first published account, to our knowledge, of an aquatic forensic food chain as depicted in Figure 6.11.

Case No. 3

A fully clothed body of a 32-year-old female was discovered by a group of boy scouts in a shallow, ice-covered creek in southeast Ohio on January 30, 1989 (Figure 6.12a). An autopsy performed by the coroner's office determined the cause of death to be strangulation. Upon examination of the body during autopsy, numerous caddisfly cases up to 15 mm long composed of multicolored particles of rock and sand were collected on the folds

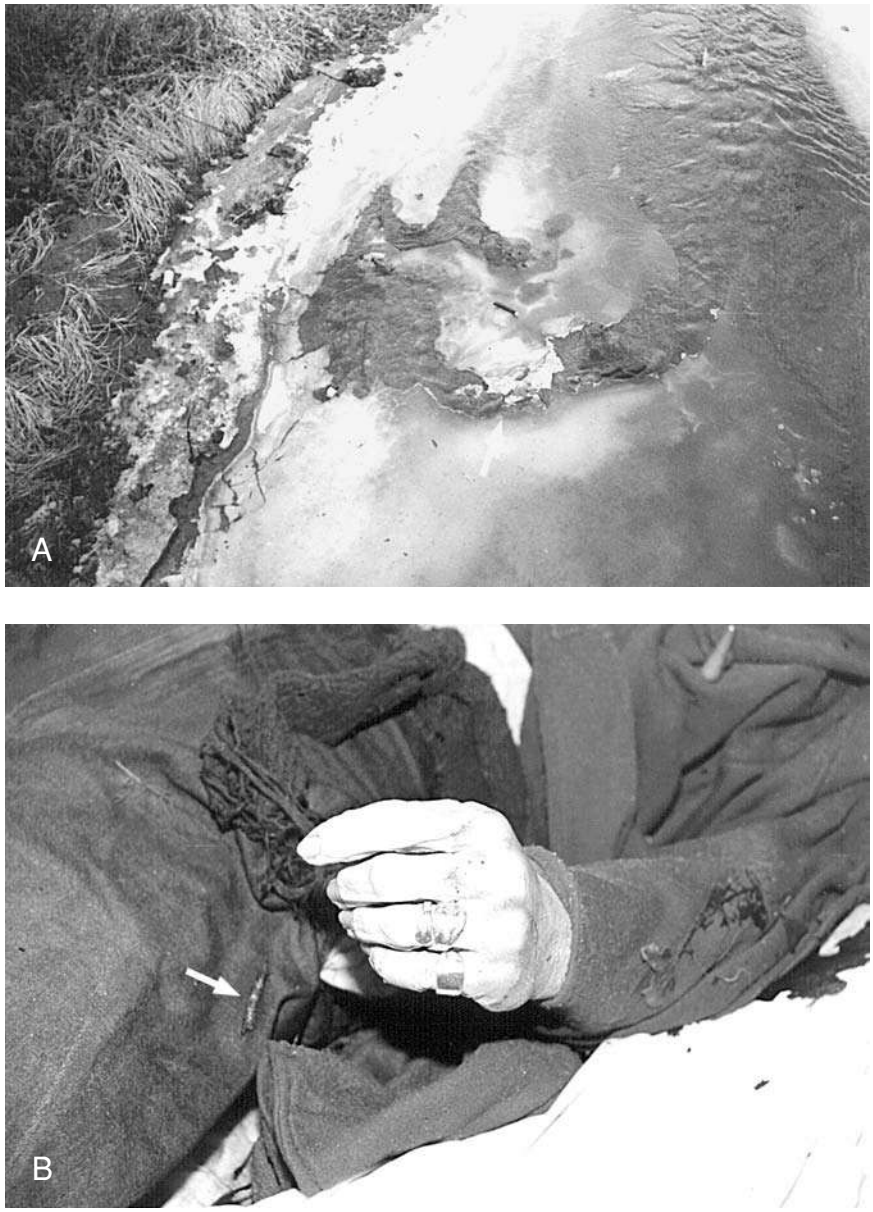


Figure 6.12. (a) Aerial photo of dead body (arrow) of a female found in an iced-over stream in Ohio during the winter. (b) Photo of corpse of above female removed from stream showing limnephilid caddisfly case (arrow) attached to clothing. (c) Photo of a piece of bark found near corpse in above stream with some of the same caddisfly cases attached that were found on clothing. (Photos courtesy of Anthony Tambasco, Mansfield Police Department, Mansfield, OH.)

of her clothing (Figure 6.12b). These same caddisfly cases and those of related species also were found on natural stream substrates near where the body was found (Figure 6.12c). The caddisflies were sent to a forensic entomologist and later identified as larvae belonging to the family Limnephilidae and the species *Pycnopsyche lepida*. The larvae spend their entire time in the water and the protective cases they construct out of sand, gravel, and other materials are used as ballast to help weight them for bottom dwelling in fast currents

Figure 6.12 (c) *Continued.*

and for protection against predators. It is possible that the type of case materials used by a caddisfly in a specific type of stream or stream reach (e.g., in a riffle zone with gravel vs. pool zone with sand and silt) could indicate the planned movement of a body or the accidental drift of the remains from one habitat to another.

In this particular case, the larvae of the caddisflies found on the victim's clothing were not instrumental in determining the PMSI or establishing the time of death. They were used to confirm that the body had been in a river for a while and probably not moved or transported there from another type of aquatic habitat. We strongly feel, as discussed earlier, that a knowledge of the life history features of caddisflies could be very important in certain aquatic forensic investigations.

Case No. 4

A missing person report was made on March 26, 1993, for a 16-year-old white female who was last seen at her place of employment in LaPorte, IN. Her empty car with the hood up and the keys in the ignition was found the following night a short distance from the above site. On April 27, fishermen discovered the partially clothed body of the missing female in a large pond near where the car was discovered. Her body was floating face down in less than 6 ft of water with some tree branches laying across part of her remains. An autopsy revealed that the cause of death was the result of strangulation. The body was in a bad state of decomposition and it was determined that it had been in the water an estimated time of 30 days.

When the body was removed from the water, numerous aquatic invertebrates were collected from the victim's body and clothing. These included species from the following taxa: amphipods (*Hyalella azteca*), snails (*Physella*, *Planorbula*, *Promenetus*), clams (*Sphaerium*), water mites (*Lebertia*), dragonflies (Gomphidae, *Gomphus*), damselflies (Coenagrionidae, *Ischnura*), chironomids (*Phaenopsectra*), pigmy backswimmers (Pleidae), crane flies (Tipulidae, *Erioptera*), and biting midges (Ceratopogonidae, *Bezzia*).

The above information collected from the remains did not provide evidence that could be used to establish a PMSI in this investigation. However, after reviewing the habitat, mode of existence, and trophic information on the invertebrate taxa collected, a few conclusions could be drawn. First, the habitat in a pond or lake where most of these invertebrates occur and where the victim was found is termed the littoral zone. This is the shallow shoreline zone where sunlight penetrates to the bottom and is sufficient to support rooted plant growth. Most of the taxa collected from the corpse are climbers on rooted vegetation growing in this habitat or burrowers in sediments, primarily silt (Merritt and Cummins, 1996c). Also, several taxa found belong to the scraper functional feeding group (Table 6.2), and feed on periphyton or attached algae. This would indicate that the victim had been in the lake long enough for these plants to colonize the surface of her body or clothing and provide a food resource for these invertebrates to utilize (J. Wallace, personal observation). This kind of ecological information would have been more useful if it was not known when the victim originally disappeared, as the presence of different invertebrate life stages are seasonal and substrate colonization rates of the different taxa varies over time. Also, based on the invertebrates found on the body at that depth and location, one could possibly determine whether there was movement of the body from one site to another within the pond (e.g., deep to shallow zone), possibly due to bloating, wind, or wave action.

Case No. 5

This case history actually describes two separate murders reported by Hawley et al. (1989) involving the same forensic insect evidence. The first case was a 21-year-old male found dead floating in a stream in an urban area. The cause of death was impalement per rectum by a 3-ft length of pipe. A preliminary examination of the body showed the presence of a few 3 to 5 mm coiled translucent red strands or "fibers" on the clothing and body, thought to be carpet fibers. These fibers were initially treated as such and placed in a dry plastic petri dish and sealed for later analysis. When analyzed, the fibers turned out to be larvae of chironomid midges or "blood worms" as they are called due to the presence of hemoglobin (Figure 6.6b).

The second case occurred a year later and involved an unidentified adult female found floating in a farm drainage ditch. She had been killed by blows to the face with aspiration of blood (Hawley et al., 1989). Examination of the partially clothed body again disclosed similar translucent red "fibers," but this time a forensic entomologist was available to recognize these as insect larvae belonging to the family Chironomidae (see Appendix, Key 4). Although it was stated by Hawley et al. (1989) that the stage of development of these larvae may be useful in estimating the duration of submersion if life cycle data were available, no information was provided as to whether or not these larvae were important in these cases. We feel as others do that chironomid midges may prove to be very useful in forensic investigations (Haskell et al., 1989; Hawley et al., 1989; Keiper et al., 1997).

Case No. 6

In most instances, it is the immature stages of aquatic insects, particularly Diptera, which are associated with a corpse in a stream or a pond rather than the terrestrial adults. However, one case involving the latter occurred in the northwestern U.S. on the lower slopes of a mountain range and proved to be interesting and unique. The story was passed on to Haskell et al. (1997) by the late Dr. Paul Catts, an outstanding forensic entomologist

at Washington State University. The body of a dead female clad in a swimsuit was found to have petechial hemorrhages arranged in clustered patterns on exposed portions of her thighs. She was found in a wet swampy habitat which was heavily forested and a major mosquito breeding area. The hemorrhages were inconsistent with the circumstances surrounding the death, and the pathologist had no explanation. A forensic entomologist who was participating in the investigation determined that these "hemorrhages" were actually multiple probing marks on her exposed skin caused by a mosquito's mouthparts (proboscis), as the insect was trying to take a blood meal on the still warm, but dead body (Figure 6.13*). Unable to bring up blood in the proboscis due to lack of blood pressure, the female mosquito probed more sites, which created the clusters of "petechial hemorrhages." When the assailant was finally apprehended, he confessed to having placed the body in the swampy habitat shortly after he had killed her (Haskell et al., 1997).

Conclusions

At the beginning of this chapter, we showed the great discrepancy between the number of forensic studies published dealing with terrestrial insects and those dealing with aquatic insects. This discrepancy has occurred for several reasons, some of which have been made evident throughout the text. First, we and others have pointed out that aquatic insects, unlike their terrestrial counterparts, have not evolved obligatory sarcophagous habits to feed on carrion in their environment. Crayfish, a crustacean found in the same habitats, have probably come the closest to this functional feeding mode. Studies on salmon decomposition in Pacific Northwest streams, where carcasses have been a natural part of the landscape for eons, indicate that some caddisflies and possibly other groups of aquatic insects have become facultative scavengers and utilize this food resource during different times of the year. However, most insect decomposers in lotic and lentic systems feed on leaf litter and similar forms of organic matter which enters the stream or lake on a seasonal basis. This is in contrast to marine systems where a wide variety of scavenging fishes and invertebrates other than insects occur.

Secondly, to date we have not discovered a successional insect model to follow in aquatic systems as exists with many blow fly species in terrestrial systems. This is partly because many aquatic insects are natural drifters and will drift onto a substrate (carcass) for attachment only, and not necessarily to feed. Therefore, when an aquatic insect is collected from victims or their clothes, it is difficult to interpret whether or not the insect in question was actually colonizing the corpse or arrived there by accidentally drifting onto another substrate in its movement downstream with the current. Even if it did colonize the corpse and was identified, it is much more difficult to trace it back to a specific instar or developmental stage knowing that some aquatic insect orders have 20 to 40 larval instars, and especially since the developmental times for the majority of species have not been worked out in detail enough to establish a PMSI.

We also noticed in recent studies that the growth rates of algae on submerged carrion over time, in conjunction with the utilization of this food resource by certain aquatic insects (i.e., scrapers), may be of significance in helping to determine the PMSI. In fact, our preliminary results indicated that algal growth on an aquatic substrate such as a pig

* See color section insert following page 78.

or even a human corpse occurs in a successional manner similar to the successional sequence we observe with terrestrial insect colonization of human corpses. In our discussion of aquatic insects of forensic importance and in the case histories given, we have provided examples of different families, based on their functional feeding groups, that could or have been of assistance in determining the PMSI. Specifically, these include larvae of mayflies (Ephemeroptera), stoneflies (Plecoptera), caddisflies (Trichoptera), midges (Chironomidae), black flies (Simuliidae), and in a unique case, adult mosquitoes (Culicidae). We know that others exist and will be found to play a significant role, but there first needs to be much more experimental research conducted on the aquatic insect colonization of submerged carrion or corpses in lotic and lentic systems and the elucidation of life history features on those specific species involved.

Another problem that has hampered studies in freshwater and marine aquatic systems has been the wide variety of confounding environmental and biological factors acting on the corpse and influencing the PMSI. These were discussed in some detail and include: current and wave action, water temperature, oxygen concentration, salinity, depth, nature of the substrate and other parameters that may influence water quality, and the diversity of aquatic insects or other arthropods colonizing the corpse. These, in addition to body factors such as whether or not it is floating or submerged, or clothed or not, make the determination of a PMI or PMSI a difficult task.

Lastly, the collection and rearing of aquatic insects from submerged corpses by untrained personnel has not been emphasized in the literature and is a more difficult procedure to accomplish than in terrestrial situations. This statement may have support from a recent study by Hobischak and Anderson (1999) who carefully examined water-related deaths reported by the British Columbia's Coroners Service over a 2-year period. After looking at reports from 47 cases, they found entomological evidence from only three cases. Of these, all the insect species reported were terrestrial, none were aquatic and no cases reported using insects in determining the PMI. As they alluded to in their study, this was probably due an ignorance by the investigator(s) as to the potential importance of the aquatic fauna present on a corpse, lack of recognition of these kinds of insects, poor collecting techniques, and/or a combination of the above.

If a corpse is found in a stream or other shallow body of water, an investigator should come prepared to the scene with waders, alcohol vials, forceps, and gloves to collect specimens off the body before it is removed from the water. This procedure is probably rarely done and is more difficult than collecting maggots with forceps off a terrestrial corpse and placing them in an alcohol vial. If one wishes to rear immature aquatic insects back in the laboratory to obtain adults for specific identifications, they generally will have to carry out the procedures described earlier, which are much more labor intensive than simply placing the maggots collected from a terrestrial corpse on a piece of fresh liver in a container with sand and waiting for a week for adult emergence. The different techniques used for collecting and rearing aquatic insects have surely prevented investigators from involving these kinds of insects more in crime scene investigations.

In summary, our chapter has reviewed the literature and presented the stages of decomposition for different types of carrion (mainly pigs) in freshwater environments and the associated aquatic insect fauna. The ecological approach we have chosen to determine which aquatic insect groups may be important in corpse decomposition is based on the functional feeding group classification. This general classification system distinguishes invertebrate taxa within aquatic ecosystems according to the different morphological-

behavioral adaptations used to harvest nutritional resources. These feeding mechanisms determine the food resources that are processed: shredders feed on CPOM, collectors on FPOM, scrapers on periphyton or algae, and predators on prey.

A simplified pictorial dichotomous key is provided to assist investigators and researchers with the identification of these different functional groups that may be found associated with or feeding on corpses in aquatic environments. As we have discussed above, determining the postmortem submersion interval (PMSI) in aquatic studies has been, and will continue to be, problematic due to several factors. However, it is critical to determining the time between death and corpse discovery in these environments. Hopefully, we have identified those groups that may be important and ones which need further research emphasis. Collecting and rearing techniques for aquatic insects have been reviewed and the logistical problems have been discussed as compared to those used in terrestrial environments. Finally, we have provided several case histories where aquatic insects have been used to either determine the PMSI or associate a body with a particular type of aquatic habitat or location within the habitat. It is abundantly clear that we still have a long way to go in determining the PMSI using aquatic insects, and it will be some time before we can approach the level of sophistication and accuracy that we have achieved with terrestrial insects. We hope that this chapter has provided a start in that direction.

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Appendix

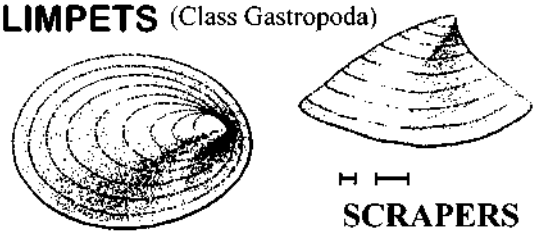
APPENDIX: SIMPLIFIED KEYS TO THE FUNCTIONAL FEEDING GROPUS OF LOTIC MACROINVERTEBRATES

KEY TO FUNCTIONAL FEEDING GROUPS

indicates size or range of sizes

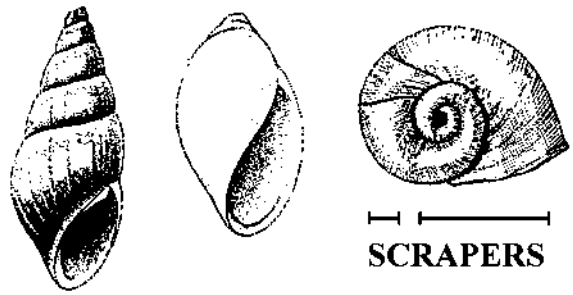
1. **ANIMALS IN HARD SHELL** (Phylum Mollusca)

a. LIMPETS (Class Gastropoda)



SCRAPERS

b. SNAILS (Class Gastropoda)



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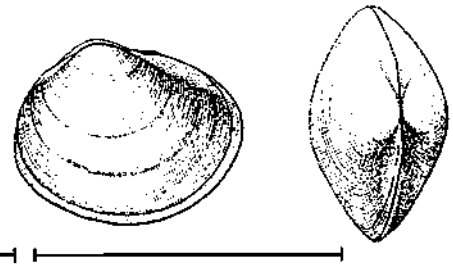
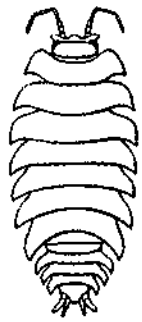
Snails are generalized (facultative) feeders and can also function as Shredders.

c. CLAMS OR MUSSELS (Class Pelecypoda)



FILTERING COLLECTORS

2. SOW BUG OR SHRIMP-LIKE ANIMALS



SHREDDERS

Generalized, can also function as Gathering Collectors.

3. LARVAE IN PORTABLE CASE OR "HOUSE"

Go to KEY 2

4. LARVAE IN FIXED RETREAT, WITH CAPTURE NET

Note: Care must be taken when collecting to observe nets.

Go to KEY 3

5. WITHOUT CASE OR FIXED RETREAT

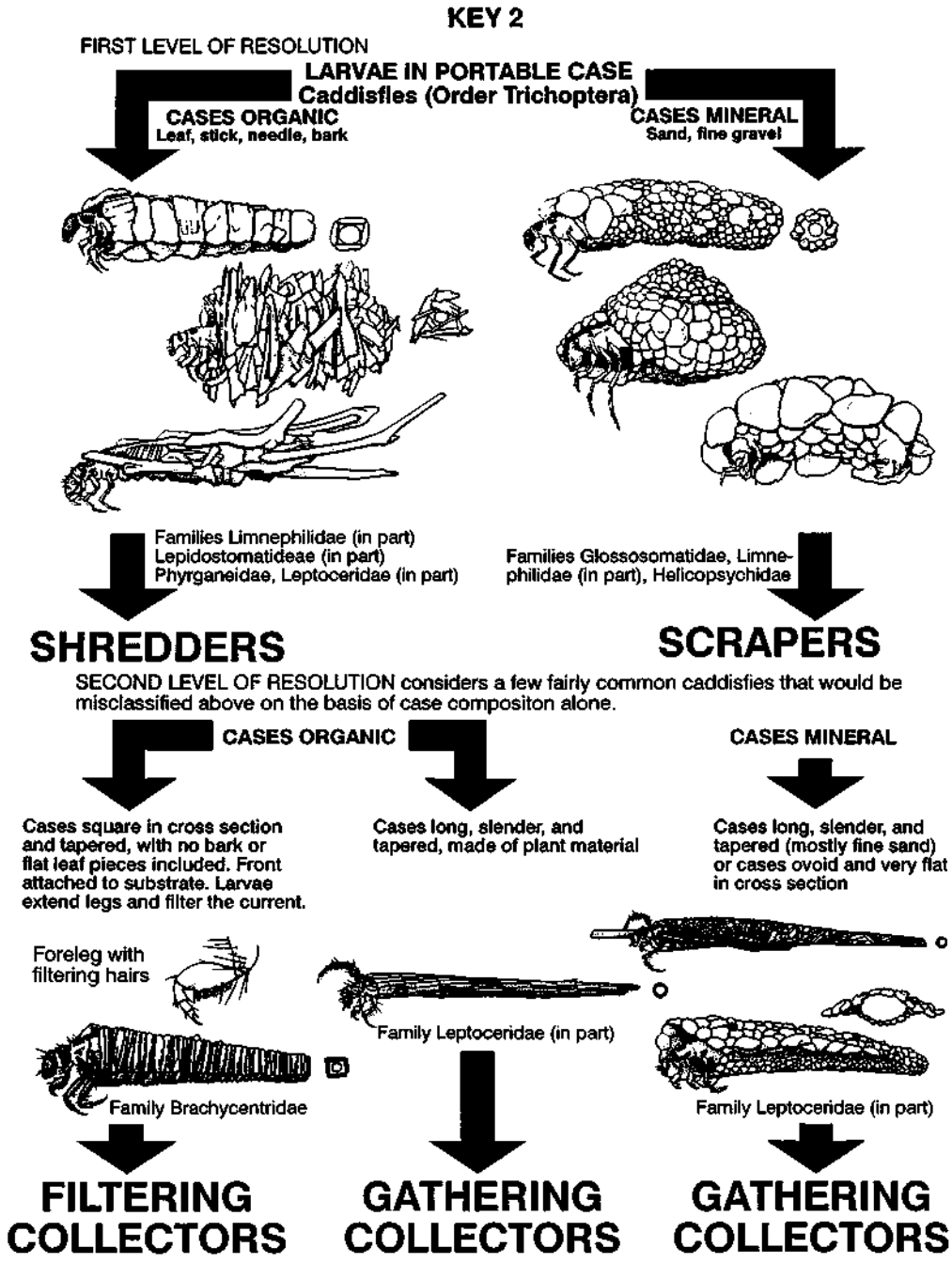
a. **WORM-LIKE LARVAE WITHOUT JOINTED LEGS**

Go to KEY 4

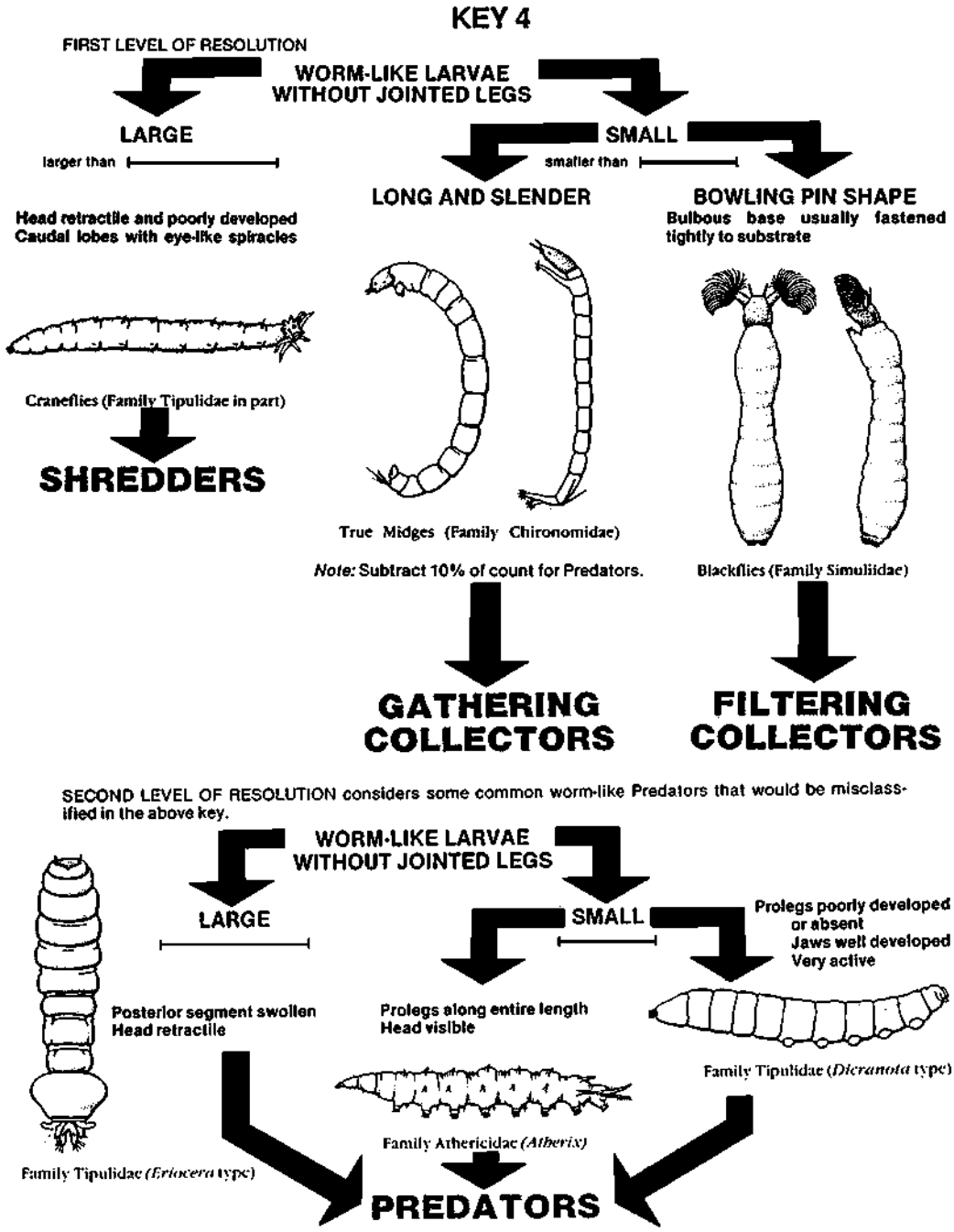
b. **NYMPHS OR ADULTS WITH JOINTED LEGS**

Go to KEY 5

6. DOES NOT FIT KEY 5 EXACTLY. GO TO KEY 6.


KEY 2
FIRST LEVEL OF RESOLUTION
LARVAE IN PORTABLE CASE
Caddisflies (Order Trichoptera)
CASES ORGANIC
Leaf, stick, needle, bark
CASES MINERAL
Sand, fine gravel
Families Limnephilidae (in part)
Lepidostomatideae (in part)
Phryganeidae, Leptoceridae (in part)
Families Glossosomatidae, Limnephilidae (in part), Helicopsychidae
SHREDDERS
SCRAPERS
SECOND LEVEL OF RESOLUTION considers a few fairly common caddisfies that would be misclassified above on the basis of case compositon alone.
CASES ORGANIC
CASES MINERAL
Cases square in cross section and tapered, with no bark or flat leaf pieces included. Front attached to substrate. Larvae extend legs and filter the current.
Cases long, slender, and tapered, made of plant material
Cases long, slender, and tapered (mostly fine sand) or cases ovoid and very flat in cross section
Foreleg with filtering hairs
Family Brachycentridae
Family Leptoceridae (in part)
Family Leptoceridae (in part)
FILTERING COLLECTORS
GATHERING COLLECTORS
GATHERING COLLECTORS


KEY 3
FIRST LEVEL OF RESOLUTION
LARVAE WITH FIXED RETREAT AND CAPTURE NET
Note: Care must be taken when collecting to observe nets.
Caddisflies (Order Trichoptera)
True Flies (Order Diptera)
COARSE NET IN "SCAFFOLDING"
FLATTENED SOCK-LIKE OR TRUMPET-SHAPED NET OF FINE MESH
TUBE WITH SILK STRANDS STRUNG BETWEEN TERMINAL PRONGS
smaller than
True Midges (Family Chironomidae)
Families Hydropsychidae, Philopotamidae, Polycentropodidae
FILTERING COLLECTORS
SECOND LEVEL OF RESOLUTION separates from free living larvae those net spinning caddisflies that may have been inadvertently collected without being associated with their nets.
NET SPINNING CADDISFLIES
Frequently separated from their nets
HEAD AS WIDE AS THORAX
Especially Philopotamidae (bright yellow) and Hydropsychidae (bright green or brown)
FILTERING COLLECTORS
FREE LIVING CADDISFLIES
Non net spinning
HEAD LONG, SMALL, AND NARROWER THAN THORAX
Rhyacophilidae (often bright green)
PREDATORS


KEY 4
FIRST LEVEL OF RESOLUTION
WORM-LIKE LARVAE WITHOUT JOINTED LEGS
LARGE
larger than
SMALL
smaller than
LONG AND SLENDER
BOWLING PIN SHAPE
Head retractile and poorly developed
Caudal lobes with eye-like spiracles
Bulbous base usually fastened tightly to substrate
Craneflies (Family Tipulidae in part)
SHREDDERS
True Midges (Family Chironomidae)
Note: Subtract 10% of count for Predators.
Blackflies (Family Simuliidae)
GATHERING COLLECTORS
FILTERING COLLECTORS
SECOND LEVEL OF RESOLUTION considers some common worm-like Predators that would be misclassified in the above key.
WORM-LIKE LARVAE WITHOUT JOINTED LEGS
LARGE
SMALL
Prolegs poorly developed or absent
Jaws well developed
Very active
Posterior segment swollen
Head retractile
Prolegs along entire length
Head visible
Family Tipulidae (Dicranota type)
Family Athericidae (Atherix)
Family Tipulidae (Eriocera type)
PREDATORS

KEY 5

FIRST LEVEL OF RESOLUTION

NYMPHS WITH JOINTED LEGS

3 (or 2) TAILS (FILAMENTS) AT BACK — NO EXTENDIBLE LOWER LIP (LABIUM)

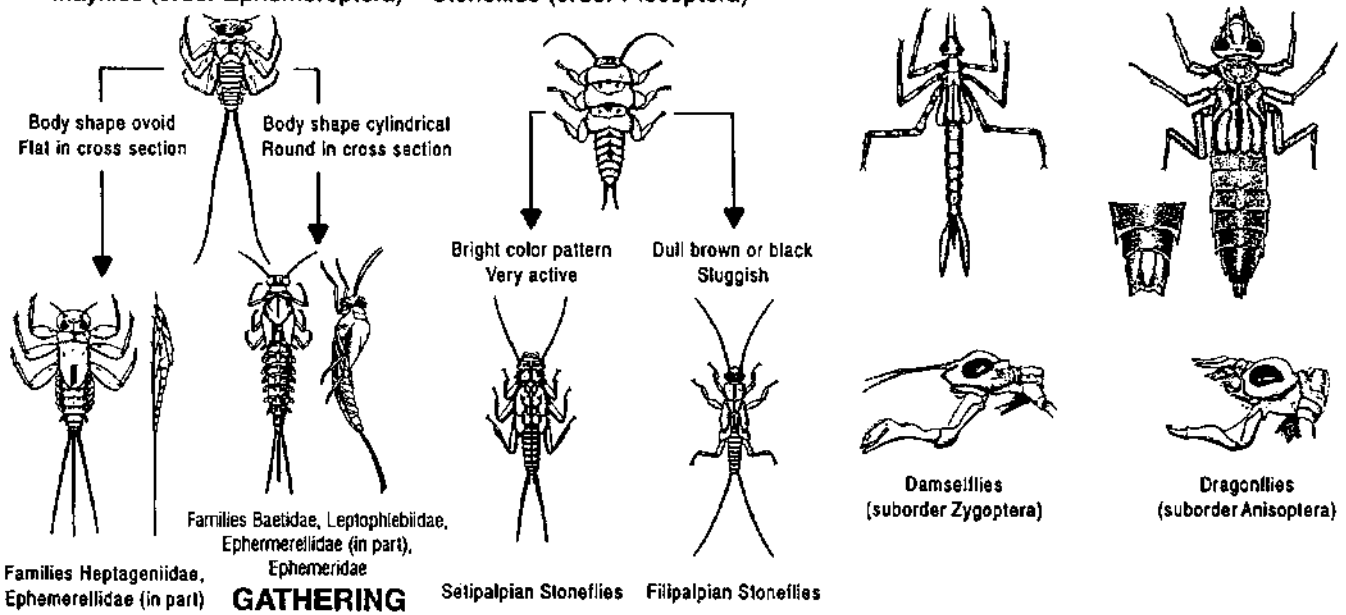
3 (OR 2) TAILS WITH LATERAL ABDOMINAL GILLS
Mayflies (order Ephemeroptera)

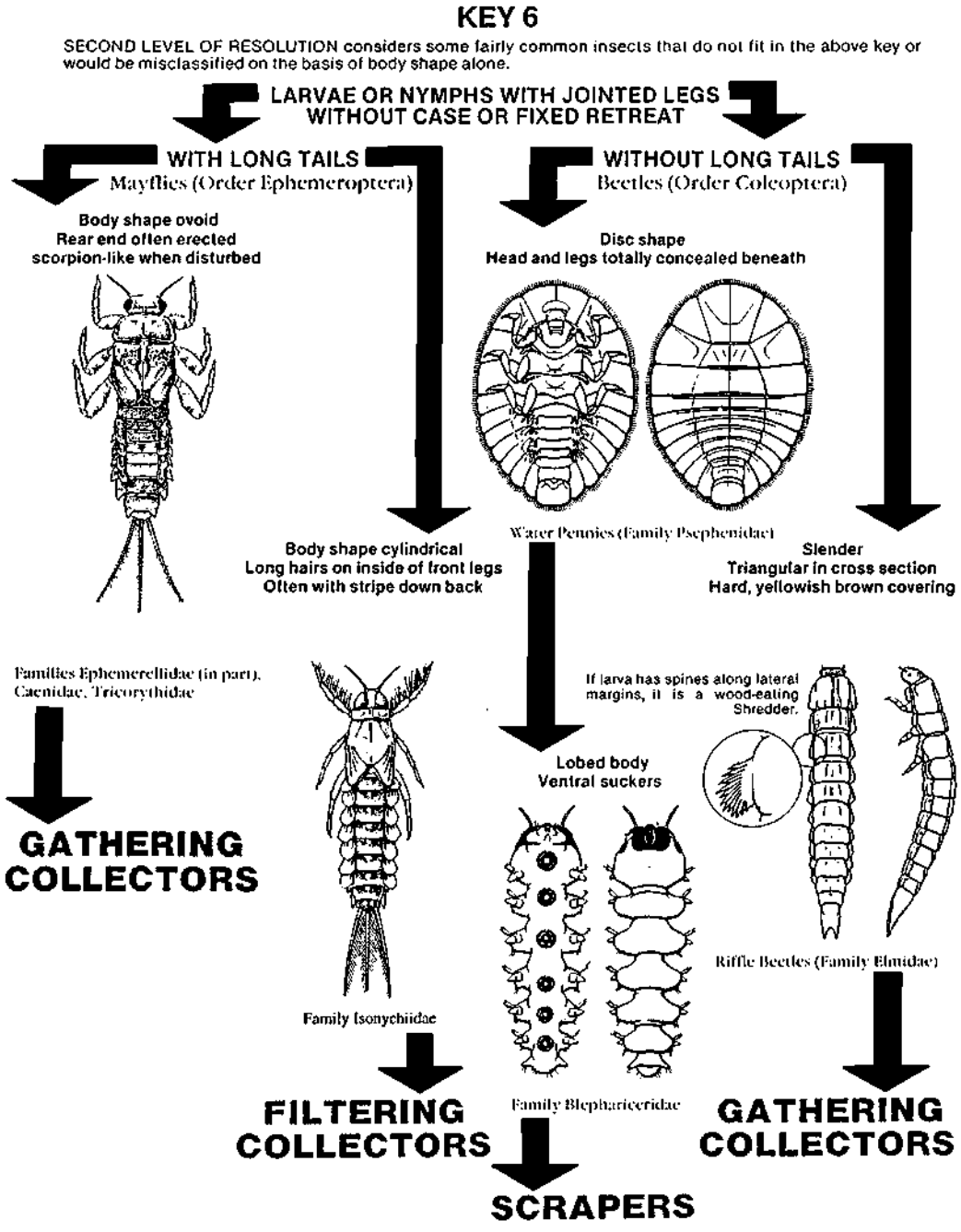
2 TAILS WITHOUT LATERAL ABDOMINAL GILLS
Stoneflies (order Plecoptera)

3 FLAT PADDLES OR POINTS AT BACK — EXTENDIBLE LOWER LIP

3 FLAT PADDLES AT BACK

POINTS AT BACK




KEY 6
SECOND LEVEL OF RESOLUTION considers some fairly common insects that do not fit in the above key or would be misclassified on the basis of body shape alone.
LARVAE OR NYMPHS WITH JOINTED LEGS WITHOUT CASE OR FIXED RETREAT
WITH LONG TAILS
Mayflies (Order Ephemeroptera)
WITHOUT LONG TAILS
Beetles (Order Coleoptera)
Body shape ovoid
Rear end often erected
scorpion-like when disturbed
Disc shape
Head and legs totally concealed beneath
Water Pennies (Family Psephenidae)
Body shape cylindrical
Long hairs on inside of front legs
Often with stripe down back
Slender
Triangular in cross section
Hard, yellowish brown covering
Families Ephemerellidae (in part), Caenidae, Tricorythidae
GATHERING COLLECTORS
If larva has spines along lateral margins, it is a wood-eating Shredder.
Lobed body
Ventral suckers
Family Isonychiidae
FILTERING COLLECTORS
Family Blepharicerida
SCRAPERS
Riffle Beetles (Family Elmidae)
GATHERING COLLECTORS

Recovering Buried Bodies and Surface Scatter: The Associated Anthropological, Botanical, and Entomological Evidence

7

ALISON GALLOWAY
HEATHER WALSH-HANEY
JASON H. BYRD

Contents

Introduction

It is often stated by archaeologists that, once excavated, a site can never be completely reconstructed and the same is true in a scene investigation where human remains are found. For this reason, it is essential that the investigators be meticulous in recording the features of the scene and all stages of the recovery process (Dirkmaat and Adovasio, 1997). The primary aim is to use these data to discern the events of the death and, if necessary, prosecute the perpetrators. This attention to detail also minimizes the possibility of

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contamination and maximizes the recovery of the skeletal material and other lines of evidence, including entomological and botanical, which may be overlooked or destroyed in a hasty collection.

The forensic anthropologist provides a number of lines of information during the investigation through their role as expert witnesses in the identification of the human skeletal material. Their analysis is a multistage process based on the close examination of the osseous material in the laboratory or morgue. First, the anthropologist provides a biological profile of the decedent (i.e., the age, sex, ancestry, and stature). Second, he or she analyzes the trauma observed on the bones, states whether this occurred in the antemortem, perimortem, or postmortem period, and discusses the mechanisms of injury which could have produced these defects. Third, the anthropologist estimates the interval since the death of the individual and discusses the probable sequence of events in the postmortem period (body movement due to scavenging, water action, etc.). Of these, the biological profile is rarely of particular interest in court although it is critical for beginning the search to identify the remains. Instead, court testimony more frequently centers on the issues of trauma and disposal, for how and when the death occurred are crucial points that may make or break the case for the prosecution or the defense.

The laboratory or morgue, however, may not be the first time the anthropologist encounters the remains. Often he or she is called to the scene to assist in the recovery of the body and associated evidence. Although such cases are only a small portion of the overall caseload, our involvement in scene recovery is increasing. Local law enforcement agencies may lack skeletal experience or adequate manpower, or they may have prior experience in working with anthropologists and appreciate their involvement. Anthropologists can play a critical role in the recovery of evidence. The anthropologist is often better equipped to locate and map the scene of initial decomposition, to eliminate nonhuman bones, to identify carnivore or rodent activity on the bones, and predict the direction of travel for missing elements. They are able to recognize skeletal elements amidst an assortment of ground cover that often closely matches the bones in coloration and shape, ensuring maximum recovery. For example, collecting bones from a heavily forested area is often complicated as the bones have taken on staining from the leaf cover and are difficult to identify, especially since ribs resemble twigs and vertebrae may be concealed under the fallen leaves.

Since the anthropologist arrives with this knowledge and often with the equipment for recovery (Appendix, equipment list), it is often the anthropologist's responsibility to organize and conduct at least the recovery of the remains themselves. Once on the scene, it is not unusual to be handed the reins and asked, "What do you want us to do?" by the investigators present. Often it is the anthropologist, entomologist, or botanist's knowledge of evidence collection and mapping within their respective discipline that provides them with the skills needed to collect evidence for their scientific colleagues in other fields. For this reason, no anthropologist should be willing to do scene recovery until they have either extensive work on prior supervised scenes or have a strong background in archaeological techniques and the ability to quickly adapt these for the forensic setting. Equipment pertaining to scene recovery should already be assembled and kept up to date.

Scene Recovery in the Caseload

Instances in which we are called to the scene are only a small portion of the overall caseload. Among board-certified forensic anthropologists, for example, scene recoveries were

performed in only 27% of the total caseload (P. Willey, 1998, personal communication). This reflects the nature of our work. About a third of our cases are nonhuman elements. Many human bones come in as isolated specimens, such as an old anatomical skull found amongst the trash at the side of the road, a femur washed up on the beach, or a pelvis found by hikers who can no longer recall where they found it. Often human remains are recovered intact in relatively early stages of decomposition by the coroner's personnel. In some instances, the remains are fresh and the anthropologist is only asked to render an opinion on the skeletal trauma. At other times, no skeletal evidence remains and the assessment is based on photographs and the reports of others.

As it becomes apparent that irregularities in the collection of evidence can undermine an otherwise apparently solid case, law enforcement personnel are turning to the forensic sciences to assist in this initial stage of the investigation. Educational programs aimed at exposing more law enforcement agencies to what anthropologists, entomologists, and botanists can offer also increases the likelihood that they will call for assistance. Although jurisdictions take pride in their own units, the mobility of officers between locations, interagency training programs, and the demand for more sophisticated work even in rural areas has increased the spirit of cooperation.

Nature of Anthropological/Archeological, Botanical, and Entomological Evidence

The anthropologist is responsible primarily for the recovery of skeletal evidence. This includes the bones of the body, the various epiphyseal portions for subadults, and the dentition and ossified cartilaginous material. During scene processing, however, the anthropologist may be responsible for the initial recovery and documentation of all other physical evidence associated with the remains such as the soft tissue, expended bullets, shotgun casings or wads, ligatures, clothing, jewelry, medications, hair, and personal items. Insects affiliated with the decomposition, including maggots and pupae, also may be collected by the anthropologist or other trained field technicians, or they must assure that such collections are made if a forensic entomologist is not available. The responsibility of collecting botanical samples such as roots within the body and broken branches also may fall to the forensic anthropologist or entomologist in the absence of the botanist. Finally, less tangible things such as the degree of leaf cover, the shape and depth of the burial, digging instrument impressions, sunlight exposure, drainage, vegetation changes or root growth through the disposal site must be recorded and documented.

The tangible and intangible items all have a role in the interpretation of the circumstances of the death and disposal of the remains. Some will pertain to the identification of the individual; who he or she was, what type of life had he/she led, and what was his/her health prior to the time of death? Others pertain to how the victims died: were they killed, how were they killed, did they arrive at the disposal site under their own power, were they clothed at the time, and did they have personal items with them such as watches and jewelry? Still others pertain to what injuries were produced around the time of death. Other lines of evidence relate to the events of the postmortem interval: how long was it between the deposition of the body and its discovery, were the remains moved, was there predation on the remains? If this case comes to trial, all of these points must be investigated and the interpretations made on them shown to have strong evidentiary support.

Finding the Location

Scene recoveries can begin slowly, building from rumors of a body, or quickly by the accidental discovery of remains. Because these events may not be predictable, it is important to have the procedures well-established, equipment and forms at readiness, supplies on hand, and personnel contact methods in place.

Once the scene location is suspected, one of the first responsibilities is determining who is in charge of the search and possible recovery. A single law enforcement agent as supervisor should be in charge of the entire investigation (Morse et al., 1976; Stoutamire, 1983; Haglund et al., 1990). Initially, this person decides what areas will be searched and how, who will be involved, where the boundaries are, and when to call off work in an unproductive search. Once remains are located, this person determines the entry routes to the scene to be taken by personnel, what should be collected, and to whom it should be delivered. Obviously, since scene recovery usually involves a number of people and often a number of agencies, these decisions usually are made after discussion and consultation. For the forensic scientist coming into this arena, it is important to "scope out" the organization and internal politics of the scene (Killiam, 1990). All affiliated personnel who will be overseeing the anthropologist, entomologist, or botanist's work or assisting in the actual recovery should be recorded (name, agency affiliation, and contact information). Exchange of business cards often facilitates this process.

Methods Used to Locate Human Remains

Human remains are often discovered casually, such as by hikers, hunters, campers, etc. who happen upon them in the course of their normal activities (Hochrein, 1998). In some cases, homeowners will discover remains in undisclosed storage areas, the backyard, or basement. In both instances, recovery usually depends upon the recall of uninvolved personnel. In most cases of this type, authorities are almost immediately notified of the grisly find. Unfortunately, in some instances, people have collected recognizable portions of the remains such as the cranium or pelvis and kept them for variable periods of time before realizing their significance and identification of the original location may be problematic.

Another scenario for the initial discovery of a human body is from information provided by an involved person. This informant may be the perpetrator or be someone who has inside knowledge of a crime or inappropriate disposal of a human body. Often sufficient time has elapsed so that their memory of the exact location is poor. At the time of the crime, they may have reached the location in such a state of anxiety that judgment of distances is wildly inaccurate. For example, Rhine (1999) recalls one case in which the informants insisted that they had driven 10 miles down a dirt road, when in actuality the remains were only a mile or so off the main highway.

Such searches often demand considerable patience as well as an understanding of the thought processes involved in depositing a dead body or in the wanderings of an individual whose death was suicidal, natural, or accidental. When moving a live or dead individual, transportation becomes a critical issue. A dead body is heavy and unwieldy so that most remains from homicides or already deceased bodies being dumped will be located near some form of road. Covering the remains is often desirable, so thicker brush or ground cover is usually sought. Victims brought live to the scene also usually require some vehicular transportation, so they are found relatively close to a road, although they may be forced

to move farther before being killed than a dead body could be carried (Duncan, 1983). In some respects, those individuals whose disappearance is due to nonhomicidal circumstances may be more difficult to predict. Their bodies may be found in extremely remote areas or in the odd nooks and crannies of even the most urban of places.

Military situations, such as recovery of remains from the Vietnam era or earlier plane crashes, require more reliance on interviews with the local population. Craters left by plane crashes often replicate those left by other military ordinance and all may have since been converted to irrigation and fish ponds. Much of the plane may have been recycled into housing or farming instruments over the course of time. People also may be reluctant to discuss the collection of material from the crash or the fate of the occupants of the plane. Diplomacy and patience are required (Finnegan, 1995).

Once a general locale has been identified, the anthropologist, entomologist, or botanist may be able to assist in the determination of the exact site. There are scene indicators in each of these respective disciplines that probably would not be noted by the casual or untrained observer. Once the scene is found, a general walk-through or drive-by is useful to determine the terrain, the most probable extent of the scatter, and the best approach (Duncan, 1983). This is done by as few people as possible, and the pathway taken should be noted in the scene recovery records.

The location techniques differ due to the nature of the remains and the substrate. Underwater recovery will not be discussed here, as these activities require specialized training (Johnson and Steuer, 1983). Terrestrial scene recoveries can be roughly divided into those involving surface remains and those in which burial occurs. In surface scatters, the terrain may indicate how extensive the distribution will be. For example, in an urban area, a body found between two fences may be confined within a narrow area, while in a rural area, where there are large numbers of coyotes, the remains may be distributed over areas $^1/_2$ to 1 mile from the initial scene. Remains in or along a creek or river may have been transported much greater distances. In one instance, a body could be traced back to a river disposal after it was recovered in ocean waters (Ebbesmeyer and Haglund, 1994). Buried remains tend to be confined to the grave, although body position varies and burials are often disturbed by scavengers or those making the discovery.

It is customary to establish a series of perimeters at the scene with varying search intensities (Haglund et al., 1990). The enclosed areas are restricted to those investigators who have assigned tasks relevant to the case. The inner perimeter is typically an area 50 to 100 ft in diameter within which the remains are concentrated and should include the perpetrator's entry and exit routes. Within this inner area is a second perimeter within which the body is concentrated. There may be a third inner area in which the search is most intense, usually shoulder-to-shoulder spacing while crawling. The outer perimeter is an area that is larger than the crime scene so as to exclude press, potential witnesses, and sightseers. Inner and outer scene perimeters are roped off and secured. Boundaries of these areas can be effectively marked by flagging tape. Peripheral searches in the outermost areas are usually less intense and may only be used when substantial portions of the evidence are known to be missing. Uniformed and armed law enforcement personnel are well trained in keeping onlookers outside of these perimeters and maintaining scene integrity. No one smokes, eats, or drinks within or near the established crime scene perimeters. Doing so will contaminate the scene and may jeopardize the integrity of the case.

Locating surface remains will require a systematic search. When searching a small constricted area, a sector or zone search is recommended (Eliopulos, 1993). Relatively little

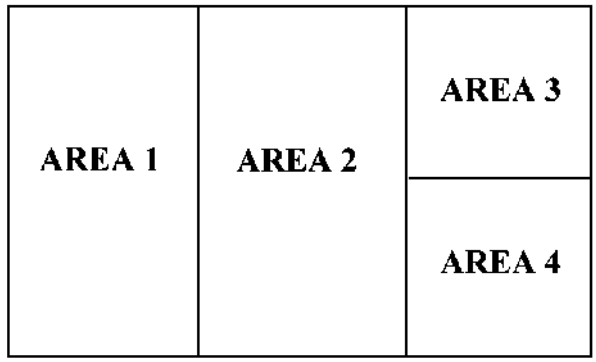

Figure 7.1 Example of a sector search plan. The area to be searched is divided into sectors which are designated by letter or number. (Adapted from Eliopulos, L. N. 1993. *The Death Investigator's Handbook,* Boulder, CO, Paladin Press. With permission.)

manpower is necessary for this type of search (Figure 7.1). Also requiring little manpower is a spiral pattern that begins at the main concentration of body parts (the center) and continues in widening circles moving away from the victim (Figure 7.2). Straight-line searches are used in very large scenes and when large numbers of people are available to search. This search technique requires that the searchers stand side by side and travel across the scene in a straight line (Figure 7.3). When little manpower is available but a large area must be searched, a line search can be used (Figure 7.4). Grid searches are usually rectangular areas (Figure 7.5) which cover the entirety of the general area. The scene is divided into multiple grid units and a search is made of each grid unit. A second search follows which is perpendicular to the first search, but within the same grid units.

Whatever the search technique, obvious accommodation is made for surface features (cliffs, trees, bodies of water, etc.) without ignoring the possibility that bodies, body parts, or other evidence may be located in poorly accessible areas. Intensity of the search varies by the visibility. When the ground cover is sparse, greater distance can be maintained between the searchers. If leaf cover is thick, closer spacing is used and the searchers may crawl while hand raking the ground cover to expose the underlying surface and examine between the leaves.

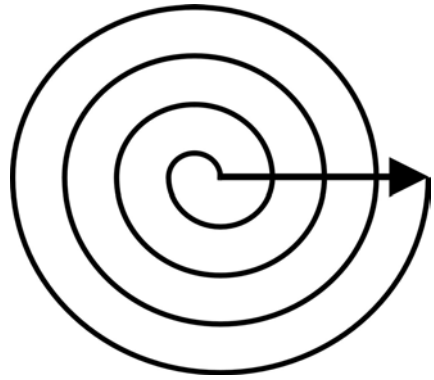

Figure 7.2 Spiral searches begin at the central location of the remains and move progressively outward. (Adapted from Eliopulos, L. N. 1993. *The Death Investigator's Handbook,* Boulder, CO, Paladin Press. With permission.)

Figure 7.3 Searchers working at approximately arms length can slowly work through leafy ground cover such as this outside of the innermost perimeters. As search intensity increases, distance between searchers decreases and they will need to work more slowly and closer to the ground. (Photo courtesy of Alison Galloway.)

Each searcher marks anything that is suspicious, usually using colored flags on thin metal strands. In addition to the obvious items such as bones, clothing, or weapons, other items such as animal scat which may contain bone fragments, trash items, and unidentified stains should be flagged. Searchers should be briefed as to what constitutes an item of interest prior to the onset of the search, especially on less obvious items such as small bones, insects and their remains, as well as vegetation damage or changes, and other signs of soil disturbance.

Color-coded flags may be used to delineate the type of material flagged. For example, orange flags may indicate possible skeletal material while red flags indicate associated physical evidence like a bullet jacket. Once all the material is flagged, the limits of the crime scene can be delineated. If there is excessive vegetation at the scene, as much as possible

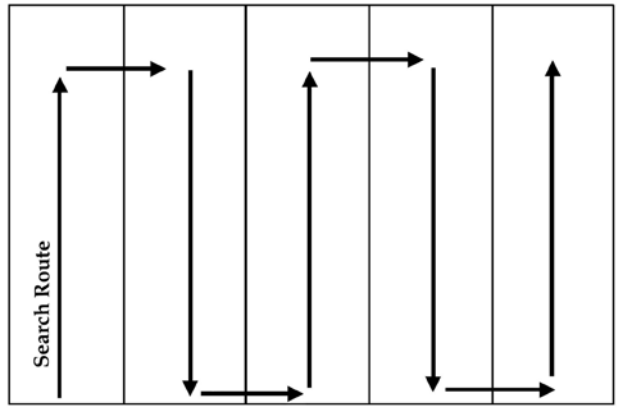

Figure 7.4 Line searches are conducted with searchers lining up and moving parallel to each other along the search route. (Adapted from Eliopulos, L. N. 1993. *The Death Investigator's Handbook,* Boulder, CO, Paladin Press. With permission.)

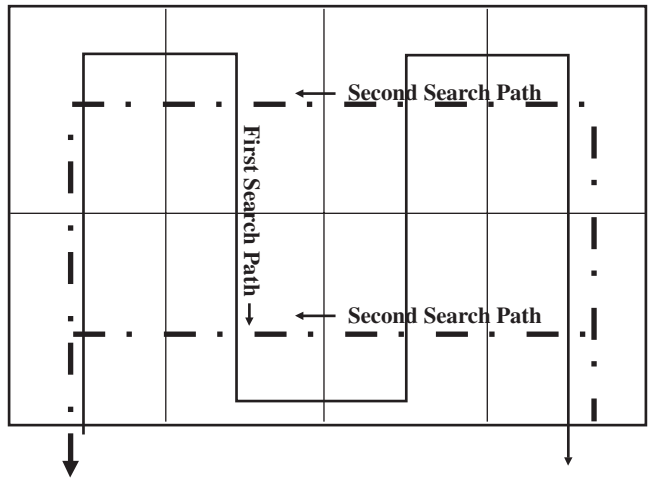

Figure 7.5 Grid searches begin with a strip search but follow this with a second search pathway which is perpendicular to the initial path. (Adapted from Eliopulos, L. N. 1993. *The Death Investigator's Handbook,* Boulder, CO, Paladin Press. With permission.)

is removed without disturbing the remains and associated physical evidence. However, this should only be done after the forensic botanist (or someone specifically trained to collect botanical evidence) has made all relevant collections and cleared the scene. All vegetation, except trees, is cut to within 1/2 to 3/4 in. of the surface, unless it will result in movement of evidence. This provides an unobstructed view of the entire scene. All vegetation that has been cut should be carefully placed in a suitable receptacle, removed from the cleared area, and examined to determine if the cuttings contain any further physical evidence. Upon completion of these operations, use a metal detector within and outside the grid (Figure 7.6). All positive recordings should be flagged.

Figure 7.6 A metal detector is useful for locating jewelry, metal items on clothing, as well as bullets and casings at the scene. (Photo courtesy of Alison Galloway.)

Since it is difficult to acquire enough manpower to conduct a search only with anthropologically trained personnel, it is useful for several trained people to be intermingled with the searchers. It is a good practice for the anthropologist, entomologist, botanist, investigator, and evidence technician to follow behind inspecting each flagged item, without moving it from its original location. This can allow for elimination of spurious evidence, such as large numbers of nonhuman bones, piles of animal feces without human bony inclusions, incidental or transient insect species associated with dung or decomposing vegetation, or naturally occurring botanical phenomena (oddly shaped sticks, decomposing wood stains, etc.). The remaining items are numbered in sequence, usually using standing plastic markers. It is not unusual to find that the entire search has been for naught in that the "body" consists of the disarticulated nonhuman remains of someone's or something's dinner.

Frequently the bulk of the human remains are located in one general area with various items transported beyond that region. As noted above, a much larger peripheral area may be systematically searched, but frequently similar results can be obtained by accommodating the search to the terrain. Game trails are obvious areas to follow as dogs, coyotes, and wolves tend to bring food away from the scene to dens or eating areas. Areas in which dens may be located should be searched and open areas are often favored as feeding areas. Nests of rodents also should be examined. Packrats, in particular, are frequent thieves of small bones that are used in the building of their elaborate nests. Birds may be attracted by glittering objects and store these in nearby nests, and they may incorporate hair into their nests (Pickering and Bachman, 1997). Finally, gravity may play a role with remains being moved downslope. Crania are notorious for "rolling away" and may be found in streambeds well below the rest of the body (Behrensmeyer, 1975).

It is often the case that a domestic dog out for its daily morning run returns with a human skull or leg (often complete with shoe). Increasingly investigators exploit this ability to locate remains by using cadaver dogs in scene investigation (Figure 7.7). These highly trained animals may be introduced in the initial search to locate the remains. Since even the general scene location is not known, cadaver dogs can be used to track along the most likely areas, such as paths of egress or entrance. Dogs also may be used following a walk-through search when smaller bones may still be missing, and are an excellent alternative when larger peripheral searches are needed. They often are useful in locating the initial area of decomposition, which may be marked by drainage of body fluids and a hair mass but often little else. This may be well masked by subsequent leaf fall or debris accumulation and effectively hidden from a walk-through search.

Cadaver dogs are usually trained with decomposing human flesh and bone although some "pseudoscents" are available (Tolhurst, 1991). Various breeds are represented and training must begin relatively early in the dog's life to achieve a well-trained animal. Trainers tend to be very cautious in the use of their animals, usually avoiding areas where they can be exposed to hazardous materials such as landfills, and preferring to work with them in the early morning when the animals are fresh and when the scents are much more keenly sensed. If the remains are covered or buried, dogs require more stringent conditions. Temperatures should be between 40 and 60°F, humidity should be 20% or higher, the ground should be moist, and windspeed should be at least 5 mph (France et al., 1992).

Buried remains typically occur as the result of human activity, such as legally sanctioned burial or the illegal burial of a crime victim, or by natural deposition (e.g., covered

Figure 7.7 A cadaver dog, such as the one shown here wearing a brightly colored coat, is useful in searching large areas for scattered remains. (Photo courtesy of Alison Galloway.)

by landslide). Buried remains usually are more confined and less obvious. One should expect that a clandestine grave will be irregular in shape and shallow. A hastily dug grave used to hide a crime victim is not going to be the classic "grave" (i.e., rectangular in shape and 6 ft deep). Initially, evidence of animal and insect activity may give clues as to the location of the remains. Animals are often drawn by the smell and will dig down into a grave, even exposing and removing portions of the body. Also, the body may have been dragged out of the grave. Flies also may be attracted to completely covered bodies with shallow internment. Aggregations of flies may be seen landing on the disturbed ground, or often clusters of small flies may be seen hovering above the gravesite. These flies are attracted to the decomposing odors emanating from fissures in the soil, and often may be found months after the remains are buried.

Burial location may be discerned by observation of differences in vegetation, soil compaction or slumping, disturbed soil (Figure 7.8). As the decomposition process continues, soft tissue will be lost and the resultant voids will be filled by overlying soils (Duncan, 1983). Rains also help dissolve the loose clods of dirt and fill the areas between the dirt clumps. The result is that the initial mound of dirt will subside leaving a slight depression. Cracks may appear as the disturbed soil pulls away from the undisturbed soil outside the grave. Water may accumulate in this area and allow for more moisture-loving vegetation to develop.

During the first year after burial, vegetation may be different in areas over the grave (Duncan, 1983; France et al., 1992; Owsley, 1995). This difference is due to the disturbance of the soil and the ability of the seeds to take advantage of the cleared and loosened soil. In most cases, seeds are available in the soil at the site, which have likely been deposited during the previous years. Additionally, seeds from plants currently fruiting can be blown by the wind or otherwise transported to the site. In some cases, no seeds are available due to placement of the soil originally located on or near the surface too deeply in the grave pit to allow for seed germination. Seasonality and exclusion of light from the soil surface also are factors that may cause the soil surface to remain bare of vegetation. The number of plants inhabiting a disturbed area such as a gravesite can vary from sparse to dense, but

Figure 7.8 A burial depression of a shallow grave approximately 8 months after the remains were placed is seen as a dip in the surface and, in this case, by a scarcity of vegetation due to destruction of the original ground cover. (Photo courtesy of Alison Galloway.)

it is usually recognizable as being different from the surrounding vegetation. The inhabitants usually seen are those recognizable as "weeds" by botanists and gardeners.

Following the arrival of these plants, the growth may occur faster and the existing vegetation may appear greener as the less compacted soil allows moisture to accumulate and penetrate farther. Seasonal differences in revegetation were noted in one study by France et al. (1992). In dry areas, revegetation was delayed so that the burial excavations persisted virtually unchanged until after the rains returned. In contrast, in moister times, the soils broke down rapidly, masking pit edges. The soils became more fine-grained and compacted. France and associates showed that the actual nature of the initial revegetation depends largely on the seed sources in the immediate area and the microtopography. After about 3 years, it is the disturbance itself rather than the presence of any decaying remains that controls the vegetation differences. These differences are seen to persist for many years after the burial.

The area around the grave also is trampled during digging, and plants may be flattened, branches broken, and leaf cover disturbed. Soils removed from a grave are usually piled adjacent to the hole and mix with the preexisting materials. Rarely can the soil be completely returned to the grave and loose dirt will be spread over a wide area. Burials in areas that are cultivated are more difficult to detect (Duncan, 1983). Typically depressions will last only for several months. Where the soil is fine-grained and loose, the depressed area may be slightly more visible. Where the dirt tends to be broken into large clumps, all traces are quickly obliterated. Flood irrigation may highlight the excavation since the more deeply

disturbed soil can catch the water, cause additional subsidence, and remain damp longer. Fortunately (or unfortunately), shallow burials in cultivated areas are often discovered in the course of plowing and disking, superimposing massive postmortem trauma over any perimortem injuries.

In sandy soil, burials tend to be shallow, as the grave walls cannot be maintained to a great depth (Duncan, 1983). Excavation also is limited by the water table at or near sandy beaches. Cadaver dogs may be particularly helpful as the porous sands allow decomposition gases to escape.

Aerial photography can be utilized in locating graves (France et al., 1992). Changes in vegetation growth, soil marks due to the excavation, settlement, and subsidence of the ground are seen best in low angle sunlight which emphasizes surface texture. Therefore, early morning and evening photography is best when the long shadows highlight even minor discrepancies in topography. Areas where there is new luxuriant growth can be more prominent from the air and may indicate disturbance of the soils.

Probes may be useful in locating graves. These are inexpensive thin metal rods that are attached to a crossbar (Owsley, 1995). Diameter is usually about $^1/_2$ in. and they can be extended to 4 to 5 ft. The probe is pushed into the ground with the crossbar being used to continue the intrusion. In most cases, even if the topsoil is relatively loose, resistance will noticeably increase at 1 to 1.5 ft below the surface. When the area has been disturbed, however, as in the burial of a body, the probe can easily be inserted to its maximum length. Additions of sensors can increase utility by monitoring changes in gases, soil pH, and subsurface temperature. The operator should be familiar with use of the probe and should "take the feel" of the area in a location away from any suspected burial before beginning to probe. Systematic probes over a large area can be useful in locating single or multiple graves. Probes also may help release odors that can be detected by cadaver dogs.

Ground penetrating radar (GPR) may be used in the location of burial sites (Miller, 1996). GPR provides both vertical and horizontal information on subsurface disturbances. The equipment is able to detect soils that have been disturbed since these are less dense and have a different mixture of soils with different moisture contents and electrical properties. Larger objects are easier to identify and the radar will not penetrate metal so this is readily apparent.

GPR is useful for broad, flat areas with little vegetation since the mobile unit rides on a sled that must be pulled over the surface of the area. Large grids may be established and then the unit drawn back and forth over the entire area. It has some limitations, however. GPR requires a machine operator familiar with the general nature of the local soils. Rocky and rough areas are difficult to analyze and soils with many natural inclusions may be difficult to interpret. Since the GPR cannot absolutely identify human remains, it will not prevent fruitless searches. Recently one author (Galloway) was involved in a search in which about 10 to 15 ft of overlying roadway was removed by backhoe in search of a buried body reported by an informant and in which GPR confirmed some disturbance. After considerable effort by the law enforcement agency, the resulting finds consisted of one artiodactyl bone, one dog bone, and one turkey bone.

Scene Constraints and Integrity

When the location has been established, whether the anthropologist was involved from the start or has only been called as human remains are located, the proper authorities must

be notified. Sometimes the local law enforcement agency will call in a forensic anthropologist, entomologist, or botanist but neglect to inform the coroner/medical examiner that human remains have been located. This leads to later conflicts over the human remains when the authority of the coroner's/medical examiner's office is effectively side-stepped. The situation may be confusing in some jurisdictions where both a coroner and medical examiner system operates. It is important to be aware of the state of affairs in terms of responsibilities prior to beginning work.

Obviously the law enforcement investigators should remain, along with their evidence technicians. It is also important to have uniformed personnel from the law enforcement agency with the recovery team, usually placed at the entry points at the outer perimeter. At times in high-tension situations or when the alleged perpetrator(s) may still be at large and potentially threatened by the recovery, having armed personnel with the recovery team is important as well.

Recovery Constraints

At the outset, it is useful to survey the constraints under which the recovery process will be conducted (Skinner and Lazenby, 1983). In some cases these will be financial where the requesting agency must support the anthropology team's time and expenses. Fees for recovery services vary considerably among forensic anthropologists with some charging by the hour, some charging a flat fee regardless of the time, and others donating their efforts to community service. Out-of-pocket expenses should be covered in any event by the requesting agency.

Unlike archaeological excavations where weeks to months may be allocated for a "dig," a forensic scene recovery normally is given less time despite its more critical nature. There is a fine balance of trying to work quickly while providing the utmost in documentation and recovery. At the beginning of the work, provide the agency in charge with an overall estimate of how long it will take to complete the work, barring unforeseen difficulties. This will allow the agency a chance to arrange for other contingencies such as meals, hotel accommodations or campsites, toilets, guards for times when the scene is left unworked, lights for night work, etc. Avoid attempting to work nonstop on a scene as all workers must be alert and attentive to detail, which is impossible to maintain without rest, food, and breaks.

The terrain and climatic conditions also may provide a number of constraints (Skinner and Lazenby, 1983). In northern climes, heavy snow cover or permafrost may prevent recovery efforts until there are more favorable conditions. In other cases, the need for additional safety equipment or specially trained personnel may necessitate delaying the recovery process. However urgent the recovery may appear, it is not worth risking the lives of the investigators. The presence of hazardous material (Galloway and Snodgrass, 1998) and unexploded ordinance are additional concerns, particularly in military or paramilitary situations.

Because it is important that the forensic anthropologist derive his or her interpretations directly from the skeletal evidence, it is advisable to avoid any information about the scene that may influence this determination. For this reason, it is best to limit conversation about the possibilities of who the body may be or how long the person has been missing. This is often difficult as the remains may be linked to a high-profile missing person, or the local law enforcement may already suspect the identity, or evidence found at the scene (purse, wallet, credit card) may point to a specific individual. If the agencies

are aware of the anthropologist's preference to remain "in the dark" about these suspicions, they usually will respect this if assured they will get a field assessment of the biological profile as soon as possible.

Controlling Access, Paths of Travel, and Scene Photography

Once the crime or body disposal scene is identified, it is important to minimize damage due to trampling. The perpetrator's route may contain evidence of their passage such as footprints, threads of clothing, hair, or discarded trash (i.e., cigarette butts, soda cans, etc.). Repeated passage would obliterate or dislodge these forms of evidence. For this purpose, it is customary to establish a line of entry, one route that is clearly marked by which all personnel enter and leave the scene. Brightly colored flagging tape is usually used to mark this route. In some cases, it is helpful to bring in long planks to use as walkways (Haglund et al., 1990). This may not be the most accessible route since the easiest way has often been the one used by those who placed the body in the area in the first place.

Written logs should be maintained for all personnel entering and leaving the scene. Similar logs are kept for all evidence found, recording how and when it is transported from the scene. This allows for a more accurate reconstruction of who was present when specific items were recovered and who could, potentially, be witnesses to the condition in which these items were recovered and what happened to them during the recovery process. These logs are but the first of a series of steps in the paper trail that should accompany the scene recovery. Often a 24-h clock (military time) is used to prevent confusion as to when specific events occurred. All of this information will be subject to subpoena if the case goes to trial.

Photographs should be made of the area that assist in explaining what happened as well as how it happened and when it may have occurred. While the scene is in as close to pristine state as possible, photographs are taken of the scene from 360°, incorporating major landmarks (e.g., road signs, bridges, buildings, etc.). If the scene must be cleared of vegetation for investigation to proceed, continue to photograph the scene changes and evidence as it is collected. Pay attention to the vegetation surrounding the subject of investigation (remains or physical evidence) to support the botanical evidence. Macro-photography of the vegetation in the immediate proximity to (or in contact with) the remains or associated physical evidence should be conducted as well.

A number of different formats are currently available with which such images can be recorded. Ideally, a dedicated single-lens reflex camera always should be available for fieldwork. Also necessary are either a set of lenses (35 mm, 50 mm, 100 mm, macro) or a macro-zoom lens. Photographs, usually 35 mm, can be produced as either prints or transparencies. While the former is often easier to incorporate into a report or use while discussing a case, the latter has the advantage of being useful in presenting to a larger audience such as the jury. It is now relatively easy to either photographically or digitally convert slides to prints. Traditionally both black-and-white and color films were used. Largely this was to minimize the expense of producing color prints and to provide material for publication. Now, however, color prints are much easier and cheaper to produce than black-and-white. Coupled with the ability to digitally convert color to black-and-white prints, the need for both formats is fading. One cautionary note is that reliable labs should be cultivated for processing this film due to both its sensitive and its graphic natures.

Digital cameras provide rapid collection of images, similar to Polaroid cameras, which can be readily transferred to the computer. The advantage of this format is that multiple images can be taken, provided there is sufficient storage capacity. Some digital cameras will even accommodate a floppy disk. A note of caution is that digital images are easily manipulated; therefore, their evidentiary quality is questionable. Some film versions should always be taken to document the accuracy of the digital work.

Video cameras also are increasingly used to document the sequence of events in the recovery process. These also may be used for training films and for other general audience purposes once the case is adjudicated, although the film quality of most hand-held cameras is relatively poor in the large format of a projection screen. Videos do allow the recovery process to be described as it is being produced and is particularly useful in complex cases where stratigraphy or taphonomic changes are more easily seen "in the field" than in charts or still photos.

The press often appears at the scene as quickly as the forensic anthropologist, entomologist, or botanist, if not sooner. In the authors' experience, the law enforcement agencies are becoming much more efficient in controlling the access to crime scenes and feeding information to the press via a spokesperson. In most cases, they will prefer that any information pertaining to a case be released via this method but, in some circumstances, may wish that one of the forensic scientists provide some air time. This approach is most often used when a body is unidentified after a period of time. Discussions about what will be said should occur behind the scenes and these specialists should consult with the agency before agreeing to do any interviews.

At the crime scene, media coverage can be extensive. Camera crews are able to provide close-up shots from great distances such as from homes overlooking the scene, or from hovering helicopters. Similarly, microphones can pick up conversations well beyond normal hearing. Since actual coverage of the human remains can be excruciating for the relatives as well as damaging for the case, it is best to hide the scene where possible with screens or covers and avoid overly loud conversations. The extent of coverage may become a factor in dictating the speed of recovery as added time can increase the press determination for more graphic footage.

Establishing the Location

As obvious as it sounds, one of the most important tasks early in the investigation is to determine where you are. When the written report is provided to the attorneys handling the case, they should be able to use it to return to the scene. This does not mean providing directions such as "take Highway 1 north to milepost 26.3, turn west …" but rather "the scene is located approximately 500 m due west of milepost 26.3 on Highway 1." The use of recognizable markers such as street addresses, road names or numbers, mileposts, and natural or architectural landmarks is helpful. An overview map placing the scene within this wider context can be useful (Figure 7.9). Computer map indexes are available to assist in this project.

GPS (global positioning system) allows for the location identification, often to within about 5 ft. Handheld or computer-linked systems are now available at a relatively low cost which enable the field researcher to locate the scene onto a topographic map or a computer atlas. Unfortunately such systems do not always function as desired due to the availability

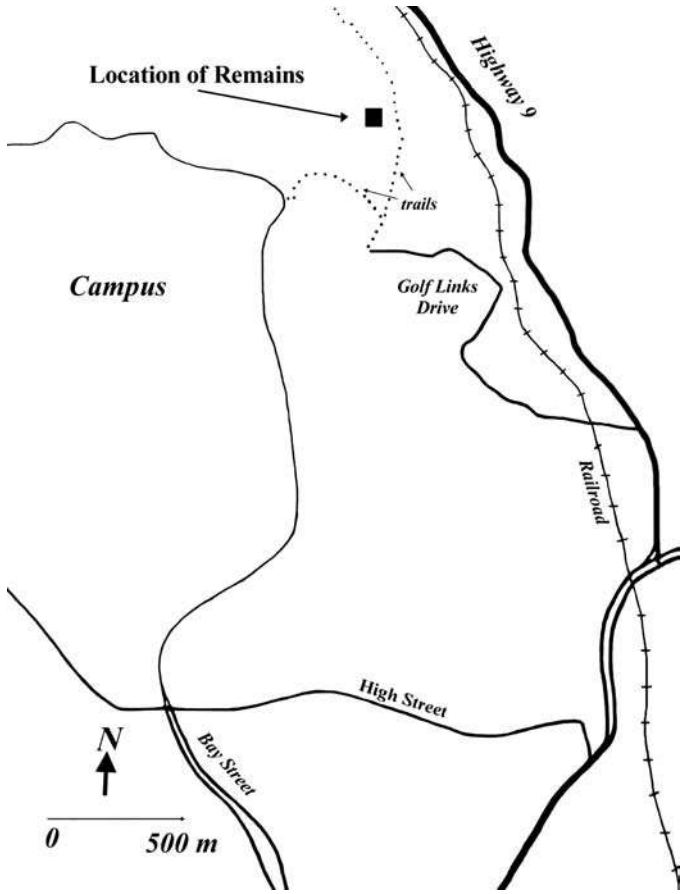

Figure 7.9 The overview map shows the location of the crime scene in relation to easily recognizable landmarks such as highways, towns, and named roads. (Figure courtesy of Alison Galloway.)

of adequate number of the satellites. GPSs are, however, ideal when working in remote areas as often the law enforcement investigators can locate the site only on the ground, which has limited use in producing the written report. However, it also must be noted that due to the "white noise" purposely introduced into the GPS units produced for public use, the signal will drift causing the recorded GPS position to vary by approximately 50 yd. Therefore, GPS units should be used in setting the datum, but are not suitable for mapping the relative location of each piece of evidence. These items should be mapped by running a tape measure from the datum to each item.

Once the general location is determined, a prominent feature at the scene is often used to provide a link between mapping scales. For example, a house may be identified on the overview map by address, then the northeast corner of the house is used to show the location of the remains (Figure 7.10), and, as importantly, the datum or data points, the point or points from which the scene is mapped. It may be helpful to think of these layers of maps as providing (1) a means to drive to the scene (overview map), then (2) the route to walk from where you are parked to the actual scene (linkage map).

In order to map a site in greater detail, a datum must be established. All other locations at the scene are tied to this point. The datum must be something which has more perma-

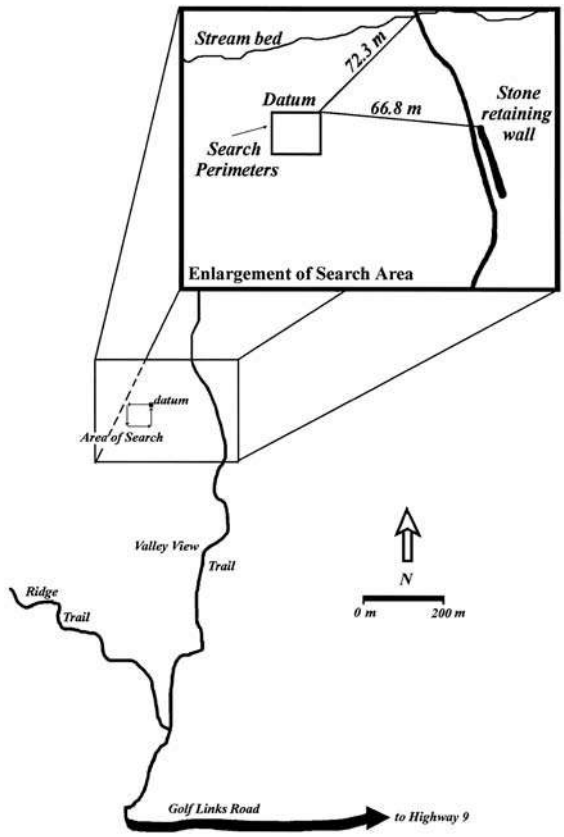

Figure 7.10 The linkage map shows more tightly defined areas and indicates the datum in relation to identifiable permanent landmarks in the local area. (Figure courtesy of Alison Galloway.)

nence than a simple flag or other location marker and it must be clear of any excavation area. Buildings, large boulders, trees, drainage structures, or communications towers and poles are often used. Structures such as the corner of a building or an electrical pole are excellent because these fixtures are usually documented in local government records. If there is no suitable item, a stake may be driven into the ground or the surface painted to denote a spot. A metal stake buried a few inches in the ground can mark the spot and can be easily found later with a metal detector. The indicated spot is then linked to other identifiable features through use of a tape measure and compass or by taping the distance from two other identifiable features (triangulation). Since often the measurements are taken over uneven terrain, the tape measures should be maintained at a horizontal level with a plumb bob used to mark the point to which measurements are taken (Figure 7.11). The datum should be established so that at least the bulk of the site can be covered from a single datum point. The use of multiple data points may be required in large scenes. Because the datum also serves as a depth indicator, it should be on a higher level than the scene and a recorded point on the datum used as the depth reference. The distance from this point to the ground is recorded and subtracted from subsequent depths to produce a profile. The datum should be photographed with a north arrow visible and the direction of the photo indicated in the log, or otherwise recorded.

Figure 7.11 When measuring over longer distances, a tape is kept as close to horizontal as possible with a plumb bob being used to sight the artifact or skeletal element being mapped. Where the plumb bob crosses the tape will indicate the distance. (Photo courtesy of Alison Galloway.)

Should all permanent fixtures be far from the site, a subdatum can be established between the remains and the nearest permanent fixture. The exact location of the subdatum should be documented the same as for the datum. Documentation should include exact distance and compass direction from your datum (Figure 7.12). Often long distances are not drawn "to scale" but are marked by discontinuities in the lines between points with the actual distance noted to the side.

Once the datum has been established, the excavation grid is created. However, a grid may be foregone with a simple site where the remains are in a confined area or in a simple grave. The grid should include all of the bones and associated physical evidence. Usually a 3×4 m^2 unit is sufficient. If scattering is severe, a larger grid or several smaller grids separated from each other may be necessary. Establish a meridian by laying a north/south line if possible and then lay a baseline (i.e., the east-west reference line). These lines should create the outermost borders of the grid (Figure 7.13). To ensure the grid is symmetrical, make sure that the corners form right triangles. This can be accomplished by using a large drafting triangle, a "3-4-5" triangle or the basic formula for the length of the hypotenuse of a right triangle ($x = \sqrt{a^2 + b^2}$ where a and b are the length of the sides of the triangle). A wooden or metal stake is placed at each corner and connected by rope and the corners are mapped in relation to the established datum. The elevation also should be measured for all four corners in order to establish ground level of your site in relation to your datum. Then, using more rope and stakes, establish transects within the grid and parallel to your meridian and baseline. These crossbars of the grid should be established at distances that best suits each particular site; however, 1 m units are commonly used. Identify grid unit

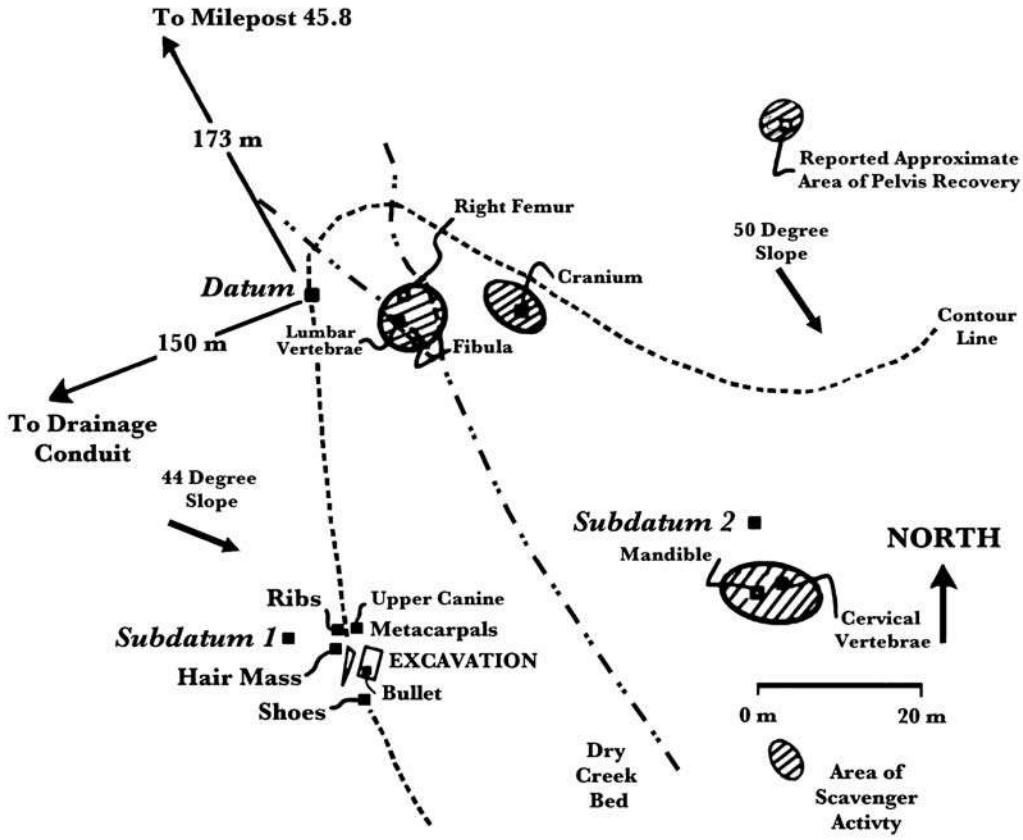

Figure 7.12 Map of scattered remains with subdatum, and discontinuities marking long distances. (Figure courtesy of Alison Galloway.)

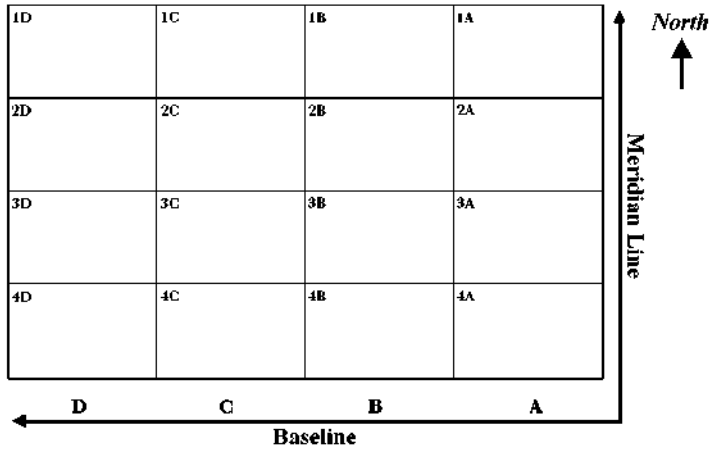

Figure 7.13 Search and excavation grids usually are laid out along a north-south axis with each square being identified by a number and a letter. All items found within one grid are labeled with this designation. (Figure courtesy of Alison Galloway.)

coordinates by using letters in the E-W direction and numbers in the N-S direction (or vice versa). This will provide quick identification of a grid unit.

Photograph the implementation of the grid at each stage, beginning with the first axes through its completion and excavation. Take general shots across the area with datum included and indicate on the photo log the direction from which you were photographing (Appendix, photo log).

Depth measurements should be taken across the grid. This allows a reconstruction of the topography of the site, such as the presence of creeks, cliffs, caves, or gullies. These features may help explain the movement of some items, which would not be understandable if the area was depicted as a flat surface. Sometimes irregular topography does not easily permit accurate depth measurement, so access to more formal mapping equipment, such as a transit or total station, is necessary.

Forensic Excavation and/or Recovery Techniques

The recovery team should aim for completeness in recording so that the original layout of the scene can be reconstructed as well as how the actual recovery process was approached. Once the recovery is completed, this information cannot be retrieved, so it is essential that it be done correctly from the start (Skinner and Lazenby, 1983). Evidence often goes beyond the scope of being physically retrievable. Important information may lie in the position of the body, its location with regard to other physical features, the relative intensity of sunlight, etc. Evidence may even be less obvious. As Skinner and Lazenby point out, scorching of nearby trees can support claims that a body was burnt in a location, and absence of such scorching would argue against such an event. To simply collect the physical remains of the victim without recording the context could substantially damage the case.

Before mapping begins, determine the spread of the remains and the scale of the drawing. Designate a notetaker, mapper, and photographer and make sure they are familiar with the equipment and recording forms. Include a scale on the map from the start and familiarize all personnel with that scale. Multiple drawings at different scales may be necessary in order to accommodate all the details. The positioning of the body in relation to magnetic north should be recorded (e.g., the head points SE, the feet point NW). Then placement of the head, hip, knees, feet, shoulders, elbows, wrists, and hands should be carefully mapped. Ensure that the remains and all physical evidence are photographed *in situ* and within the grid. When each item is mapped the following information should be recorded on data sheets (Appendix, evidence log):

- Specimen number
- Description of item
- Grid unit in which the item was found
- Distance from the datum
- Compass direction in degrees (in relation to the datum)
- Elevation/depth

Voice-activated tape recorders are often useful in providing more complete detail than can be easily done on paper where dirt, wind, and biohazards interfere with moving between excavation and paper recording.

Photographs should be made frequently during the process of excavation. Inclusion of a photo board with the case number and date is helpful for later establishing the case identity. Cameras that include a date stamp also allow for some validation of the association. The victim and associated physical evidence must be photographed from many different directions. In general, when taking wide angle shots of the subject, try to include a previously noted landmark for scale, or some other object of known size and dimension. If possible, include a metric scale on any macro shot. If this is impractical, take a larger photo with a scale to encompass the area later taken in extreme close-up. Additional specimen photography most likely will be necessary at the morgue or laboratory.

Surface Remains Recovery

In recovering surface scattered remains, each item must be numbered, photographed from above with a scale and north arrow in the photograph, and mapped onto the scene map. One useful item that assists in mapping is a large 360° scale that fits over the datum point. Using a compass, orient this to north, then the entire scatter of remains can be plotted by running a tape measure from the datum to the item to be recorded. If this is not available, triangulation from two permanent points is used to place the location of the remains on the scene map.

Notes should record the position of all skeletal items, orientation, and degree of articulation. The loose leaf cover and topsoil may contain loose bones, jewelry, hair ornaments, ties, insect fragments, botanical evidence, or other evidence that could easily be lost if this layer is simply discarded. Such small items will often be covered over and may "self-bury" in that the lighter soils will be washed over them and the evidentiary material will sink into the ground. Use of a metal detector is extremely helpful in such scattered scenes. A version that can distinguish between ferrous and nonferrous material is the most practical as often bodies are deposited in areas already extensively used as trash sites. This type of machine will ignore the odd bits of barbed wire, rusted auto parts, or wrought iron but will allow location of bullets and jewelry that could otherwise be easily lost.

Once photographs and notes are completed, the remains can be removed. Relatively complete remains are usually placed in body bags, with a body board or plank placed underneath to provide support and minimize shifting of the remains. Labels should include case number and the contents. Once the remains are lifted, it is often helpful to excavate several inches below the remains to recover any small bones, teeth, and physical evidence that have fallen from the body (Lipskin and Field, 1983; Wolf, 1986). This loosened soil should be screened.

Because remains are often scattered over a wide area and there is always the possibility of multiple victims present, running notes on the inventory are helpful. Charts are useful for a quick reading of which bones are still to be recovered. These charts also allow the anthropologist to record which portions of a bone are recovered and may prevent some confusion, such as when two right proximal tibial fragments are found which are actually portions of one bone rather than of two individual bones.

Buried Remains Recovery

In the recovery of buried bodies, it is helpful to include an archaeologist with your recovery team (Morse et al., 1976), or to have significant excavation experience yourself. Excavations may require some additional manpower to assist with the multiple tasks of digging, remov-

ing soil from the site, and screening, as well as mapping and photography. However, usually only about three to four people are needed to work a single grave and excessive manpower becomes unwieldy. It also is important to be aware of local conditions. Excavation techniques that work well in desert areas may be inappropriate in waterlogged areas where lowering the water level becomes critical.

Once the prospective burial site is located, the area should be carefully photographed. Depict the overall area prior to and after the removal of soil and vegetation. As exhumation begins, show any sign of possible tool used by the grave digger during the original interment. Be sure to depict the sunken soil and soil color changes. Depth markers (meter or yard stick) and a north arrow should be included in each photo. Notes should be made with regards to vegetation, condition of the soil, contours of the presumed pit, odor and insect activity, and signs of disturbance such as animal excavations. As scavengers may partially disinter and scatter the remains, the recovery should include a surface search, followed by excavation of the grave (Lipskin and Field, 1983).

Grids outlined with tightly drawn string tied to large nails are established to enclose the entire visible pit (this can be expanded if needed). It is best to use two nails to form each corner so that the nails are not positioned directly on the edge of your plot where they can be undercut and prone to dislodging (Figure 7.14). Again photographs should be taken after the vegetation is removed. Loose leaf cover over the grave should be carefully examined. This layer also may conceal footprints or other evidence that may be useful for the investigation.

Often the outline of the grave can be estimated at this point. The edge may be highlighted by lightly tracing the line with the point of a trowel. In some cases use of a

Figure 7.14 Large stakes or nails are used to form the string grid over the area to be intensively searched or excavated. Offsetting the nails prevents undercutting during excavation or clearing of topsoil. String should be set close to the ground to prevent tripping. (Photo courtesy of Alison Galloway.)

Figure 7.15 Artifacts such as pieces of jewelry (above), bones, or other evidence is mapped by taking readings perpendicular to two of the grid lines. These are then drawn to scale on the site map. (Photo courtesy of Alison Galloway.)

short probe can be helpful in determining the edge, working from the outer nondisturbed soils in toward the grave. This also can be used as a reference for modifying the placement of grids and the excavation approach. It should be remembered that graves are often irregular in shape, having been dug in haste, with poor equipment, and in awkward circumstances such as under low-hanging branches (Wolf, 1986).

At this point the map of the excavation usually consists of the placement of a grid on the linkage map and a separate map of the grid. This second map should be at a large enough scale that legible notes can be made as to the identification of items and depths. As each item is found, map its position with the tape measure using the two grid sides to measure *into* the grid with the tape measure (held horizontal to the contiguous line from which the measurements are being taken) (Figure 7.15). Indicate the grave outline on the map and any exposed items or bones. Depth measurements also should be taken of the pit and the surrounding area. This allows the grave and, eventually, the remains to be shown in profile. To obtain depth measurements, a line level is attached to the string from the datum. The string is held taut over the item or area to be measured and tape measure used to obtain the distance between the string and the surface (Figure 7.16). Be sure to note how far above the ground the string attaches to the datum.

Because of the danger of contamination, loose soil is then removed from within the grid. First, any soil that appears to have been displaced by either animal predation or human discovery should be taken and screened. Then loose soil from around the presumed site is cleared and screened. Finally, the loose soil within the grave outline is swept up and screened.

Photograph frequently during the excavation process. Photos should be identified as to the case and orientation and recorded in the photograph log. Autofocus cameras are often useful for overhead shots since they can simply be held out and the photograph taken

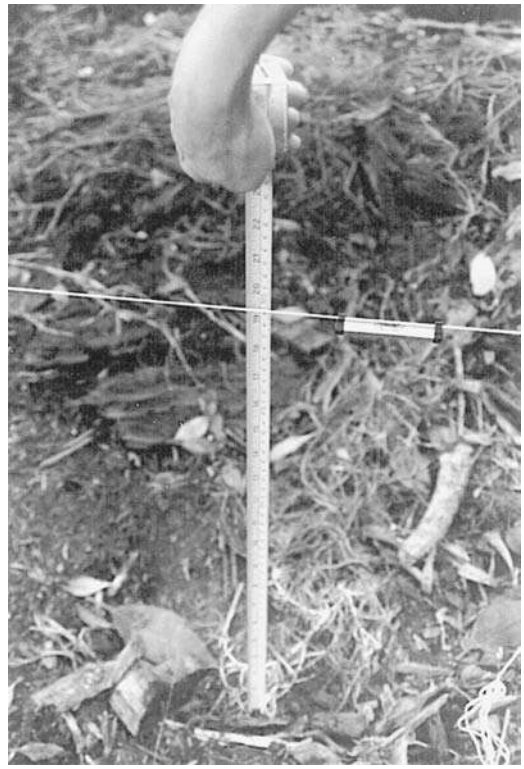

Figure 7.16 Depth readings are taken using a string from the datum. A tape measure is held vertically and the reading taken where the leveled string crosses the tape. Placing the level close to the string allows one person to level and read the tape and prevents false levels which can occur when the level is at the midpoint of the string. (Photo courtesy of Alison Galloway.)

without requiring the operator to view the image. Some shots also should be taken without any identification that can be used for public presentations after the case is adjudicated.

At this point excavation may begin but the approach often depends on the anticipated depth of the burial. The aim is to preserve as much of the pit outline as possible without endangering the remains or associated evidence. In shallow graves, it is possible for excavators to work from the surrounding areas by leaning into the pit. As the depth increases, however, leaning into the pit will endanger the sides and there is the danger that either the sides or excavator or both will fall into the grave, possibly damaging the remains. In these cases, parallel pits are usually advisable so that the excavators can work at a safe and comfortable level with relation to the remains and complete full exposure of the body prior to removal of any portions (Figure 7.17). While this will eventually destroy one side wall, the outline of this wall should be recorded as the excavation moves downward. The side pit also facilitates the removal of the remains by providing space in which a support plank may be placed onto which the body is moved.

Excavation should proceed in levels of approximately 10 cm within the grave. Hand trowels are used in a "scraping" manner to cut off broad, thin layers of dirt. Frequent brushing exposes features. If possible, it may be useful to excavate only half of the grave (lengthwise). This exposes a profile of the feature, showing the pit length and any disturbances within the soils overlying the body. Often, however, the pit is relatively small and

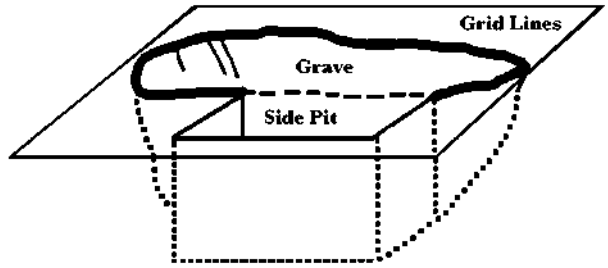

Figure 7.17 In excavations that exceed the excavator's ability to work safely from the edge, a side trench is dug. While sacrificing the integrity of one wall, this prevents edge damage from leaning into the grave site, the danger of falls into the pit, and facilitates removal of the remains once excavation is completed. (Figure courtesy of Alison Galloway.)

adherence to archaeological standards are of less practical use. Screening, of course, should be used for all the soils because small bones, entomological, and botanical evidence (i.e., grass, leaves, and broken twigs interred while filling of the grave) can be found at all grave depths. In particular, adult insects emerging from their pupal cases will burrow up through the soil in order to reach the surface. Since many insects die in their attempt to reach the surface, it is not unusual to find adult insects distributed throughout the soil between the body and the ground surface. Be careful to place the screening area well away from any potential excavations. For instance, if you find that the body is in an unusual position, the grid may have to be expanded necessitating moving the backdirt you had already accumulated.

The edges of the pit can be "felt" with the trowel or digging implement. Because this outside soil has not been disturbed, it is more densely compacted making a noticeable difference in the difficulty of digging (Pickering and Bachman, 1997). Experience in excavation teaches this. If the anthropologist lacks any archaeological training, it may be useful to take an archaeological field class if only to acquire the skill of feeling the differences in the soil. Careful excavation along with light brushing can, in more clayey soils, reveal indications of the instruments initially used to dig the grave (Hochrein, 1998). The curved profile of a shovel, the narrow band of a pick, the repeated grooves of a gardener's trowel can all be retained in the pit outline.

Once a bone is exposed, the body can be followed using careful excavation techniques. Many texts advocate switching from trowels to wooden or plastic implements. Where practical, this should be followed but, in many areas, reliance on wooden implements would be an exercise in futility — you could not move earth. Use of smaller tools (dental picks and probes) and frequent brushing of the surface are essential.

All remains must be mapped in three dimensions (Figure 7.18). This means that measurements are taken not only from the sides of the grid, but also depth measurements are taken from the datum. This information permits a scene profile to be drawn. As described above, bring the string with the line level taut across the vertical tape measure. For large objects, such as the skull, measurements are taken on the top and bottom at a minimum, and unusual orientations may require additional measurements.

As much as possible it is best to expose the entire scene for photographs prior to the removal of any of the body. In fact, hasty removal of the remains can be one of the primary errors in scene recovery and it can be safely lifted only when its relationship to the other

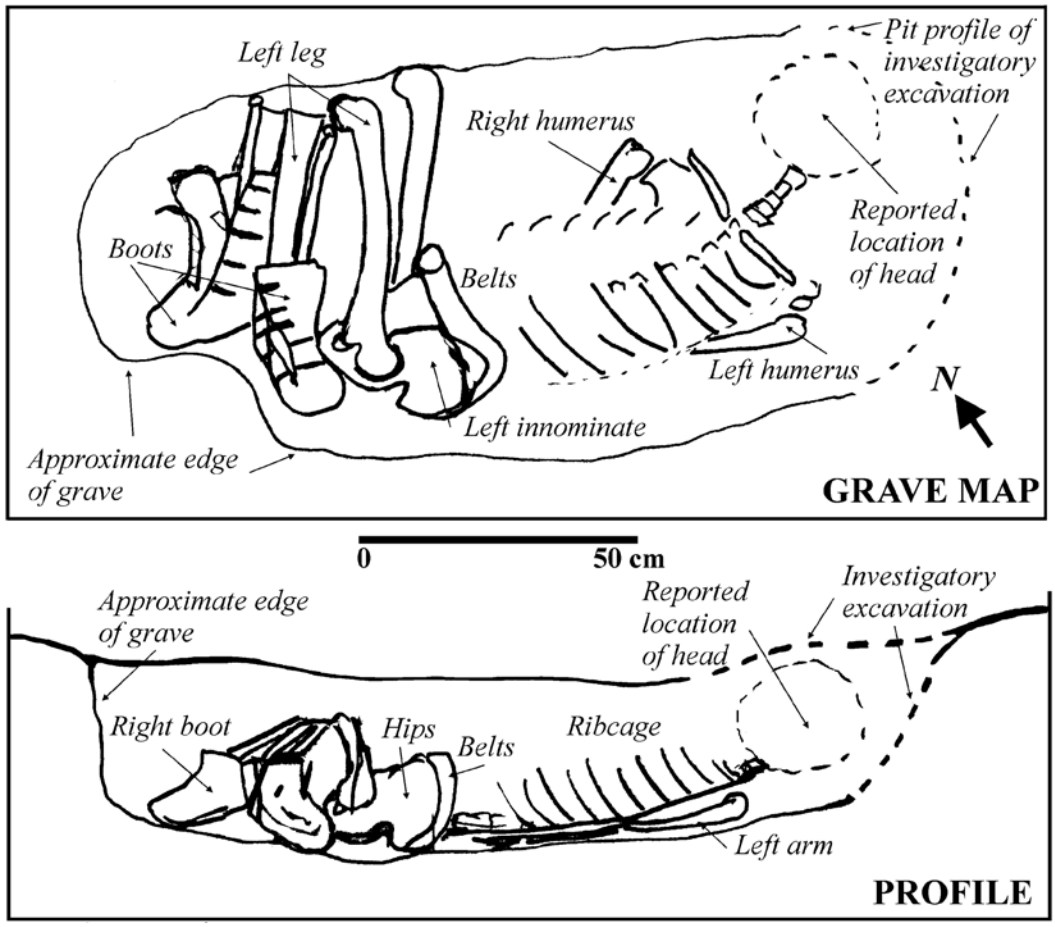

Figure 7.18 The scene is mapped from directly overhead and a profile view is also drawn which shows the excavation along the midline. Such maps become extremely important when presenting the case to a jury and require accurate in-field recording of all items. Map and profile relate to Figure 7.19. (Figure courtesy of Alison Galloway.)

features of the scene are fully realized (Pickering and Bachman, 1997). Photograph all physical evidence detailing clothing color, condition, size, and brand name. Macro photos must be taken of injuries and indications of restraints.

After the body is exposed, conduct a final check to make sure everything that is present at the scene is recorded on the map. Working systematically from one end of the scene to the other with two people, one calling out each item and the other checking the maps, ensures that there will be no errors in recording. Remember that not only location but also depth may be needed.

In many cases the remains will not be completely skeletonized. This is particularly true in cases where the body has been wrapped in plastic or some type of container or when there are multiple, stacked victims. Often the intent of the perpetrator is to accelerate decomposition but instead this inadvertently, dramatically decreases the rates of decay. The remaining flesh may be sufficiently intact even years afterward for at least a partial autopsy. In such cases the odor usually is a warning to proceed carefully with the excavation

Figure 7.19 In this excavation, an individual is recovered whose head was removed during the initial search by police personnel. The remains lay supine, with the hands tied behind the back. Expended bullets were located in the thoracic cavity. (Photo courtesy of Alison Galloway.)

and avoid any intrusive digging. Gentle scraping of the surface accompanied by frequent passes with the brush will expose the covering. Such remains can be lifted directly into a body bag if it is possible to provide sufficient support underneath the wrapping so that the body is not supported by the original container. Lifting by the wrapping could superimpose additional damage that may interfere with interpretation of any other indicators of tearing or stretching from the time of disposal.

When the remains are completely skeletonized, there are two approaches. In both cases it is important to completely expose the remains prior to removal of the body. In many cases, once exposed, photographed, mapped, and recorded, individual bones can be removed. In many cases, portions of the body will cover other body portions. For example, a body may be in the grave in a supine position but with the hands tied behind the back (Figure 7.19). In this case separate photographs of the hand positions will be required. A second method, which also has advocates, is to completely pedestal the remains. This means that the body is left on a "stand" or pedestal of dirt. The pedestal is then undercut and the body, still encased in dirt, is slid onto a wooden support board for transport to the laboratory where the excavation process is finished. This method is particularly helpful when the bones are in very fragile condition or when staining and other less tangible features can be recorded more carefully than in field conditions.

Soil samples from the body, particularly the abdominal and chest areas may be helpful. On bodies buried close to the surface, large quantities of pupal cases are often found within or on the body and associated clothing, bedding, or other material, as the maggots were unable to migrate before pupation. Loose soil at the bottom of the pit also must be checked for evidence.

Once the body is removed, a profile of the grave (Figure 7.18) should be completed by taking depth measurements of the floor of the pit. Using the midline of the overhead map and the same scale for depth, mark the depth to which the grave was originally dug.

Collection Techniques

If material is to be transported directly back to the laboratory for analysis, then one must establish the chain of custody, recognizing that the material is coming from the supervision of the primary investigator to the anthropologist, entomologist, or botanist. This documentation is essential for showing the court that the material has been safely and securely protected from the time of the excavation until it is presented in court. Since it is not unusual for the local agency to fail to have such a form, it is helpful to have a supply (Appendix, transfer of evidence). This form includes places for the case number(s), agency, items, the person's name and signature releasing the material, and the person's name and signature obtaining custody. Each transfer should be dated and timed, with both agencies retaining a copy for their records. When the material is returned to the agency, the next transfer should be noted on the same forms. This is true also for any transfers of evidence away from the laboratory, such as samples taken and sent for DNA sampling, dental arcades sent to the forensic odontologist, or prosthetic devices sent to an orthopedic consultant or manufacturer.

Anthropological Evidence

Bone does not preserve well in plastic bags. The moisture present in the bones along with the nutrients present in the soil and bone provides a perfect medium for molds and mildew. Left unchecked, this will lead to the breaking apart of the bone so that even basic morphology can be lost. Instead, bones should be packed in clean paper bags labeled with the case number, agency, item number keyed to the site map, date, contents, and the initials of the person responsible for collection. Identification of the element by the anthropologist also should be included. Since bags may differ in size and shape, it is useful to have a moving box into which to pack the bags of bones for transport to the morgue. Make sure that heavy bones (i.e., pelvis or femur) are not put on top of smaller, delicate bones. For bones that are less sturdy, bubble-pack or foam-pack can be wrapped around them. Cotton batting is difficult to extract from the bones and leaves excessive fibers and, therefore, is less useful.

Human remains that are only partially skeletonized with soft tissue and body fluids present should be packed in heavy plastic airtight containers or evidence bags, labeled, and then placed inside "hazardous biological specimen" bags or body bags. If the soft tissues are still intact on the hands, place the hands in paper bags prior to moving the body. Watertight "hard-sided" equipment bags function as airtight (and odor tight) containers for the transfer of this type of decomposing material. All the same, these remains should be immediately transferred to the morgue or laboratory for processing.

Bones collected from a single area, such as a burial, can be collected together, depending on the quality of the bone. For scattered remains, however, each area should be bagged separately and labeled as to its location within the overall scene (Howard et al., 1988).

Do not clean the bones in the field (Haglund et al., 1990). Since there may be trace evidence retained with the bones, cleaning may dissociate it and raise questions as to whether the evidence was from the body or surrounding area. For example, a bullet retained within the cranium could be lost or questions raised as to the possibility that it was only an inadvertent association actually due to hunting or target shooting.

If material is dry, or will dry during transportation, then special care must be given to the cranium (Bass, 1987). The drying soils will create a clump of dirt that forms a

"bomb" within the cranial vault. Left intact, this will bounce around within the vault and can break the bones apart. The cranium should be examined for presence of dirt and carefully packed to minimize jostling during transport. Obviously the dirt should not be removed at the scene but packing material can be placed into the vault to control the movement of the clod. Handling the skull also requires care. No skull, in any circumstances, should be held by fingers in the orbit or nasal aperture, and forensic cases are no exception (Wolf, 1986). The fragile bones in these areas are easily broken and almost impossible to reconstruct. Because the excavated skull may be severely fragmented by peri- or postmortem trauma, it also should not be lifted by the foramen magnum. Instead it should be cradled in both hands until it can be packed for transport. Care also must be taken not to lose the teeth, especially the anterior ones.

Some more friable material requires special handling. With authorization, burned bones can be consolidated by using a diluted glue which is dribbled onto the bone/teeth using a pipette. This works well for preserving dentition during transportation to a morgue and allows gentle handling. Only those areas critical to analysis should be treated and carefully recorded. These substances may interfere with chemical, serological, genetic, or other laboratory results if treated material is unknowingly submitted (Wolf, 1986).

Botanical Evidence

Botanical samples from the scene can include macrobotanical samples as well as pollen. The larger samples include roots that have grown through the remains, ends of limbs that have been cut or broken to be used as weapons or coverings, or leaves that have fallen over the body. Analysis of new plant growth and dead plants at the scene may be used to establish the postmortem interval (Lipskin and Field, 1983). In addition, because many plants have parts that are easily detached (i.e., seeds, spines, hooks, or hairs) and that can inadvertently attach themselves to the clothing or hair of the victim or perpetrator, combined with the fact that plants are habitat specific, botanical evidence can be used to place the victim and/or perpetrator at a particular location (Lane et al., 1990; Hall, 1988; 1997). Pollen samples also are particularly useful in establishing associations between, victim, suspect, and crime scene.

The macrobotanical evidence should be documented and collected before the scene is processed for anthropological and entomological evidence. The habitat around and including the scene should be assessed. Broken branches, crushed plants, overturned planters, and places where the suspect(s) may have walked through or by a hedge or other dense vegetation should be photographed (with macro and normal lenses). Samples from the major trees and shrubs from around the scene are collected to help the botanist reconstruct the scene's habitat. Preferably, at least one 12-in. branch (with its leaves and fruits) from each woody plant and examples of all herbaceous (nonwoody) plants also should be collected. Also, collect a handful of leaf litter and other vegetative detritus. After the samples for habitat reconstruction are collected, then proceed to collection of samples from around, on, and under the body. Collect all vegetative material on the victim and other objects associated with the crime. Because identification of species may be crucial for interpreting the information, it is best to collect samples of branches with leaves from the same tree as the specimen taken into evidence or from surrounding vegetation if the plant of origin is not immediately evident. Searchers should take precautions against handling poison oak and poison ivy.

When collecting the herbaceous samples, include the plant's roots and be sure to shake out most of the dirt clumps. Plant material in body fluids or other muck should be put into plastic containers and be kept cool, but not frozen. All other samples are to be put into paper bags or they can be wrapped in newspaper and allowed to fully dry in order to prevent decay from molds and mildew. The specimens must lie flat. On the bag or newspaper write the case number, sample number, date and time of collection, and name of collector in indelible ink. In addition, the following relevant data should be recorded on ancillary sheets (Appendix, collection sheet) and should include the following information:

- Where the sample was taken in relation to the established datum and GPS coordinates
- Where on the victim or object the sample was taken (i.e., adjacent to the left elbow)
- The type (as in grass, tree, or shrub) and size of plant from which the sample was taken (i.e., an approximately 25 ft tree)
- The plant's color (i.e., leaves are green on top and white with red veins underneath and purple fruit is present)
- The relative frequency of occurrence for the plant within the scene (i.e., frequent or infrequent — percentages if possible)

Most importantly, a forensic botanist must examine the collected material and photographs immediately. It is prudent to maintain an on-call list of forensic experts with whom your agency wishes to work.

Entomological Evidence

The collection of entomological evidence should occur after the botanical evidence has been collected. The proper collection of entomological evidence is dealt with extensively in Chapter 3. What follows, however, is a brief discussion concerning the alteration of normal insect activity on buried human remains. It has long been observed that buried human remains decompose at a much slower rate than those placed above ground. The fact that the act of burial slows decomposition is due to a number of factors. These factors include the exclusion of some bacterial organisms and vertebrate scavengers, and the cooler temperatures that decrease with soil depth. However, the most influential factor that slows the decomposition of buried remains is the exclusion (either partial or total) of insect activity and the alteration of normal insect succession (Lundt, 1964; Payne et al., 1968). Mégnin (1887) and Motter (1898) were the first to formally report the alteration of normal insect succession of buried remains. Schmitz (1928) and Leclercq (1975) supported and enhanced earlier works by showing that buried remains have their own unique insect fauna that varies with the habitat and type of burial.

With buried remains, the insect fauna is generally limited in direct proportion to the amount of soil or debris covering the body. However, Rodriguez and Bass (1983) noted that as little as 1 in. (2.5 cm) of soil would exclude the majority of blow fly species. This is due in part to the fact that many adult female blow flies require tarsal contact with the remains before oviposition will commence. The blow flies are stimulated to oviposit by the free moisture on the surface of the remains, as well as by oviposition pheromones released from other nearby female flies when a suitable oviposition substrate is found. Exclusion of tarsal contact, even by a thin layer of soil, may be enough to inhibit or delay oviposition. Colonization of a buried body by insects requiring direct access to the body

is an indication that the remains were unburied and exposed to the environment for a period of time. Such a fact can be crucial in reconstructing the sequence of events leading to the burial of the corpse.

Flesh flies (Sarcophagidae) and various relatives of the ubiquitous house fly (Muscidae) are not as readily deterred by thin layers of soil. An evolutionary divergence from the blow flies has lead to sarcophagid flies not requiring tarsal contact with the remains, and their unusual ability to deposit an active larvae instead of an egg. The first instar larva hatches from the egg while it is inside of the female's body. Thus, it can immediately crawl and burrow into the decomposing tissue upon being deposited by the female. Flesh flies that are in flight also have been observed to deposit larvae onto carcasses, or on the soil surface directly above buried remains. These newly deposited larvae can rapidly burrow through several inches of soil to reach the remains. Colonization of buried remains by these flies is more common during the later stages of decay when bloat may produce fissures in the soil surface and allow closer access by the adult flies. In most cases, it is generally assumed that bodies buried 1 ft (0.3 m) or less will be colonized by one or more species of dipterous larvae (Rodriguez and Bass, 1985). The adults of smaller flies such as those of the Phoridae (coffin flies) will burrow into the soil to oviposit directly on the remains. This adult burrowing behavior also is seen in several beetle families such as the rove beetles (Staphylinidae) and burying beetles (Silphidae), all of which have been recovered from remains buried from 10 to 100 cm deep.

Some insect species (such as *Conicera tibialis*) that successfully colonize decomposing remains underground may undergo several generations and continue to live for extended periods of time on the buried remains. However, the typical scenario is that the larvae which were deposited on the soil surface and burrowed down to the remains will continue to develop, pupate underground (either on the remains or in cavities in the soil created by the decomposing tissue), and undergo adult emergence while still confined underground. The newly emerged adults will then burrow upward through the soil and reach the surface. However, many die while attempting to reach the soil surface. Therefore, it is not uncommon to find dead adult insects at all levels in the soil directly above the remains. Thorough sifting of all the soil excavated from the defined burial site (from surface to grave floor) should be sifted and examined by an entomologist for the recovery of entomological evidence. In addition to finding adult insects in underground locations, larvae, pupae, pupal cases, and insect body fragments can be expected. All stages of the insect's life cycle should be actively sought during the excavation process. If living insects are recovered from the burial site, live and preserved collections should be made as described in Chapter 3.

It is impossible to overstate the simple fact that photographs of the scene are needed to augment the insect samples. The forensic entomologist is trained to observe small fragmentary remains of insects and their castings that most individuals on the forensic science team will overlook. Therefore, it is beneficial to take numerous photographs of the colonized body in reference to its surroundings, photographs depicting the location of the insect colonization on the remains and overall body shots which depict the body's state of decomposition. Always include macro photographs of the insects that have colonized the body.

Field Assessment

A brief assessment of the biological profile of the victim, any obvious defects, and the length of the postmortem interval is appreciated at the scene. It is best not to be too

restrictive on the ranges and temper any interpretation with the precaution that this is a preliminary assessment and may be expanded during closer examination. Such information, however, allows the law enforcement personnel to begin amassing the information that may determine the positive identification.

In cases that were well publicized, it is not uncommon for an impromptu memorial to be established by the public as the sympathetic and the curious visit the crime scene. This frequently leads to a rash of submittals in the subsequent weeks as every bone or oddly shaped rock or stick is brought to the sheriff's office by concerned citizens convinced they have found part of the victim's body. To avoid lengthy trips to confirm or deny these items, transmission of pictures by e-mail has proven quite effective and efficient.

Preservation Techniques

Anthropological specimens are best preserved in dry, well-aired conditions. Bones will need to be cleaned in the laboratory in most cases. This process can range from allowing dermestid beetles to consume remaining desiccated tissue, cooking in detergent-treated water, defleshing of significant amounts of soft tissue, or a light brushing under running water. Cleaning occurs only after there has been a close examination of the remains by the anthropologist and, of course, after the pathologist has completed his or her analysis and the remains have been inspected for trace evidence. If identification is in question, untreated portions of long bones and teeth should be retained for DNA analysis.

During the processing and analysis and until the remains are returned to the coroner/medical examiner, all human remains are to be treated as evidence. This requires that they only be handled in secure conditions. Only individuals directly associated with the investigation should be given access to the remains and they should never be used for demonstrations or teaching purposes. When the bones are no longer being actively examined, they should be securely locked in storage cabinets or safes. Storage in archival-quality, acid-free boxes and paper bags are preferable to plastic containers that frequently encourage the formation of mold on bones. Containers should be clearly labeled with the case number and contents.

Summary

The resolution of civil and criminal trials depends upon the continuity and protection of evidence, whether skeletal, entomological, or botanical. In the best of circumstances, an experienced evidence response team, such as those of the F.B.I, will work together to systematically search and recovery the evidence including the human remains. However, it is best to be prepared for the worst situation, where the forensic scientist is responsible for a larger share of the roles. Preparation in terms of equipment and forms and in terms of excavation skill, knowledge of evidence collection procedures, and the rationale behind recovery techniques is essential. To include the forensic anthropologist early in the scene investigation and recovery operations helps to ensure maximum recovery and preservation of both the tangible and intangible evidence (Galloway et al., 1990). Together we can strive to obtain the best recovery of evidence that may be the victim's last chance for justice to be brought forth.

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Appendix

Anthropology, Entomology, and Botany Equipment Lists

Recommended tool kits for use during field recovery are items listed below. Add or delete items depending upon agency budget, preference, and time allowances.

Anthropology

Search Equipment

Probes	Flags	Walkie talkies
Rope	Leather gloves	Knee pads

Excavation Equipment

Pointed and square shovels	Mason's trowels (4.5 or 5-in.)	Pruning shears
Saw	Metal stakes	Probes (2)
Evidence flags (various colors)	Dustpan (2–3)	Small "camper's" shovel
1/8 and 1/4 in. screens	5-gal buckets (3–4)	Visual markers
Tarps (for excavated soil)	Metal detector	Wheelbarrow
Flashlight(s)	Paint brushes (1/2 to 4 in. wide)	Rake (2–4)
Dental picks	Wooden picks	Scene tape

String, twine, rope or surveyor's tape
Brightly colored spray paint (red is suggested because it contrasts well with grass and shrubbery)
Thin wooden sticks with one end painted to mark positive metal detector readings

Supplies for Transportation

Paper evidence bags (various sizes)	Plastic evidence bags (various sizes)
Cardboard boxes	Foam wrap/bubble wrap (acid free)
Plastic containers with lids	Integrity tape
Indelible markers	Biohazard waste disposal bags
Body bags	

Mapping Equipment

Compass	50-m tape measure	10-m tape (2)
Two-way line level, transit or farmer's level with stadia rod	Graph paper	GPS equipment
	Stakes	Protractor
Plumb bob	Erasers, pencil sharpener	String (heavy gauge)
Pencils	Flagging tape	Metric straight edge
Flagging pins	Drafting compass	Long nails (6–8 in.)
Nails for stakes		

Documentation and Field Analysis

Notepaper	Transfer of custody forms	State map
Anthropometer	Dental charts	Inventory form and chart
Sliding calipers	Spreading calipers	Osteometric board
Stature chart	Soil cards	Photography log
Indelible pen (2–3)	Indelible marker (2)	Pencils
Pencil sharpener	Clipboard	Hand lens
UTK databank forms		

Photographic Equipment

35 mm camera (2)
Slide film
Extra camera and flash batteries
North arrow
50 mm macro lens
Digital camera
Color print film
Metric scales
Ladder
Flash unit
Normal lens
Photo board or slate board with chalk
Video camera

Personal Equipment

Disposable gloves
Sunscreen
Cell phone
Toilet paper
Boots
Antimicrobial cleansing soap
Ivy block
First aid kit
Transportation vehicle
Insect repellent
Drinking water
Tarps for protection from the elements
Identifying clothing

Entomology

Collapsible insect net and 12-in. extension handle
Mason's trowel
"Whirlpack" specimen bags, 6 × 9 in.
Fine point forceps, curved tip
Fine point forceps, straight tip
Soft, featherweight forceps
Glass vials with polyseal caps
Surgical gloves
Dual scale thermometer, 6 in.
Self-adhesive labels
Nonadhesive labels
Preservation and collection chemicals
Paper towels
Disposable gloves
1/4 in. screen
Styrofoam shipping containers
Death scene form
Aluminum foil
Vermiculite
Liquid approved container with 70–80% ethyl alcohol
Distilled water
Indelible marker
Pencil
Pencil sharpener
Eraser

Botany

Pruning shears (2 pair)
Sharpening stone
Trowel (one per collector)
Pointed shovel
Indelible marker (2)
Paper bags (various sizes)
Plastic containers with lids
Cooler
Ice packs
Surgical gloves
Gardening gloves (leather)
Ivy block

Photography Log

Case Number: ______________________ Date: ______________________

ME Number: ______________________ Time: ______________________

Photographer: ______________________ Recorder: ______________________ Page ___ of ___

Film Roll No.	**Item No.**	**Description**	**Location**	**Compass Direction *(degrees)***

Evidence Log

Case No.: ______________________ Date: ______________________
ME No.: ______________________ Time: ______________________
Recorder: ______________________ Location: ______________________
Collector: ______________________ Page ___ of ___

Item No.	**Item Description**	**Distance from Datum *(meters)***	**Compass Direction *(degrees)***	**Grid Unit *(if applicable)***	**Elevation/ Depth *(if applicable)***

Transfer of Evidence

Case No.: _____
ME No.: _____

The following items:

Item No.	Description

Transfered from **Time/Date** **Transferred to:** **Time/Date**

Botanical Evidence Collection Sheet

Recorder: _____ Case Number:_____
Agency: _____ Date: _____ Time: _____ Page ___ of ___

Item No.	Tree/Grass/ Shrub/Other	Color Description	Distance from Datum *(meters)*	Compass Direction *(degrees)*	Location Relative to Body or Other Physical Evidence	Relative Frequency
1.						
2.						
3.						
4.						
5.						
6.						
7.						
8.						
9.						
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24.						
25.						

Estimating the Postmortem Interval

8

JEFFREY D. WELLS
LYNN R. LAMOTTE

Contents

Introduction

The time elapsed since death, or postmortem interval (PMI), is a matter of crucial importance in investigations of homicides and other untimely deaths. Such information can help to identify both the criminal and the victim by eliminating suspects and connecting the deceased with individuals reported missing for the same amount of time (Catts, 1990; Geberth, 1996). Even when the cause of death is natural, the time of death can have important implications for legal matters such as inheritance and insurance (Henssge et al., 1995). Crucial information such as when the deceased was last seen alive may indicate a maximum possible PMI, but this can occur only if he or she has been identified. Often it is the condition of the body itself that must tell us when death occurred (Henssge et al., 1995).

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Postmortem changes in a body depend upon many factors (Micozzi, 1991), and the PMI can be a remarkably difficult thing to determine (Bass, 1984). Obviously, any physical or biological change that is a function of time since death provides a potentially useful clue in this matter. Initially, the predictable physical and chemical consequences of death are usually the most reliable PMI indicators (Henssge et al., 1995). But, as the time since death increases, the above methods become less useful and more accurate results are often obtained using ecological information. A decomposing body can dramatically alter both the behavior and composition of species at a site. A cadaver attracts a variety of vertebrate and invertebrate scavengers (Putman, 1983), while the products of decay can produce changes in the underlying soil flora and fauna (Bornemissza, 1957).

It has long been observed that insects associated with vertebrate carrion display PMI-dependent processes (Hall, 1990). One of these processes is the development of insect species whose larvae consume dead tissue. Flies in the families Calliphoridae, Sarcophagidae, and Muscidae are the most noticeable because of size, number, and ubiquity. These are frequently succeeded on carrion by beetles in the families Silphidae and Dermestidae (Smith, 1986). For convenience, we will use terms in this chapter appropriate for the most common forensic situation. Typically, this is a case in which the evidence consists of "insects" collected at a "crime scene" from the body of what is suspected to be a "victim" of a homicide. The discussion, however, applies equally well if noninsect arthropods are collected, or if no crime is thought to have been committed.

The estimated age of an immature insect that has fed on a body provides a minimum PMI because, with very rare exception (see below), adult females do not deposit their offspring on a live host. In its most conservative form, such an approach does not estimate the maximum PMI because an unknown period of time may elapse between death and the deposition of eggs or larvae. Depending on the insect species and conditions at the scene, the degree of development can indicate a PMI from less than 1 day to more than 1 month (Smith, 1986).

Another PMI-dependent process is the succession of arthropod species found on and within a body (Schoenly and Reid, 1987). In contrast to larval development, a succession model includes information about the time elapsed between death and the appearance of a particular arthropod species and stage. Therefore, it can be used to estimate both the minimum and maximum PMI (Schoenly et al., 1992). According to our definition, the simplest succession model is used when an investigator estimates both the age of a larva and the time interval between death and that individual insect's arrival at the body. Succession data have been used to very accurately calculate a PMI as large as 52 days (Schoenly et al., 1996), and could be applied to a much greater interval.

Reference Data

Faced with the need to estimate the PMI from entomological data, the investigator must select a model of insect development or succession. This can involve a comparison to other death investigations, or experimental data may be generated *a posteriori* to match a case (Goff, 1992; Introna et al., 1989), but most often previously existing experimental data are consulted. Larval growth rates are usually studied using small containers in the laboratory. Larval growth in such an artificial setting suitably mimics growth in the field as long as certain other conditions match those of the scene (see below). Developmental data have

been gathered for a large and constantly growing list of species and conditions (e.g., Byrd and Butler, 1998; Goodbrod and Goff, 1990; Greenberg, 1991; Greenberg and Wells, 1998; Introna et al., 1989; Nishida, 1984; Reiter, 1984; and many others).

Carrion succession is a classical subject in ecology (Fuller, 1934; Payne, 1965; Reed, 1958), and much of the recent work has had a forensic objective. While some experiments have involved human corpses (Rodriguez and Bass, 1983; Rodriguez and Bass, 1985), these are extremely difficult to obtain legally, and most published information on insects involved in human decay comes from case studies (Lord, 1990; Smith, 1986). As useful as these may be, they are usually just a "snapshot" of the process. Therefore, researchers usually employ nonhuman carcasses (such as pigs) in order to include replication and repeated sampling in a succession study (e.g., Anderson and VanLaerhoven, 1996; Hewadikaram and Goff, 1991; Tantawi et al., 1996; and many others).

Factors that Influence Carrion Insect Development and Succession

In order to choose an appropriate model of growth or succession, conditions at the crime scene and the manner in which the specimens were handled must be determined. The closer the match between conditions at the scene and those used to generate reference data, the less margin of error in estimating the PMI. If possible, the entomologist should visit the site, consult the reports of other investigators, and obtain the most reliable weather records (Haskell and Williams, 1990).

Many biotic and abiotic factors are known to influence carrion insect growth and activity. Determining these factors and their effects has been the most active area of research in forensic entomology. The following factors are of particular importance. This is, however, not meant to be a comprehensive list, and investigators should carefully consult the primary literature and consider all biological information about any species used for analysis.

Individual Species Characteristics

Accurate identification of samples is usually the first priority in a forensic analysis of the entomological evidence (see Chapter 2). The most important implication for PMI estimation is that carrion insect species differ in terms of growth rate, arrival time, and position within the order of succession. However, other relevant physiological or natural history factors can only be considered following proper identification. For example, although it is generally known that Sarcophagidae deposit only live larvae (Shewell, 1987), certain common Calliphoridae show a limited version of this behavior. Erzinclioglu (1990) described the retention of a single fertilized egg by a gravid *Calliphora vicina* Rodineau-Desvoidy. This egg can hatch immediately after oviposition and the resulting larva will be "precociously" developed compared to its siblings. Such precocious eggs have been found to be common among blue bottle (Calliphorini) species, but not other calliphorid taxa in northern California (J. D. Wells and J. King, unpublished).

Weather and Seasonality

Temperature has a profound effect on insect metabolic and development rate (Adrewartha and Birch, 1954; Chapman, 1982). Generally, within a certain range of temperatures, development is accelerated as temperature is increased, but this does not hold true at temperature

extremes that may prove lethal to the insect. Both air temperature and exposure to sunlight will affect corpse temperature.

Some species enter larval or pupal diapause (arrested development) in response to seasonal cues (Denlinger and Zdarek, 1994), and this can greatly increase the time spent in that particular life stage even if temperatures are relatively warm. Closely related species may display quite different diapause behavior (Ash and Greenberg, 1975), thus illustrating the need for comprehensive knowledge of insect life history for PMI estimation.

Presence of a Maggot Mass

Carrion fly larvae can have a metabolic and feeding rate greater than most immature insects (Hanski, 1976; Levot et al., 1979). A large number of larvae in the same location can generate a temperature substantially higher than ambient, causing what is commonly termed the "maggot mass" effect (Cianci and Sheldon, 1990; Goodbrod and Goff, 1990; Greenberg, 1991; Turner and Howard, 1992). Because larval growth is inhibited as lethal high temperatures are approached, the difference between ambient and maggot mass temperatures is likely to be greatest during cold weather (e.g., Deonier, 1940). However, this is a complicated phenomenon, since the oldest larvae on the body may have developed prior to any elevation in temperature. The temperature and size range of larvae in the mass should be recorded at the scene before the body is disturbed.

Food Type

Some carrion fly larvae can develop on a range of food types. The most extreme example is the phorid *Megaselia scalaris* (Loew), which can eat live or dead invertebrates, as well as a variety of other organic materials, and has even been found feeding on paint (Disney, 1994). *Lucilia sericata* Meigen grew more slowly in a vegetable medium compared to when larvae were fed meat (Povolny and Rozsypal, 1968). Therefore, it seems likely that an extreme change in diet would affect development rate in other species as well. Occasionally, a forensic entomologist may be asked to age larvae from something other than a corpse. For example, a vendor may be liable if larvae in contaminated food are shown to have been present at the time of sale, and larvae from feces in a diaper may suggest a case of abuse or neglect (Goff et al., 1991). Development rates observed for a meat medium may not be appropriate for such cases.

Drugs and Other Toxins

Chemicals in or on the victim, such as might accompany drug overdose or suicide, may have a variety of effects on carrion insects (Goff and Lord, 1994) (see Chapter 11). Insect-mediated decay processes can be accelerated or decelerated depending on the substance and concentration. The presence of such substances may be indicated by a toxicological analysis of victim or insect tissue, or by other evidence such as a container at the crime scene.

Geographic Region

Little information is available on possible regional variation in forensic entomological phenomena. Although the development of some common species has been studied by laboratories in widely separated locations (e.g., see discussion of *Chrysomya megacephala*

(Fabricius) in Wells and Kurahashi, 1994), differences in the methods used and in presentation of the data make it difficult to interpret and compare the results. In one study designed to test the possibility of regional variation, Cyr (1993) found no statistically significant difference in the duration of developmental stages of *Phormia regina* (Meigen) from the states of Washington, Indiana, Texas, and Louisiana.

In contrast, a number of observations suggest that carrion fly behavior can indeed vary according to region. For example, throughout most of its distribution, *Chrysomya rufifacies* (Macquart) invades carrion or live vertebrates only after other larvae are present (Bohart and Gressitt, 1951; Wells and Greenberg, 1994; Zumpt, 1965), but in particular sites it has been observed to lay eggs on fresh carrion (O'Flynn and Moorehouse, 1979) and on uninfested new-born calves (Shishido and Hardy, 1969).

Whether any regional differences, if they exist, have a genetic basis or simply reflect a behavioral response to different environmental conditions is unknown. Although it is possible for a phenotypic gradient to develop from natural selection (one well-studied example is the diapause response) (Kurahashi and Ohtaki, 1989), any regional genetic variation is unlikely to be the result of isolation. The larger carrion flies are extremely mobile, and within the limits set by preferences for general types of habitat, they are commonly observed to disperse several kilometers in 1 day (Baumgartner and Greenberg, 1984; Norris, 1965). *Cochliomyia macellaria* (Fabricius) typically survives the winter only as far north as southern Texas and Florida, yet it spreads throughout almost the entire contiguous U.S. and into Canada each year (Hall, 1948). Such behavior should promote extensive gene flow between locations, and the limited number of molecular genetic studies done so far suggest that widespread carrion-feeding species encounter no barriers to gene flow over distances of thousands of kilometers (Stevens and Wall, 1997; Taylor et al., 1996).

Preservation Method

Most plots of larval growth (growth curves) describe the change in body length with age. Typically, larvae are killed and preserved in some fluid prior to measurement. The type of fluid and the method of processing influences the length of the preserved larva (Tantawi and Greenberg, 1993). These differences must be taken into account if the insect evidence is preserved using methods other than those of the reference study.

Insect Colonization Preceding Death

There are exceptions to the rule that carrion insects are only attracted to a dead body. Infestation of a live vertebrate by fly larvae is called myiasis (James, 1947; Zumpt, 1965). Although this situation is extremely rare, if a person was already infested when death occurred, then insects present at that time could cause one to overestimate the PMI. The larvae of many carrion fly species have occasionally been found feeding in necrotic wounds (where they can actually promote healing (Sherman and Pechter, 1988)). Investigators should be alert to contributing factors, which include a medical history of a persistent wound such as might result from surgery, cancer or diabetes, and physical or mental incapacitation (Greenberg, 1984; Hall et al., 1986; Seaquist et al., 1983).

Deviations from the common decay pattern in which the head is the first site of infestation *may* indicate antemortem trauma and insect activity. As mentioned above, larvae may initially feed on feces within clothes. This would be suggested if the deceased wore

diapers, or if larvae in the anal region were the oldest present. However, we stress that the typical "head down" pattern of decomposition documented on humans provides only a suggestion of normal decay. Postmortem insect feeding can begin anywhere other than the head, such as when insects are attracted to a wound, and that antemortem activity also can occur in the head. Some very close relatives of carrion flies, the "screwworms" *Chrysomya bezziana* Villeneuve and *Cochliomyia hominivorax* (Coquerel), will only deposit eggs on a live host. A nonspecialist may find it difficult to distinguish the immature stages of these species from those commonly used as forensic evidence (Spradbery, 1991). Humans may be killed by an infestation that started in the nasal sinuses (Spradbery, 1994), and this is a situation that could be misinterpreted as the normal pattern of decay if discovered after death, although we are not aware of this having happened. In most cases, the proper identification of specimens will prevent such a mistake.

Estimating the Postmortem Interval (PMI)

In almost all cases, a sample of the collected insects are killed and preserved prior to being used to estimate the PMI, while others within the sample or similar sample may be reared to the adult stage for identification. The moment of preservation is the point in time from which one calculates backward to the time of death, and it is of critical importance to document this time for the evidentiary record. The circumstances of every death investigation are unique, and there is no single best algorithm for PMI estimation. Both the conditions at the scene and the quality of the data available to the entomologist vary widely.

It almost goes without saying that one must make certain assumptions in order to reach any estimation of PMI. These may reflect some obvious lack of information, such as whether there was a maggot mass or exactly how the remains were handled between the time of discovery and the time insect samples were collected. Frequently collection of the insect samples occurs during the autopsy, which may take place many hours after the discovery of the body. However, even if the circumstances of the case appear to be easily reconstructed, one must usually assume that information such as that contained in other investigators' reports is correct. An opposing attorney may challenge these assumptions, however reasonable, and the forensic entomologist may be required under cross examination to describe how the PMI estimate would be different if different assumptions were made (see Chapter 14). It is best to think of these things in advance rather than while on the witness stand.

As a general approach, the analysis should begin by reaching those conclusions that require the least amount of knowledge of the scene. Perhaps the most conservative conclusion is an absolute lower limit on specimen age. Each insect species has a range of conditions under which development rate is highest. If known, this optimum rate provides a lower limit on age, i.e., a larva of a given size or instar cannot be younger than the time it takes to reach that state under optimal conditions.

Estimating the PMI from Degree of Development

If the reference model for species development is a growth curve, then the best estimate of age for a larva is the value corresponding to its size on the curve. That is, a horizontal

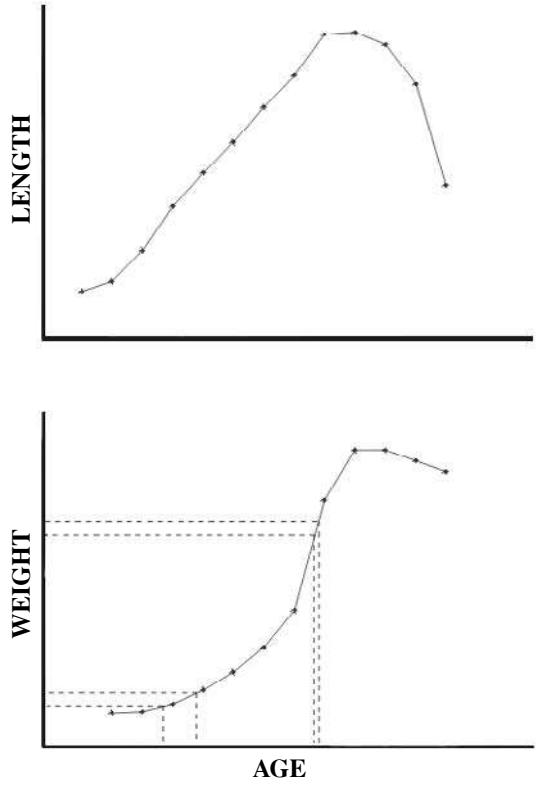

Figure 8.1 Hypothetical maggot growth curves showing the shapes often observed for the change in length or weight between egg hatching and pupariation. Dashed lines illustrate the fact that a prediction of age based on size is likely to be most precise where the curve is most steep, because at that point an error in measurement results in a relatively small change in the estimate of age.

line from the value of the length or weight of the larva will intersect the growth curve directly above its age. This calculation is likely to be most precise if the intersection occurs where the growth curve is most steep, because a small change in size results in only a small change in the estimation of age (Figure 8.1). Maggot growth curves, particularly those showing weight as a function of age, may form an "S" shape, with slow growth during the first two larval intars and a slow decrease in size between the cessation of feeding by the third instar and the onset of pupariation. Within these "flat" regions of the curve, other information is likely to be as useful as size for estimating age.

Larvae of the same age hatch and molt in relative synchrony (Davies and Ratcliffe, 1994; Kamal, 1958; Wells and Kurahashi, 1994; Wells and LaMotte, 1995), and these qualitative changes in form are easily recognized. The age of an individual insect from the crime scene is likely to fall within the range of age for which the same instar has been observed in the model. Other information such as the presence of a pharate larva (the next growth stage that is visible through the cuticle immediately prior to molting) will narrow the range. Unfortunately, the postfeeding stage can last as long as the rest of larval development, and lacks such abrupt changes in appearance. Postfeeding larvae undergo an

emptying of the crop, and the physical length of the crop provides some information about the time since feeding ceased (Greenberg, 1991). However, pupariation, metamorphosis, and adult emergence produce dramatic morphological changes that may be used to estimate age (Greenberg, 1991).

Although many development studies employed constant temperatures, thermal conditions at the scene will almost certainly have fluctuated to some extent. Data from growth at constant temperatures may be used for such situations by dividing the time period under consideration into short intervals (e.g., 12 h (Williams, 1984)), and then applying a model of development that is closest to the mean temperature during each period. Williams (1984) recommended a 10% correction factor for this method to accommodate the fact the *Lucilia illustris* (Meigen) grew more slowly under fluctuating temperatures than was predicted by growth at constant temperatures (Hanski, 1976). It appears, however, that this is not a universal effect, and development at fluctuating temperatures can be accelerated, retarded, or the same as development at a constant temperature with the same mean value (Davies and Ratcliffe, 1994). Some development studies have included temperature fluctuations designed to mimic a typical diurnal cycle (Byrd and Butler, 1996; 1997; 1998; Davies and Ratcliffe, 1994).

Another approach has been to model development in terms of accumulated degree hours or degree days, a process known as thermal summation (Wigglesworth, 1972). This method is described in greater detail in Chapter 9 and is the method used to create the computer model of insect growth discussed in Chapter 10.

Estimating PMI from Stage of Succession

The number of arthropod species collected from a corpse may number in the hundreds (Schoenly and Reid, 1987), and the number of individuals of the most common species can easily be in the tens of thousands. Furthermore, many of these can be incidental species with no particular ecological association with carrion. Clearly one must focus on only a subset of the total fauna in order to find patterns that may be analyzed for forensic purposes. Reoccurring taxa or life stages, those in which individuals may visit and leave a corpse several times (e.g., ants or adult flies), are considered relatively poor indicators of time since death (Schoenly, 1992).

Schoenly et al. (1992) introduced the concept of recording succession as an "occurrence matrix." In this system, a species or stage is noted as being either present or absent at a given point in time following death (i.e., the PMI value). Using this system, the assemblage of species collected from the victim is compared to the occurrence matrix, and those PMI values for which one would find that assemblage are identified. Although no information about the relative abundance of each taxon is included, this system has the advantage of being easily stored in machine-readable files, and Schoenly et al. (1992) developed software capable of calculating the PMI using a large reference data set (provided that one accepts the assumption that the succession pattern shows no random variation for a given set of conditions; see the following). Furthermore, adoption of a standardized recordkeeping method such as an occurrence matrix would prevent the difficulties we have found in comparing the results of succession papers that present their results in narrative form (see the following).

When Conditions from the Scene Don't Match any Experiment

Conditions at the crime scene sometimes do not closely resemble those of any experiment on development or succession. In such a case, one must use qualitative judgement as to whether entomological processes proceeded at a faster or slower rate than for a chosen model. Investigators should develop their own baseline data for the species and conditions likely to be encountered at their location.

Dealing with Inherent Uncertainty in Growth and Succession

As with any natural phenomenon, there is random variation in forensic entomological phenomena. The common practice of using only larvae that are relatively big for their age to construct a growth curve suggests to us that most forensic entomologists realize that, even under the same conditions, not all larvae grow at the same rate. However, only a few laboratory studies have reported the complete range in size found among larvae of equal age (Wells and Kurahasi, 1994; Wells and LaMotte, 1995). These studies have shown that the larval size distribution is skewed toward the low end of the scale (Figure 8.2). In other words, there are a few extreme "runts," and this is particularly noticeable with third instars. Similar measurements of wild populations are almost nonexistent, but the single sample we have taken fits this same pattern (Figure 8.2D). It may be that the stunted individuals are moribund (Davies and Ratcliffe, 1994), and the fact that they have a relatively small cephalopharyngeal skeleton (J. D. Wells, unpublished) indicates that they are already undersized at the time of the final larval molt.

Similarly, little is known about random variation in succession rate. Perhaps this is not surprising given the need for replicate bodies in order to measure such variation and the effort that succession studies can entail. The replication of experimental conditions quickly leads to a huge amount of work, and the intensive sampling and analysis of even a couple of large carcasses can produce enough data for a Master's thesis (Hewadikaram and Goff, 1991). Although some ecological studies of carrion involved a large number of carcasses, the authors did not describe their methods in enough detail to know what conditions might have been replicated (Abell et al., 1982; Fuller, 1934; Johnson, 1975). Other authors, who clearly used more than one carcass under the same conditions and sampled in the same manner, presented their results as a composite "typical" succession pattern with no information about random variation (Bornemissza, 1957; Cornaby, 1974; Hall and Doisy, 1993; Nabaglo, 1973; Reed, 1958).

Despite some difficulties in interpreting the older papers, there are strong indications that dramatic variation in succession patterns can occur. It is interesting that Reed (1958) excluded some carcasses from analysis because they were "atypical" (his quotation marks), while Nabaglo (1973) mentioned that some carcasses decomposed more slowly than the reported succession model if they were invaded by fungi, or more quickly if they were monopolized by silphid beetles. What was probably an extreme example of variation in succession was reported by Tantawi et al. (1996). During one of their experiments, four rabbit carcasses placed side-by-side displayed two distinct patterns of succession. The biggest difference was shown by larvae of *Ophyra ignava* Harris, which was found in two rabbits from 9 to 23 days following death, and in the other two rabbits from 16 to 91 days

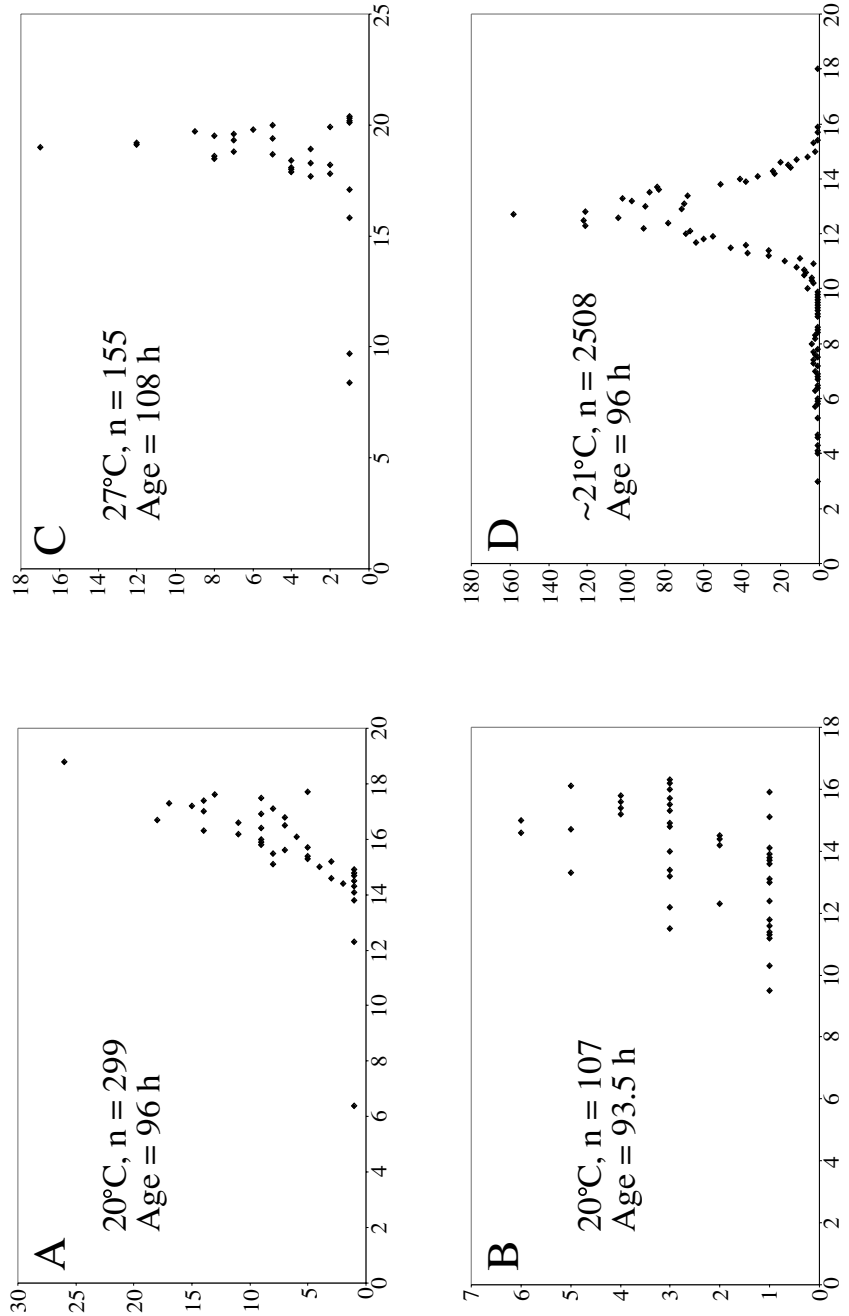

Figure 8.2 Size distribution for calliphorid larvae of equal age. (A) *Calliphora nigribarbis,* Tokyo, Japan. (B) *Aldrichina grahami,* Tokyo, Japan. (C) *Chrysomya megacephala,* Bangalore, India. (D) *Chrysomya megacephala,* Okinawa, Japan. (A–C) were produced in the laboratory using the methods of Wells and Kurahashi (1994). Larvae for (D) were produced by exposing a rat carcass to oviposition by wild flies for a 2-h period. The carcass was then held at a shaded outdoor location, and the larvae were preserved by flooding the carcass with ethanol.

following death. Although the reason for this divergence is not known, a possible explanation is that a small initial difference in succession rate between the two sets of rabbits was magnified by weather patterns (Tantawi et al., 1998). In particular, rehydration of tissues by heavy rain on days 14 and 15 may have led to the second wave of *O. ignava* oviposition that was observed on the slower decaying carcasses (which may have had more tissue to "revive" than did the faster decaying carcasses). Dropping temperatures that occurred after all larvae had left the faster decaying carcasses then slowed the development of this second cohort of larvae and, therefore, extended the time that they were present in the remaining carcasses.

However, such variation is not always observed. Early and Goff (1986) and Braack (1987) used more than one carcass at a site in order to compare their rates of decomposition. Although these authors did not use a common sampling technique for all carcasses, they indicated that succession patterns did not appear to vary among bodies. Anderson and VanLaerhoven (1996) simultaneously exposed seven pig carcasses. Arthropods were collected from three of these, and the other four were visually inspected. Anderson and VanLaerhoven stated that there were no differences among the pigs in the daily changes in gross morphology (e.g., bloating), and that the timing of colonization by individual arthropod species varied by no more than 1 day.

The implications of such variation for the conclusions reached by forensic entomologists must be addressed. More specifically, we believe that the field of forensic entomology will benefit from the development of the statistical reasoning that is so common in other areas of science. Statistical methods are essential for establishing the precision of a PMI estimate and for evaluating any conflict of opinion among experts (i.e., different PMI estimates may not be, in fact, significantly different). Furthermore, we believe that they will help to direct forensic entomology research, because different techniques for PMI estimation may more easily be evaluated according to the precision of the conclusion. In the following sections, the efforts made by the authors to develop statistical approaches to PMI estimation based on insect evidence will be described.

Statistical Considerations and Methods

The technical problems involved in estimating PMI and constructing a confidence interval on it are different when dealing with development data or succession data. Within life stages, development occurs smoothly and continuously with age, while the set of species present is categorical and changes discretely with time. These differences correspond to distinctly different sets of statistical procedures, those dealing with quantitative or continuous measurements and those dealing with categorical measurements.

In spite of these fundamental differences, the underlying rationale is the same in both settings. The factual basis is similar: in both, experimental material is measured at several points in time. Denote this reference data symbolically as $\boldsymbol{Y}(t_1)$, $\boldsymbol{Y}(t_2)$, …, $\boldsymbol{Y}(t_k)$, where $\boldsymbol{Y}(t_i)$ denotes the reference data gathered at time t_i. Information (call it $\boldsymbol{y}$) is collected at the crime scene, such as weight of a maggot or a check list of species present or absent. The datum $\boldsymbol{y}$ is compared to the reference data $\boldsymbol{Y}(t_i)$ to assess whether $\boldsymbol{y}$ and $\boldsymbol{Y}(t_i)$ are consonant. This assessment is in terms of the question, "What is the probability that results as different as $\boldsymbol{y}$ and $\boldsymbol{Y}(t_i)$, or more so, would occur if experimental material for both were measured at time t_i?" Times for which this probability (called a p-value) are not small (a 5% level of

significance often is used as the cutoff between small and not small) are tenable in light of the data, while times for which this probability are small are untenable. Under appropriate assumptions, the set of times found tenable in this way constitutes a confidence set on the time $\boldsymbol{t}$ from which $\boldsymbol{y}$ resulted.

Gaps between times for which reference data are available may be wide. Despite the lack of information in such gaps, it may be reasonable to assume that development or succession characteristics change smoothly within this time interval, so that some form of interpolation can be justified. This will always require models and assumptions, the validity of which may or may not be possible to assess. For the sake of this discussion, though, denote this interpolated information for time t by $\hat{Y}(t)$; in general, $\hat{Y}(t)$ depends on all the reference data, but it may interpolate only information from the two times that bracket t.

Now the statistical method for constructing an interval estimate on t may be seen in general. For each t in some range of possibilities, assess the statistical significance of the difference between y and $\hat{Y}(t)$. Those values of t for which y and $\hat{Y}(t)$ are significantly different are rejected as being untenable in light of the data; those for which the difference is not significant form a confidence set on t. This process is described separately for development data and succession data.

An Interval Estimate of Age from Development Data

We assume that among the population of subjects of age t the distribution of the development characteristic y (such as weight or length) is approximately normally distributed with mean μ_t and variance σ_t^2. The reference data comprise measurements of y on independent samples of n_i subjects at times t_i, $i = 1, \ldots, k$. Denote the sample means by $\bar{Y}_1 \ldots \bar{Y}_k$ and the sample variances by $s_1^2, \ldots, s_k^2$. From this data set, estimates of $\hat{\mu}_t$ and μ_t and $\hat{\sigma}_t^2$ of σ_t^2 can be calculated for each t. We shall assume that $\hat{\mu}_t$ is a linear combination of the sample means $\bar{Y}_1, \ldots, \bar{Y}_k$:

$$\hat{\mu}_t = \sum_{i=1}^{k} a_{ti} \bar{Y}_i .$$

Then the estimate of the variance of $\hat{\mu}_t$ is

$$\hat{\mathrm{V}}\mathrm{ar}(\hat{\mu}_t) = \sum_{i=1}^{k} \frac{a_{ti}^2}{n_i} \sigma_{t_i}^2 = \sum_{i=1}^{k} \frac{a_{ti}^2}{n_i} s_i^2 .$$

We assume that $\hat{\sigma}_t^2$ is a linear combination of the sample variances:

$$\hat{\sigma}_t^2 = \sum_{i=1}^{k} b_{ti} s_i^2 .$$

Given y from a mystery specimen, the test of the hypothesis that y came from a population with mean μ_t and variance σ_t^2 is based on the difference $y - \hat{\mu}_t$ and

$$\hat{\mathrm{V}}\mathrm{ar}(y - \hat{\mu}_t) = \hat{\sigma}_t^2 + \hat{\mathrm{V}}\mathrm{ar}(\hat{\mu}_t) = \sum_{i=1}^{k} \left(b_{ti} + \frac{a_{ti}^2}{n_i} \right) s_i^2 .$$

The test statistic is

$$T = \frac{y - \hat{\mu}_t}{\sqrt{\hat{\sigma}_t^2 + \hat{\mathrm{V}}\mathrm{ar}(\hat{\mu}_t)}} .$$

Unless the population variances are equal (an unrealistic assumption in this setting), T does not follow a Student's t distribution. Satterthwaite's approximation (Kotz, 1988) can be used, in which probabilities for T are approximated from a Student's t distribution with degrees of freedom computed as

$$df_t = \frac{\left(\sum_{i=1}^{k} c_{ti} s_i^2 \right)^2}{\sum_{i=1}^{k} \frac{(c_{ti} s_i^2)^2}{df_i}}$$

where $c_{ti} = b_{ti} + a_{ti}^2/n_i$ and $df_i = n_i - 1$, $I = 1, \ldots, k$. The hypothesis is rejected if the p-value computed from T is less than the chosen level of significance (say 5%); otherwise the proposition is tenable in light of the data. By a result in Lehmann (1986), the set of times t, for which this proposition is tenable, forms a 95% confidence set on the age of the mystery specimen.

Example. Data for this example were simulated to correspond to the data in Wells and LaMotte (1995). They represent dry weights (mg) of larvae of *Cochliomyia macellaria*, 25 which were each collected at ages 2.5 days and 3.0 days under controlled laboratory conditions. Figure 8.3 shows scatterplots of the two sets of weights, plotted as +. A linear interpolation model is assumed for the means μ_t (corresponding to the middle line in Figure 8.3 joining the two sample means) and variances σ_t^2 of weights for ages between 2.5 and 3.0 days. It is assumed further that weights at time t are normally distributed with mean μ_t and variance σ_t^2. Based on the data and these assumptions, a 95% prediction interval on weight y at time t can be constructed. This interval is shown in Figure 8.3 for $t = 2.75$ days. The shaded area is the area swept by all such prediction intervals for t between 2.5 and 3.0 days.

Summary statistics for the simulated reference weights of *C. macellaria* larvae are listed in the following table:

t_i	n_i	$\bar{Y}_I$	s_i^2	df_i
2.5	25	3.4880	0.77110	24
3.0	25	19.6160	9.08723	24

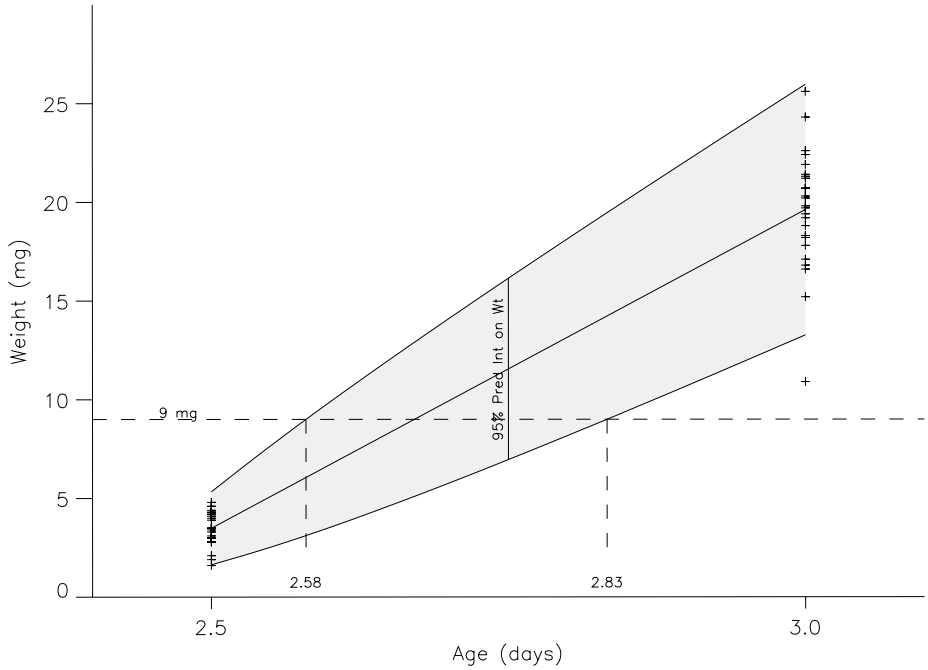

Figure 8.3 Simulated dry weights (mg) of *Cochliomyia macellaria* larvae by age (days), 25 each at 2.5 days and 3.0 days, plotted as +. The region swept by 95% prediction intervals on weight at each age is shaded. Means and variances are interpolated linearly. The ages 2.58 days and 2.83 days, at which the horizontal line at the weight 9 mg of the mystery specimen intersects the curves of upper and lower prediction limits, are the endpoints of an approximate, 95% confidence interval on the age of the mystery specimen.

Suppose the weight of a mystery specimen of *C. macellaria* is 9 mg. Is it reasonable to think that such a larva might be 2.75 days old? Denote by μ the mean weight in the population from which the mystery specimen came. The *t*-statistic for testing H_0: $\mu - \mu_{2.75} = 0$ is constructed as follows:

1. Estimate $\mu - \mu_{2.75}$ by $y - \hat{\mu}_{2.75} = 9 - (0.5\bar{Y}_{0.25} + 0.5\bar{Y}_{3.0}) = -2.552$.
2. Express the variance of this estimator in terms of the population variances:

$$\begin{aligned}\mathrm{Var}(y - \hat{\mu}_{2.75}) &= \sigma^2_{2.75} + (0.5)^2\frac{\sigma^2_{25}}{25} + (0.5)^2\frac{\sigma^2_{3.0}}{25} \\ &= 0.5\sigma^2_{2.5} + 0.5\sigma^2_{3.0} + (0.01)\sigma^2_{2.5} + (0.01)(\sigma^2_{3.0}) \\ &= 0.51\sigma^2_{2.5} + 0.51\sigma^2_{3.0}.\end{aligned}$$

3. Estimate the population variances:

$$\begin{aligned}\hat{\sigma}^2_{2.5} &= s_1^2 = 0.77110, \\ \hat{\sigma}^2_{3.0} &= s_2^2 = 9.08723.\end{aligned}$$

4. Estimate the expression from (2) and calculate *df* with Satterthwaite's approximation:

$$\hat{\mathrm{V}}\mathrm{ar}(y - \hat{\mu}_{2.75}) = 0.51\hat{\sigma}^2_{2.5} + 0.51\hat{\sigma}^2_{3.0} = 5.02770$$

$$df_{2.75} = \frac{(0.51s_1^2 + 0.51s_2^2)^2}{\dfrac{(0.51s_1^2)^2}{df_1} + \dfrac{(0.51s_2^2)}{df_2}} = 28.04.$$

The test statistic is

$$T = \frac{-2.552}{\sqrt{5.02770}} = -1.138,$$

from which a two-tailed *p*-value from Student's *t* distribution with 28.04 degrees of freedom is 0.26. H_0 is not rejected at the 5% level of significance, and so $t = 2.75$ days is a tenable age for a larva weighing 9 mg. This procedure can be repeated for other times *t*, replacing the coefficients of $\bar{Y}_{2.5}$ and $\bar{Y}_{3.0}$ by $1 - r_t = 3.0 - t/3.0 - 2.5$ and r_t, respectively, and following those substitutions through the rest of the formulas. Tedious by hand, these calculations are easy to program on a statistical computing package. The least and greatest values of *t* for which this null hypothesis is not rejected are 2.58 days and 2.83 days, respectively. Thus, given the mystery specimen weighing 9 mg and the reference data, a 95% confidence interval on the age of the mystery specimen is the range from 2.58 days to 2.83 days, a span of 6 h.

Comments

The "inverse prediction" approach we have described depends on the assumption that *y* is (at least approximately) normally distributed, and it depends on the assumed form of the relation between the population mean μ_t and time *t* and between the population variance σ_t^2 and *t*.

The closer together are the time points for which reference data are available, the less important will be the assumed relations for μ_t and σ_t^2, and linear interpolation should be sufficient. However, if μ_t and σ_t^2 change rapidly and nonlinearly with *t*, then some other sort of interpolation should be undertaken. It is appealing at first to consider fitting growth-curve models or to use smoothing or nonparametric regression techniques here. However, each such approach involves its own set of assumptions and arbitrary modeling choices. We feel that the approximations we have used are generally sufficient, and they are easy to understand. In a case where linear interpolation is troublesome, one might use three-point quadratic interpolation. Otherwise, if it is practicable, reference data could be gathered at one or more intermediate time points.

If the distribution of *y* at time *t* is not reasonably bell-shaped, so that an assumption of normality is unreasonable, it may be possible to transform *y* so that its distribution is more nearly normal. There is an extensive literature on classes of such transformations (see, for example, Atkinson, 1987).

Other approaches are possible that do not involve distributional assumptions. As an example, prediction bounds might be approximated by upper and lower percentiles (2.5%,

for example) of the empirical relative frequency distributions, interpolated linearly between reference data time points. We have experimented with this approach, and its results were not strikingly different from results of the approach we described in the preceding section.

The approach we have described can be extended to use multivariate development data, such as weight and length, to construct a prediction interval on age (Oman and Wax, 1984).

Estimating Age from Succession Data

The statistical questions involved in estimating PMI from successional data can be described in terms of an example. Consider a hypothetical field experiment in which n = 10 carcasses were exposed for 7 days. Each day, the presence or absence of each of two species, A and B, was recorded for each carcass. The occurrence data are shown in the following table. Denote the four possible species combinations as follows: A and not B = AB′; A and B = AB; B and not A = A′B; and not A and not B = A′B′. On Day 3, for example, nine carcasses had AB, one had A′B′, and none had either AB′ or A′B.

	Day						
Species	1	2	3	4	5	6	7
AB′	9	1	0	0	0	0	0
AB	1	8	9	5	0	0	1
A′B	0	1	0	4	10	4	0
A′B′	0	0	1	1	0	6	9

In a population of subjects in which each subject falls into exactly one of c disjoint and exhaustive categories, if n subjects are sampled independently and at random, then the joint probability distribution of the frequencies with which the n sampled subjects fall into the c categories is a multinomial distribution. Denote the set of observed frequencies by $f = (f_1, \ldots, f_c)$; each f_i is a nonnegative integer, and the sum of the c f_is is n. Over all possible samples, the expected value of f/n is $\pi = (\pi_1, \ldots, \pi_c)$, where π_i is the proportion of the subjects in the population that are in category I.

On Day 4, the observed frequencies of the four species combinations were 0 for AB′, 5 for AB, 4 for A′B, and 1 for A′B′; denote this set of frequencies by $f_4 = (0,5,4,1)$. Suppose a mystery carcass is found and on it species A is found and species B is not found, so it has species combination AB′; denote its set of frequencies by $y_* = (1,0,0,0)$. The comparison between the reference data f and the mystery datum y_* can be represented as a contingency table, shown below.

	Reference		
Category	Data	Mystery	All
1	0	1	1
2	5	0	5
3	4	0	4
4	1	0	1
All	10	1	11

The question, whether the PMI of the mystery carcass could be 4 days, becomes a question, whether it is reasonable to think that y_* and f_4 could have resulted from random samples from the same population.

Denote the set of species-combination proportions in the Day 4 population by π_4 and in the population from which y_* came as π_*, so that we want to know whether $\pi_4 = \pi_*$. Standard methods for testing this hypothesis in the contingency table lead to a χ^2 statistic of 11, with a p-value of 0.012 (from the χ^2 distribution with three degrees of freedom), and a p-value for Fisher's exact test (FET) of 0.182. These tests are easy to compute, even by hand, but unfortunately they appear to lead to contrary conclusions. With a sample of size one, it is unreasonable to expect the χ^2 approximation to be good. The FET p-value, although it is exact, is a conditional probability, fixing the row and column totals, and it tends to be conservative when compared to corresponding unconditional probabilities (McDonald et al., 1977). LaMotte and Wells (2000) present exact, unconditional p-values for this setting, one by "unconditioning" the Fisher exact test and the other based on a likelihood ratio (LR) test. They are more difficult to compute than the FET p-value or the χ^2 approximation. Our intent here is to describe the nature of these tests, but without going into all details of the calculations (see Lamotte and Wells for those).

The set of frequencies $f_4 = (0,5,4,1)$ is one possible outcome from a sample of 10 subjects. Any other set of frequencies that sum to 10 is also a possible outcome. There are 286 distinct such outcomes. Given the probabilities π_4, a multinomial probability can be computed for each outcome. For the mystery subject, there are four possible outcomes; given the category probabilities π_* in the population from which the mystery subject came, the probability of each outcome y_* is just the probability π_{*I} for the category into which the mystery subject falls. Assuming that the 11 subjects are sampled independently, the probability of the joint outcome f_4 and y_* is the product of their respective probabilities. Calculating the probability of any particular outcome, or any set of outcomes, is straightforward, and that probability depends on π_4 and π_*.

We want a p-value to assess the proposition H_0: $\pi_4 = \pi_*$. Given the outcome (f_4, y_*), suppose that we can list all the other outcomes that are as unfavorable to H_0 as, or more so than, this outcome is. Call this set of outcomes, including the realized outcome (f_4, y_*), the *extreme set* for (f_4, y_*), and denote it by $C(f_4, y_*)$. The probability of this set of outcomes, computed as if H_0 is true, is a p-value, but it depends on the particular value of the set π of category probabilities. A conservative approach is to present the greatest value this probability could be over all possible values of π_4. Finding the maximum value of the probability of $C(f_4, y_*)$ is where the computational difficulties begin. This is a multidimensional, constrained, nonlinear optimization problem. We have found an efficient way to perform this computation when extreme sets are defined in terms of likelihood ratios. For extreme sets corresponding to the FET, though, we have not found any such solutions, so we have had to rely on general-purpose computational algorithms, and they can be prohibitively slow for practical applications.

Different definitions of "outcomes as unfavorable to H_0 as the observed outcome" lead to different p-values. "Unfavorable" is defined in terms of an ordering, usually by a real-valued function that assigns a value to each possible outcome. The orderings corresponding to the likelihood-ratio statistic and the χ^2 statistic are equivalent in the current setting, while the ordering corresponding to the FET conditional p-value is different.

Figure 8.4 shows four p-values for the hypothetical data given above, for each day separately. The "True" p-value is included for comparison. It is calculated from the known

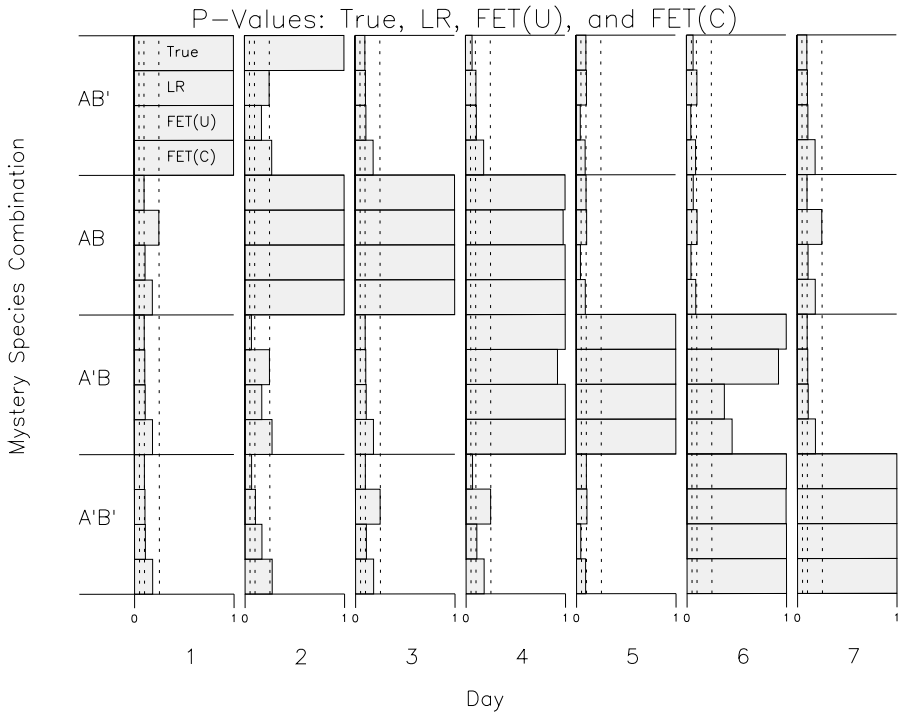

Figure 8.4 *P*-values for each possible species combination from a mystery specimen, by PMI (days), computed from the occurrence data. Vertical dotted lines are drawn at 5, 10, and 25%. In each of the four panels for each day, the top bar depicts the "True" *p*-value; the second bar (LR) depicts the likelihood-ratio *p*-value; the third (FET(U)), the unconditional Fisher's exact test *p*-value; and the bottom (FET(C)), the conditional Fisher's exact test *p*-value.

probabilities from which the data were simulated, and it is defined, for mystery category ℓ, as the sum of the category probabilities π_i that are less than or equal to π_ℓ. The LR *p*-value is the probability of outcomes with likelihood ratio statistic greater than or equal to the likelihood ratio statistic of the observed outcome. FET(C) is the two-sided Fisher exact test *p*-value. FET(U) applies the FET ordering to all outcomes, not just those with the observed marginal totals. Vertical dotted lines show 5, 10, and 25%. Looking at the top row, corresponding to a mystery species combination AB′, the LR, FET(U), and FET(C) *p*-values are large for Day 1, moderate for Day 2, and fairly small for Days 3 to 7. The FET(C) *p*-value is generally greater than the LR *p*-value and the FET(U) *p*-value. Comparisons over the contents of the figure reflect our experience: the FET(C) *p*-value is consistently greater than the FET(U) *p*-value, hence the FET(C) *p*-value is unnecessarily conservative. The FET(U) *p*-value is a little more refined than the LR *p*-value for outcomes that have somewhat small *p*-values. However, the as yet unsolved computational difficulties of the FET(U) *p*-value make it unattractive for now. The FET(C) *p*-value is so simple to calculate that it can easily be done by hand. The LR *p*-value can be calculated easily, but it requires a short computer program to perform the optimization.

Comments

Other than a basic independent-sampling model, no assumptions are required to justify the *p*-values described in the last section. The number of categories *c* required to describe

the species combinations for m species is 2^m. An alternative to keeping a category for every possible species combination is to group them into a more manageable number of categories. That the number of categories may be large, coupled with the expense and difficulty involved in getting relevant reference data in this setting, emphasize the need to select the species and define the categories carefully so that the probability distribution π_t of the c categories at PMI t changes discernibly with t.

These analyses have yet to be put into practice. We hope that forensic entomologists will evaluate them using their own experimental and case data. The authors also have found that a close collaboration between an entomologist and a statistician is particularly helpful when grappling with the basic methodological issues of a rapidly developing science.

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Insect Development and Forensic Entomology

9

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Contents

Introduction

When using insects as indicators of postmortem interval, two major approaches are possible. The first is to use the presence or absence of a species as an indicator of time of death, based on understandings of insect successional patterns. The second is to consider the degree of development for insects found on the cadaver. These approaches are complementary, although measuring insect development requires that immature insects are still present on the body. This latter approach depends on backtracking from the observed degree of development to the time of oviposition, to convert insect development into a time estimate. The time of death then can be estimated by adding the interval between death and oviposition for the species in question (recognizing that various factors can influence initial oviposition). Measuring insect development is a powerful method for providing estimates of postmortem interval, but there are many crucial considerations and potential limitations in making such estimates. In this chapter we will explore these details of estimating insect development and their implications for forensic entomology. Some general reviews on temperature and development include Wagner et al. (1984), Higley et al. (1986), and Higley and Peterson (1994). Also, Catts and Goff (1992) includes a discussion of age determination of maggots by temperature for forensic entomology.

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Temperature and Insect Development

The key observation regarding insect development is that the time of development depends on temperature. This point was first recognized in the early 1700s by the French scientist Reaumur (Reaumur, 1735), but methods for using this understanding to describe or predict insect development mostly date from the 1950s (Arnold, 1959; 1960) through to the present. Of course, the growth and development of all organisms is temperature-dependent, but for organisms with constant body temperature, we can speak of development merely in terms of time without explicitly considering temperature. However, organisms in the size range of insects cannot maintain constant body temperature; these are what are called poikilothermic animals. Larger organisms can maintain a constant (or at least more constant) temperature through metabolic heat, but this is not possible for even the largest insects. Also, for some organisms (plants for example), other factors such as photoperiod may be almost as important as temperature in determining the sequence of developmental events.

All growth depends on temperature because the biochemical reactions that are the ultimate basis for growth are themselves temperature-dependent. At this level, heat of reactions, enzyme thermal properties, and membrane permeabilities all contribute to the temperature requirements for growth. In a very influential paper, Sharpe and DeMichele (1977) proposed that temperature limits on growth were a consequence of rate-limiting reactions controlled by corresponding rate-limiting enzymes. From theoretical arguments, practical models for determining insect development rates based on the notion of rate-limiting enzymes have been developed and used for many insect species (Wagner et al., 1984). Unfortunately, the idea that development is only a function of rate-limiting enzymes ignores the potential importance of other factors such as membrane permeability, and experimental evidence does not support many assumptions that follow from this model (Hilbert and Logan, 1983; Lamb et al., 1984; Higley and Peterson, 1994). Consequently, there is no single biologically based model to account for how temperature alters growth rates.

In the absence of a definitive theoretical understanding of the relationship between temperature and growth, empirical understandings of these relationships must be used. The general relationship between development rate and temperatures is described by the temperature development curve indicated in Figure 9.1 (Higley and Peterson, 1994). The relationship is curvilinear at low and high temperatures and linear in between. The lowest temperature at which development can proceed is called the developmental minimum (or minimum threshold) and the highest temperature is called the developmental maximum (or maximum threshold). Threshold temperatures can be difficult to estimate (as we will discuss shortly), and the upper threshold typically occurs near the upper lethal temperature for a species. The temperature development curve exists for all insect species, although the specifics of the curve will vary by species. Additionally, within species variation occurs, particularly at the extremes of the temperature range of a species. Why the low temperature portion of the development curve is curvilinear has been the topic of some speculation. Although some had argued that this part of the curve merely reflects genetic variation among insects, in an elegant experiment using pea aphid *Acyrthosiphon pisum* (Harris) clones, Lamb (1992) demonstrated that the curvilinear relationship between temperature and development rate is a reflection of the underlying physiology of development and not genetic variation among individuals.

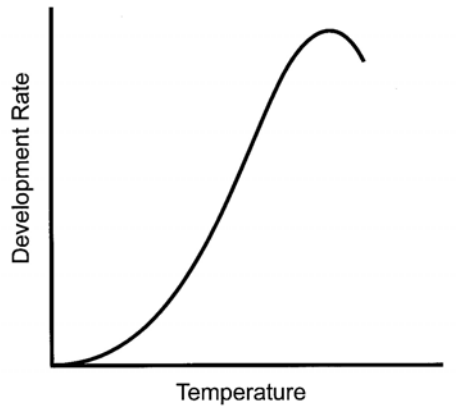

Figure 9.1 The thermal development curve — the generalized relationship between temperature and rate of development.

Describing the temperature development curve for an insect species is difficult, in that it requires substantial replication of development with many individual insects at multiple temperatures. Also, obtaining reliable development rates at low and high temperatures is particularly challenging, because these often are close to or at lethal temperatures for the species.

Measuring Insect Development

Broadly speaking, there are three approaches for measuring insect development: physiological, curvilinear, and linear (degree-day). However, the physiological approach (based on the Sharp and DeMichele model) seems to have no more theoretical validity than the curvilinear approach, so it might be more accurate to speak of only two approaches: curvilinear and linear. Essentially, the curvilinear approach seeks to describe the temperature development curve and determine insect development from this. The linear approach approximates most of the development curve as a line, with cut-offs at high and low temperature. Both methods are essentially regression approaches.

Because the temperature development curve is undoubtedly curvilinear, it seems logical that a curvilinear approach should be better than a linear approach. However, this does not necessarily seem to be the case. First, there is no consensus around a single curvilinear equation to use to describe the temperature development curve (see Wagner et al., 1984 for a review of various approaches). Second, determining a curvilinear model for the temperature development curve, requires a great deal of research and is relatively complicated to use in practice. Finally (and perhaps most importantly), various field studies have shown either no improvement of curvilinear models over linear models or better predictions from linear models for several insect species (e.g., Hochberg et al., 1986; Stinner et al., 1988; McClain et al., 1990). Perhaps the failure of curvilinear models to outperform linear models in some instances is related to accumulation of errors in the estimates of parameters needed to drive such models. In any event, on a practical level, linear models often seem to perform adequately (and occasionally better than their curvilinear counterparts).

Linear models of development assume a constant increase in development rate with increasing temperature. They further assume no development occurs below the minimum developmental threshold and assume a constant development rate occurs at or above the maximum threshold (in reality the reverse may be true, although these are probably at species lethal limits so the issue of development rate becomes moot). Linear models are most commonly called degree-day models because development is regarded as a combination of temperature above the minimum developmental threshold multiplied by time. Thus, 5 degrees above the minimum developmental threshold for 2 days represents 10 degree days (5 × 2), just as does 1 degree above the minimum developmental threshold for 10 days (1 × 10). An accumulated number of degree-days are associated with specific developmental events like egg hatch, stage transitions, oviposition to adult emergence, etc.

Determining the developmental thresholds and degree-day accumulations for specific developmental events is an important prerequisite to using degree-days. Various approaches are available for estimating the minimum developmental threshold (see Higley et al., 1986 or Higley and Peterson, 1994 for more details). The most commonly used method is the *x*-intercept approach, in which development rates are measured in the low temperature range and results are fit in a linear regression (Arnold, 1959). The linear regression can then be extrapolated to the *x*-axis where the development rate is zero. Although the *x*-intercept method is clearly an approximation, it seems to work for many, even most, species. Unfortunately, to the best of our knowledge calculated estimates of developmental minima are not yet available for forensic insect species.

Once developmental minimum and degree-day accumulations for life history events are determined, all that remains is to calculate degree-days with actual temperature data. It is critical to use a minimum developmental threshold temperature even if temperatures are above this threshold. More specifically, a given degree-day or degree-hour accumulation must be based on an associated developmental minimum. Occasionally one finds accumulations reported without an explicit developmental minimum (e.g., Greenberg, 1991); presumably such accumulations are based on an implicit developmental minimum of 0°C (32°F). Because few insects are likely to actually have a developmental minimum of 0°C (32°F), there is little or no biological basis for using 0°C (32°F) as a default developmental minimum. Calculations without an appropriate threshold will overestimate degree-day accumulations. When daily temperatures are below the developmental minimum, it is important that degree-day accumulations are set to zero (otherwise, you would have the absurd situation of calculating negative degree-days, implying an insect is growing younger).

As a practical matter, using an incorrect developmental minimum is not a problem as long as daily temperatures are above the actual developmental minimum. However, when daily temperatures do span the developmental minimum, failure to use the correct developmental minimum can result in significant errors in estimating accumulated degree-days. Based on personal observations (N. H. Haskell), developmental minima of related fly species and some data in the literature (e.g., Byrd and Butler, 1996), flies of forensic importance likely have developmental minima between 6°C (42°F) and 10°C (50°F), which are useful approximations at present. Obviously, determining precise developmental minima for forensic species is a crucial research need.

This degree-day (or degree-hour) process involves estimating the area under a daily temperature curve that is above the minimum developmental threshold (Figure 9.2). Continuous, or even hourly, temperature records would provide the most accurate degree-day determination, but these are rarely available in practice (particularly for forensic

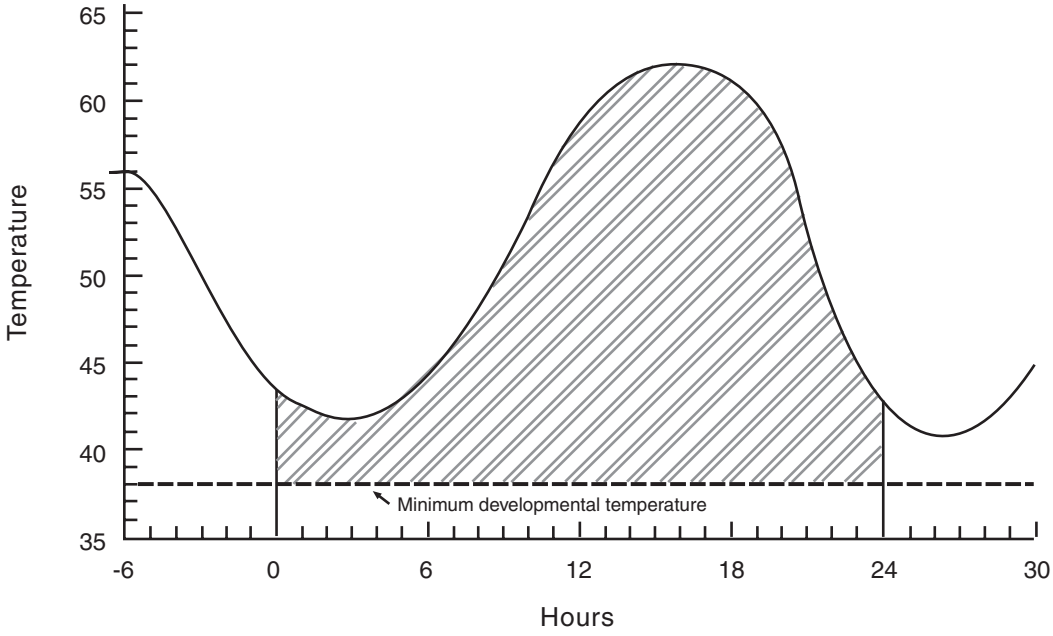

Figure 9.2 Degree-days, a measure of physiological time, indicated as the area under a daily temperature curve and above a minimum developmental temperature for a species.

determinations). However, hourly temperatures may be available from class I weather service stations, and can be useful if the station is not too far from the crime scene. Higley et al. (1986) describe the various estimation methods, compare the results for different locations in the U.S., and offer recommendations on appropriate methodology. Briefly, the standard technique is called the rectangle method because daily degree-days are measured as a box with a length of one day and a height of the mean daily temperature minus the minimum developmental threshold (Figure 9.3). As illustrated in Figure 9.3, in principle the area of the daily temperature curve above the mean daily temperature corresponds to those areas below the mean daily temperature and above the daily temperature curve. Consequently, it is possible to calculate the area under the curve as a rectangle, as shown in Figure 9.3. Arnold (1960) discusses this method in detail and points out limitations in estimating the mean daily temperature by averaging the daily high and low temperatures, including possible seasonal bias. Other approaches for measuring the area under the curve include estimating the daily temperature curve on a half-day basis with triangles or as a sine wave (Allen, 1976). Probably the sine wave method is the most accurate approach, although Higley et al. (1986) found that the gain in accuracy of the sine wave over the rectangle approach was negligible.

In making development estimates, the resolution of our estimates can be an important issue, particularly in forensic estimates. By resolution, we refer to differences between degree-days and degree-hours. If the data for calculating development are limited to daily maximum and minimum temperatures, then estimates should be limited to degree-days. Alternatively, if hourly temperatures are available, then degree hours may be calculated. It is not appropriate to convert degree-days to degree-hours because this implies a level of precision that does not exist, unless development actually is determined by hour. One can convert degree hour computations to degree-days because the definition of degree-days

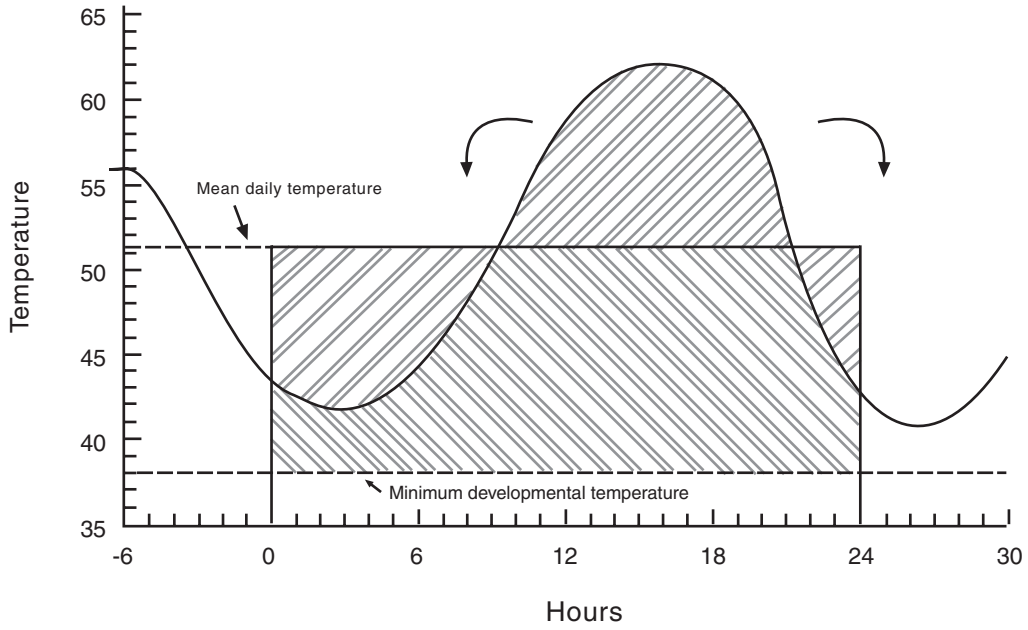

Figure 9.3 Rectangle calculation method for determining degree-days. The area calculated is that of a rectangle 1 day long and the mean daily temperature less minimum developmental temperature wide. As indicated in the figure, this method works because the area of the rectangle not under the daily temperature curve is equal to that portion of the curve above the rectangle (see text or Arnold (1960) for additional explanation).

uses a daily mean temperature for the calculation. A similar argument applies in calculating fractional degree-days. Under most instances, the thermal accumulation from midnight to noon will not equal the thermal accumulation from noon to midnight (typically noon to midnight is warmer). Also, the movement of frontal systems over a location can dramatically alter thermal accumulations, because the daily pattern of temperature change is radically altered. However, in the absence of radical temperature change, it may be possible to calculate degree days by half days, by using the daily high and low coupled with the previous day's low (see Allen, 1976 or Higley et al., 1986 for more information). When fractional degree-day accumulations are significant for determining insect development, it is essential to have data on weather patterns (frontal systems), precipitation, and (most importantly) temperatures at intervals more frequent than once or twice a day.

Limits to Estimates of Insect Development

A great many factors can influence estimates of insect development. Frequently errors will occur in both directions (retarding or accelerating development), so the net effect is to cancel each out (Higley et al., 1986). However, some factors affect development in only one direction and can systematically bias results.

Perhaps the most obvious issue is that insects actually experience fluctuating temperatures; however, most developmental thresholds and accumulations are determined under constant temperature. This area has received much study and debate. On a practical level, the influence of fluctuating temperatures seems to be more important for some species

than others (e.g., *Phormia regina* (Meigen)) (Greenberg, 1991). On a theoretical level, the issues of fluctuating temperature are more predictable. For simple mathematical reasons, fluctuations in the lower temperature portion of the development curve will allow development to proceed more rapidly than predicted from a constant temperature; whereas fluctuations in the upper temperature portion of the development curve will allow development to proceed more slowly than predicted from a constant temperature. This phenomenon is called the rate summation effect, and it is a reflection of the fact that contributions to development around a mean in the curvilinear portions of the temperature development curve are not equal (see Figure 9.4 for a graphical explanation of the rate summation effect). Specifically, in the lower (concave) portion of the temperature development curve, the differences in development at an interval above and below a mean

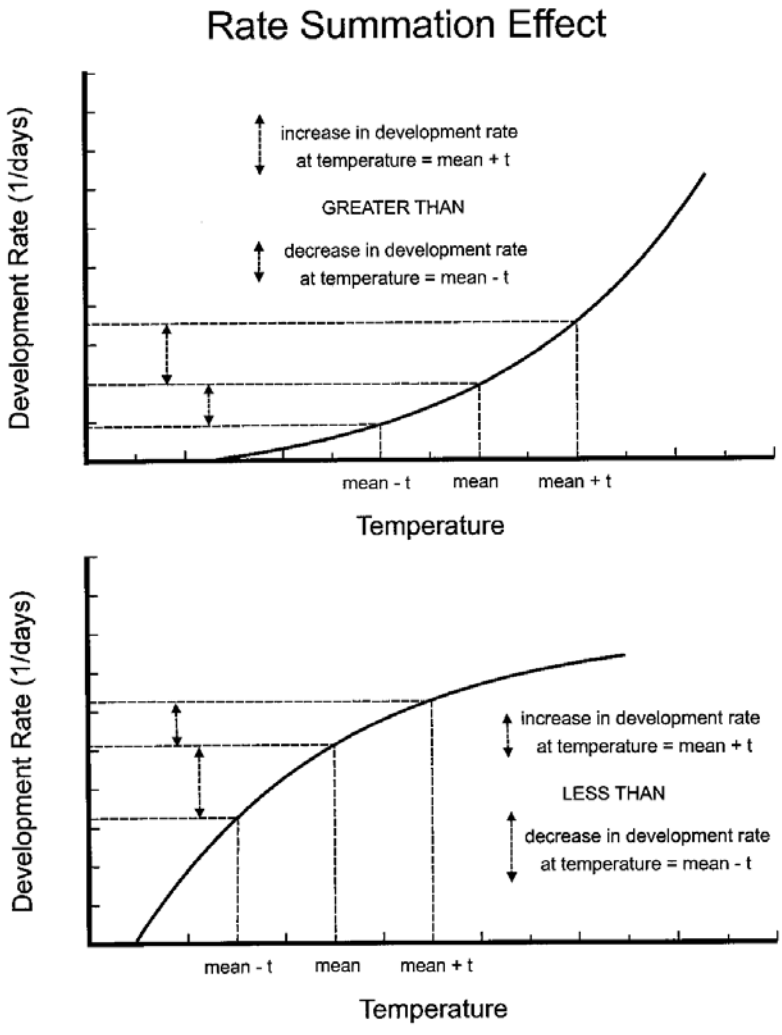

Figure 9.4 The rate summation effect. Low temperature fluctuations (t) above and below a given mean produce a development rate greater than that predicted by the mean temperature because fluctuations above the mean increase development rates proportionally more than fluctuations below the mean reduce development rates. The reverse is true for fluctuations at high temperatures.

temperature are not equal (greater development occurs above the mean). The reverse is true for the high temperature (convex) portion of the curve. Tanigoshi et al. (1976) and Worner (1992) provide more comprehensive descriptions of this principle. Worner points out that it is not clear if there are effects on development under fluctuating temperature beyond the rate summation effect. Implications of fluctuating temperatures on development of forensic fly species are offered in Catts and Goff (1992) and Greenberg (1991).

Obviously, errors or approximations in the estimates of developmental thresholds can significantly bias degree-day estimates. Additionally, nutritional status and hormonal regulation of development can alter development (typically prolonging it). However, arguably the single most important factor is the thermal environment in which insects occur. Degree-day estimates typically are based on weather service data, but these data can vary strikingly from the thermal environment occupied by insects, particularly forensic species. Baker et al. (1985) provide a valuable summary of many factors resulting in discrepancies between weather service and actual temperatures in the field, including such issues as observation time, surface structure, topography, and urbanization. Also, insects themselves can have some degree of thermoregulation (May, 1979), and this is especially pertinent for masses of maggots that can generate large amounts of metabolic heat.

Considerations Surrounding Insect Development and Forensic Species

Almost all work surrounding insect development has focused on agricultural or medically important insect species. In these instances, the question typically involves estimating time of potential occurrence of a pest species. In contrast, for ecological research and forensics, development is typically important in backtracking to determine time of occurrence of a specific event (oviposition).

Agricultural Species vs. Forensic Species

Are there differences between agricultural and forensic species regarding development? Obviously all insects are poikilotherms, and there is no reason to think these types of insects should differ. Moreover, "agricultural" and "forensic" designations are arbitrary human designations, not indications of biological difference, per se. A more telling question is to consider if herbivorous species and saprophagous or necrophagous species are different regarding thermal development. Given that some agricultural pests are also saprophagous (for example, the anthomyiid fly, *Delia platura* (Meigen)), direct evidence indicates that the same principles apply to both groups. The important observation is that differences in the thermal environment rather than differences intrinsic to species ecological roles are likely to have the greatest impact on development.

A more significant issue is the differences in uses and expectations for insect development in a forensic setting as compared to an agricultural application. Development estimates in agriculture are typically associated with making predictions about occurrence of an injurious insect population (Higley and Peterson, 1994). This prediction is for a widely distributed population or populations of an insect species over an equally wide geographic range. In contrast, forensic uses of insect development are related to a very small population in a very specific site. Ideally, we want forensic estimates to be as accurate and precise as possible; certainly much more than is necessary for agricultural predictions.

Developmental Parameters for Forensic Species

Ironically, current insect development information is much more precise for agricultural pests than for forensic species. There is a critical need for thorough determinations of developmental thresholds and stage-specific degree-day accumulations for all major forensic species. Currently, many, even most, of our estimates of developmental thresholds and stage-specific accumulations for forensic species are based on only a few studies with relatively limited data sets. Work by Kamal (1958) on developmental thresholds for 13 carrion-feeding flies has been a standard reference in forensic entomology, although some arithmetic errors in that publication have been noted (Catts and Goff, 1992). Subsequent examinations include work by Nishida (1982), Nishida et al. (1986), Vinogradova and Marchenko (1984), Introna et al. (1989), Greenberg (1991), and Byrd and Butler (1996, 1997, 1998).

In Table 9.1, we used data from Kamal (1958), Greenberg (1991), Byrd and Butler (1996, 1997, 1998), and Greenberg and Wells (1998) to develop estimates of accumulated degree-days for the egg-to-adult development of various forensic species. Given the absence of calculated minimum developmental thresholds, threshold data in Table 9.1 are based on personal observation (N. H. Haskell). Some of the source data are based on limited temperatures and replications, which accounts for much of the variability seen in the calculations. More reliable estimates of degree-day accumulations clearly are possible with more comprehensive experimentation, as evidenced by the outstanding data for *Cochliomya macellaria* (Fabricius) from Byrd and Butler (1996). However, even with ideal data, variation in estimates of about 10% seems likely for any given insect population.

Despite their limitations, values in Table 9.1 provide some practical basis for making development estimates, particularly in estimating potential minimum development times. Nevertheless, there is a continuing need to refine and improve these values. Precise values for developmental minima and degree-day estimates by stage (specifically egg, total larva, pupa, and, ideally, individual larval stage) are important areas for improvement. Additionally, studies characterizing variation in these parameters between geographically distinct populations of the same species would be of great value. Although it may be true that "most workers favor the use of locally generated development-rate data" (Catts and Goff, 1992), ultimately the forensic entomology community must move to consensus regarding temperature development parameters for forensic insect species. If significant geographic variation in development parameters exist, these need to be quantified. Similarly, clear expressions of variability in these parameters are essential to provide meaningful indications of variation in estimates of insect development.

Development and Maggot Mass Temperatures

A related issue of particular importance to forensic uses of insect development information is the importance of maggot mass temperature. The metabolic heat generated by a mass of maggots on dead tissue can be sufficient to raise the temperature of the mass well above ambient temperatures. Maggot mass temperatures may routinely occur over 20°C (68°F) above ambient (Deonier, 1940; Catts and Goff, 1992; Haskell, 1989). At moderate ambient temperatures, about 25°C (77°F), maggot mass temperatures can exceed 45°C (113°F), and in cool or cold ambient conditions (even below freezing), maggot mass temperatures may be sufficient to allow continued development. This also is true when a heavily infested

Table 9.1 Various Forensic Fly (Calliphoridae, Phoridae, and Sarcophagidae) Species with Their Corresponding Minimum Developmental Thresholds and Experimentally Determined Centigrade Degree-Day (CDD) and Centigrade Degree-Hour (CDH) Accumulations for Egg-to-Adult Emergence, Calculated from Data in Six Sources

Species	Dev. Min. (°C)	Dev. Environ. (°C)	Total Dev. Time (h)	CDD	CDH	Data Ref. in Source
Source: Kamal (1958)[a]						
Calliphora terraenovae	6	26.7	551	475	11406	Table 1
Calliphora vicina	6	26.7	508	438	10516	Table 1
Calliphora vomitoria	6	26.7	854	737	17678	Table 1
Cynomyopsis cadaverina	6	26.7	439	379	9087	Table 1
Eucalliphora lilaea	10	26.7	330	230	5511	Table 1
Phaenicia sericata	10	26.7	348	242	5812	Table 1
Phormia regina	10	26.7	309	215	5160	Table 1
Protophormia terraenovae	10	26.7	301	209	5027	Table 1
Sarcophaga bullata	10	26.7	498	347	8317	Table 1
Sarcophaga cooleyi	10	26.7	402	280	6713	Table 1
Sarcophaga shermani	10	26.7	382	266	6379	Table 1
Source: Greenberg (1991)[b]						
Calliphora vicina	6	10	1647	275	6588	Table 3
	6	12.5	1069	290	6949	Table 3
	6	19	583	316	7579	Table 3
	6	25	460	364	8740	Table 3
	6	10	1676	279	6704	Table 7[d]
	6	12.5	1063	288	6910	Table 7[d]
	6	19	562	304	7306	Table 7[d]
	6	22	446	297	7136	Table 7[d]
			Mean	302	7239	
			SD	29	685	
Chrysomya rufifacies	10	22.5	362	189	4525	Table 6[e]
	10	22	369	185	4428	Table 7[d]
	10	29	306	242	5814	Table 7[d]
			Mean	205	4922	
			SD	32	774	
Cochliomya macellaria	10	22.5	285	148	3563	Table 6[e]
	10	22	320	160	3840	Table 7[d]
	10	29	234	185	4446	Table 7[d]
	10	35	193	201	4825	Table 7[d]
			Mean	174	4168	
			SD	24	572	
Megaselia scalaris	10	22	527	264	6324	Table 4
	10	29	266	211	5054	Table 4
			Mean	237	5689	
			SD	37	898	
Phaenicia sericata	10	22	345	173	4140	Table 1
	10	29	296	234	5624	Table 1
	10	22.5	359	187	4488	Table 6[e]
	10	22	349	175	4188	Table 7[d]
	10	29	272	215	5168	Table 7[d]
			Mean	197	4722	
			SD	27	650	
Phormia regina[c]	10	22	336.5	168	4038	Table 2
	10	29	279	221	5301	Table 2

Table 9.1 Various Forensic Fly (Calliphoridae, Phoridae, and Sarcophagidae) Species with Their Corresponding Minimum Developmental Thresholds and Experimentally Determined Centigrade Degree-Day (CDD) and Centigrade Degree-Hour (CDH) Accumulations for Egg-to-Adult Emergence, Calculated from Data in Six Sources *(continued)*

Species	Dev. Min. (°C)	Dev. Environ. (°C)	Total Dev. Time (h)	CDD	CDH	Data Ref. in Source
	10	22.5	358.5	187	4481	Table 6[e]
	10	22	344	172	4128	Table 7[d]
	10	29	264	209	5016	Table 7[d]
	10	35	244	254	6100	Table 7[d]
			Mean	202	4844	
			SD	33	789	
Source: Byrd and Butler (1996)						
Cochliomya macellaria	10	15.6	588	137	3293	Table 2
	10	21.1	297	137	3297	Table 2
	10	25	240	150	3600	Table 2
	10	26.7	177	123	2956	Table 2
	10	32.2	170	157	3774	Table 2
			Mean	141	3384	
			SD	13	315	
Source: Byrd and Butler (1997)						
Chrysomya rufifacies	10	15.6	612	143	3427	Table 2
	10	21.1	314	145	3485	Table 2
	10	25	289	181	4335	Table 2
	10	26.7	222	154	3707	Table 2
	10	32.2	180	167	3996	Table 2
			Mean	158	3790	
			SD	16	378	
Source: Byrd and Butler (1998)						
Sarcophaga haemorrhoidalis	10	15.6	802	187	4491	Table 2
	10	21.1	504	233	5594	Table 2
	10	25	456	285	6840	Table 2
	10	26.7	252	175	4208	Table 2
	10	32.2	360	333	7992	Table 2
			Mean	243	5825	
			SD	66	1595	
Source: Greenberg and Wells (1998)						
Megaselia abdita	6	10	3462	577	13848	Table 2
	6	12.5	1428	387	9282	Table 2
	6	23	415	294	7055	Table 2
			Mean	419	10062	
			SD	144	3463	

Note: Because experimentally determined developmental minimum thresholds are lacking, values listed here should be regarded as approximations (see text for additional discussion).

[a] Hours reported here are recalculated from Kamal (1958) Table 1 by summing individual stages, because totals reported in Kamal (1958) do not match sum of reported development by stage.

[b] Data with only one replication not included. Degree hours from Greenberg (1991) were presented without a reported developmental mimimum temperature, therefore we assume 0°C was used as the (implicit) developmental minimum.

[c] Although a developmental minimum of 10°C is indicated for *Phormia regina*, some evidence (Deonier, 1940) suggests that *P. regina* may be more tolerant to cool temperatures and a lower developmental minimum may be appropriate.

[d] Converted from CDH.

[e] Mean of two replications.

body is placed into a morgue cooler over a weekend. Maggot growth and development may not be arrested by the cool temperatures and feeding can continue with additional destruction of tissues. Even with temperatures in the morgue cooler at approximately 38°F (3°C), temperatures have been recorded as high as 90°F (32°C) within the maggot mass on a body which had been in the cooler for over 48 h (Haskell, personal communication). Obviously, it is essential to account for maggot mass temperature in determining insect development. Principally, this is an issue for later stage maggots, rather than eggs or earlier stages that do not generate substantial metabolic heat. Consequently, ambient temperatures may be used to determine development for earlier stages and maggot-mass temperatures for later instars.

How can we account for maggot mass temperature? The maggot mass temperature will be a function of the size of the maggot mass, stage of development, location on corpse (which affects the relationship of mass temperature to ambient temperature), and ambient temperatures (Catts and Goff, 1992). A common approach is to measure the maggot mass temperature and use this as a constant in determining development. This temperature can be well over 100°F (37°C) and may be at or near the lethal high temperature for a species. Obviously other influences are occurring because maggots remain alive in the mass at such a high temperature. A key principle here is that maggot mass temperatures are not uniform. From the center of the mass to the outside edge, a temperature drop of 6°C (42°F) or more is possible. Because maggots in the mass move throughout the mass for feeding, breathing, and thermoregulation, maggots will be exposed to this range of temperatures. Also, because mass temperatures often are near the upper temperature limit for a species, it is likely that using a mean mass temperature may overestimate development (based on rate summation effects in the upper portion of the development curve). A reasonable alternative to raw maggot-mass temperatures would be to use a temperature that would provide the fastest growth of maggots (around 90 to 95°F or 32 to 35°C) for most species in the U.S.). This temperature would provide the minimum time for growth and development. Another more quantitative approach is to base an estimate of development on an average maggot mass temperature. In determining the appropriate temperature to use, it is helpful to have maggot mass temperatures from the outside edge as well as the center of the mass. Improving our estimates of insect development in maggot masses is another important research issue in forensic entomology.

Developmental Delays and Confounding Factors

At the beginning of this section, we highlighted the common need for backtracking from a given insect development stage to the time of oviposition. Such an approach provides a method for using insect development to help estimate the postmortem interval. Beyond the details and limitations in making development estimates, there are additional considerations that can influence those estimates.

To provide the most accurate estimate of insect development times, it is essential that we have an accurate understanding of the developmental history of the insect. Much of this relates to the thermal environment of the insect (as we have discussed), but other factors also are important. Often estimates are based on maggots collected from the corpse and maintained under constant (known) environmental conditions until pupation. One potential source of error is in the interval between collection and maintenance under controlled conditions. Handling maggots does not delay development, but chilling at 3°C

(37°F) does arrest development without increasing mortality (Johl and Anderson, 1996), provided the maggots are in low numbers and maggot mass heating is not present. Unless maggots are maintained in conditions to explicitly stop development, a precise record of the thermal environment of the insect, from collection to deposition in a controlled rearing environment, is essential for accurate development estimates.

Factors affecting the condition of a corpse also may affect insect development. Anything that might alter oviposition (such as coverings on the corpse, deposition of a body at night, etc.) will obviously influence developmental estimates, because initial oviposition may be delayed. Similarly, physical factors that constrain larvae migration, such as when the corpse is tightly wrapped, also may delay pupation and, therefore, development (Wells and Kurahash, 1994; Byrd and Butler, 1996). Physical features (such as black cloth or plastic on or over remains) can increase the temperatures the larvae experience, which will influence development estimates. More subtly, the presence of some antemortem drugs has been reported to alter insect development rates (Catts and Goff, 1992). It may be possible to account for the potential influence of these various factors in making development estimates if these factors are recognized, which highlights the importance of having comprehensive information about conditions in and around remains where insects are collected.

Temperature Data

Although we have emphasized a number of potential problems and limitations in the use of temperatures for determining insect development, perhaps the key limitation is using the appropriate temperature data to determine development. In reviewing degree-day calculation methods, Higley et al. (1986) concluded that the source of temperature data used in degree-day calculations was likely the most significant single source of error. They also pointed out that variation in degree-day estimates would be most significant when ambient temperatures spanned the developmental threshold. Both of these conclusions seem particularly relevant in determining development of forensic insect species.

In practice, obtaining temperature data for determining insect development is a crucial need. Given that most situations will require use of data from the nearest recording site, direct comparisons are vital for daily maximum and minimum temperatures from the crime scene with recording stations temperatures to allow for possible adjustments in estimates. Assuming weather conditions are not highly variable, temperature comparisons between the crime scene and weather service station should be conducted for 3 to 5 days (Haskell and Williams, 1991). If such direct comparisons cannot be made, details on as many features of the death scene (topography, vegetation, covering, clothing color) are vital to allow for informed adjustment of weather station data.

Describing Variation in Estimates

Another important consideration in forensic estimates is the need to provide courts with statements of variation in the estimate. This has not been done in agricultural estimates, because in making predictions it is possible to move forward the prediction to accommodate variation (Higley and Peterson, 1994). In contrast, forensic estimates need to be as accurate as possible and have a clear, objective statement of variation in the estimate. Although no approach for degree-days currently is available, we are exploring the use of Monte Carlo simulations in making such estimates of variation, and it may be other

approaches also can be used as other investigators consider this issue. Wells and coworkers (Wells and Kurahashi, 1994; Wells and LaMotte, 1995) have considered variation in age estimates from length and weight measures of fly larvae, and offer some approaches for developing confidence intervals in such estimates. Despite the many sources of variation possible in making insect development estimates, undoubtedly many errors cancel out. This is another arena in which standard methods, agreed upon by the forensic entomology community, are clearly needed.

Conclusions

Insect thermal development is a powerful tool of forensic entomology and additional research will improve its usefulness. Given the complexity of insect development and the many factors that influence development, the determination of insect development is necessarily an estimation process. With more research, we can expect the variation in our estimates to decrease. But thermal development estimates are unlikely to ever provide precise estimates of postmortem interval, except for those situations where we have very accurate temperature data. Instead, thermal development estimates are most useful in establishing a range for the postmortem interval, helping to indicate when death might or might not have occurred. Also, thermal development estimates are a valuable line of independent evidence when used in conjunction with other data, such as successional patterns or direct age grading of insects. Consequently, as our understandings and methods improve, it seems certain that estimates of insect thermal development will become increasingly useful in forensic entomology.

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Computer Modeling of Insect Growth and Its Application to Forensic Entomology

10

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Contents

Introduction

It is obvious that temperature is of critical importance to insect development and much research has been devoted to this area. Developmental theories, mathematical equations, and even computer simulations of insect growth have all been created and published within the relevant literature. However, the use and application of computer modeling in forensic entomology is largely unexplored. The reason for this is that many widely used entomological computer models do not provide acceptable results for predicting development times of insect field populations under variable temperature (Stinner et al., 1974), and this is a critical drawback for forensic applications. The only published accounts of established statistical protocol or computer simulation of the arthropod fauna inhabiting carrion is by Schoenly (1992) and Schoenly et al. (1992). Schoenly et al. (1992) developed a computer algorithm in the BASIC language to calculate the postmortem interval (PMI) from arthropod succession data. The required input for this program is the identity of arthropod taxa recovered from the death scene, data on carrion-associated arthropod taxa, known succession patterns, and data on developmental duration of the immature stages.

Schoenly (1992) also developed a set of statistical protocols proposed for analyzing carrion-arthropod succession in forensic entomological investigations. This protocol analyzed data in three ways: (1) patterns of arthropod visitation (arthropods divided into reoccurring or nonreoccurring taxa), (2) temporal changes in taxonomic composition of

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the carrion-arthropod community, and (3) applying sampled random tests (Monte Carlo simulation) to community-wide arthropod visitation times.

Most published computer-assisted entomological techniques use succession (= species replacement) timetables of certain taxa to estimate the upper and lower limits of the postmortem interval in days. Williams (1984) published a model for aging fly larvae in which a method is described for determining the time of egg hatch based on larval weight, with respect to temperature. Two programs were developed, one computes the parameters for logistic equations and the other estimates the time elapsed from when a sample is removed from a body to the estimated egg-hatch time. However, in this model, temperature and the insect species serve as independent variables while the growth rate and parameters of a logistic growth curve are the dependent variables.

In order to advance the applicability of computer simulations to forensic entomology, the baseline understanding of adult behavior and its effect on oviposition patterns needs to be expanded and adult emergence patterns need to be charted. Our objectives were to determine the mean and standard deviation of the development of immature life stages, compare the development of immature insects under constant and cyclic temperature regimes, and compare the various development model theories with the baseline data to determine the optimum model format. In addition to these goals, we also feel that advanced computer models should be developed that compensate for adult activity periods: ambient temperature fluctuations, inter-species competition, and the excess metabolic heat generated by actively feeding second and third instar larvae. With such technology, it is hoped that increased accuracy and understanding of the use of entomological evidence in crime scene investigation will be developed.

Computer Modeling Theory

The need to predict insect development time has lead to many mathematical models that describe the effects of temperature on insect development. Of these mathematical models, two general types are relevant to this work. These are the degree-day summation method (first introduced by Candolle (1855)) used in a nonlinear model by Sharpe and DeMichele (1977), and the nonlinear temperature inhibition model (Johnson and Lewin, 1946).

Linear Development Rate Models

The most common development rate model, often called degree-day summation, assumes a linear relationship between development rate and temperature between thresholds (Allen, 1976). This method works well for intermediate temperatures, but because of nonlinear effects at high and low temperatures it does produce some errors at temperature extremes (Stinner et al., 1974). The linear model assumes that rates are proportional to temperature, and since amounts are integrals of rates, the amount of development is the integral of temperature (or a linear function of it) along a time axis and has units of temperature-time (e.g., degree-days). Temperature-dependent development in poikilothermic insects can be approached using either time or rate of development. Rate of development is traditionally utilized because rate models were created from biochemical and biophysical properties (Sharpe and DeMichele, 1977; Wagner et al., 1984). Kramer et al. (1991) addresses several of the complications that arise when using rate instead of time. However,

Allen (1976) presented a method for calculating degree-days and allowing for an upper and lower threshold.

Nonlinear Development Rate Models

Since some temperatures are lethal to organisms, it is obvious that development must be a nonlinear temperature function at the temperature extremes. Nonlinear development rate functions based on enzyme kinetics were developed to describe high temperature inhibition by Johnson and Lewin (1946) and low temperature inhibition Hultin (1955). Sharpe and DeMichele (1977) described a general model incorporating both high and low temperature inhibitions based on control enzyme inactivation. Another nonlinear model of temperature-dependent development (Stinner et al., 1974) utilized a function that is a simple sigmoid curve with an inverted relationship when the temperature is above the optimum. This model, as originally given, assumed symmetry about the optimum temperature, but can be easily modified for asymmetry. A nonlinear model by Logan et al. (1976) uses an equation that is asymmetric about the optimum, but becomes negative for very high temperatures.

Of the Sharpe and DeMichele model, Wagner et al. (1984) states that there is no other poikilotherm development rate model with greater flexibility, goodness of fit capability, or stronger theoretical foundation. However, this model is a bit complicated. It possesses six constants that must be determined by nonlinear least squares and, in some situations, it may be "overkill" (particularly in cases which temperature extremes are not often encountered). Recently, Schoolfield et al. (1981) modified the Sharpe and DeMichele model to enhance its overall utility and simplify parameter estimation. As pointed out by Worner (1992), the interaction of cyclic temperatures with nonlinear development can introduce significant deviations from the linear development rate model, especially in the low and high temperature regions of the development rate function.

To accomplish our objectives of enhancing postmortem interval estimation techniques, an insect phenology model with particular reference to the unique problems of forensic entomology has been developed using Simulink flow diagram software for IBM-compatible PCs. The development of this model employed a linear chain of differential equations to represent each of the insect life stages. This technique, called a distributed delay, is broadly used in engineering to model delay processes (Manetsch, 1976; Vansickle, 1977) and has been used by others to model insect developmental delays (Gutierrez et al., 1975; Gutierrez et al., 1984a; 1984b). Distributed delays as models of biological time lags also are described in Cushing (1977) and MacDonald (1978) who referred to it as the "linear chain trick." A distributed delay is simply a linear chain of rate (i.e., differential) equations representing flow through a system of "boxes," each having the same flow rate (a) in Equation (10.1).

$$\begin{aligned} \frac{dx_1}{dt} &= f(t) - ax_1 - \mu x_1 \\ \frac{dx_2}{dt} &= ax_1 - ax_2 - \mu x_2 \\ &\vdots \\ \frac{dx_n}{dt} &= ax_{n-1} - ax_n - \mu x_n \end{aligned} \tag{10.1}$$

This chain of equations represents an entire life stage (e.g., the egg stage) in which the x_is are the number of eggs in each substage, and the flow rate, a, is the same for all substages, giving the name *distributed* delay. Some mortality is indicated from each substage at rate $-\mu x_i$. The "input" is represented as $f(t)$, i.e., some general function of time. In forensic cases involving entomological evidence, $f(t)$ would be oviposition by adult flies. This method has two major advantages: (1) it is easy to model such systems with simulation software (e.g., MATLAB/Simulink (The MathWorks Inc.), which is a "boxes-and-arrows" modeling environment; and (2) everything in the model can be obtained from the mean time in a life stage and the variance of the time. These things are usually reported with the relevant developmental literature since they are easily measured empirically. Thus, by simply measuring the mean and standard deviation of the time to complete a stage, one can obtain the data needed to construct a stage model using a distributed delay. The number of stages required, n, is given by $n = \tau^2/s^2$ (rounded to the nearest whole number) where τ and s are the mean and standard deviation of the time required to complete a life stage (e.g., the egg stage). The flow rate a in the distributed delay Equation (10.1) is equal to n/τ. A single differential equation flow diagram from Simulink is shown in Figure 10.9, while a cascade of these diagrams is shown in Figure 10.10 representing the equations in (10.1) above. The useful "single substage" formula is shown below in Equation (10.2) where we have included mortality with the per capita rate μ.

$$\frac{dx_i}{dt} = ax_{i-1} - ax_i - \mu x_i \tag{10.2}$$

Development rate functions were obtained at different temperatures under controlled conditions and through comparison with larval development under field conditions. Field comparison was based on insect collections made from two *Sus scrofa* (L.) (domestic pig) carcasses, less than 24 h postmortem, obtained as mortality from local hog producers. These carcasses were placed outdoors simultaneously under identical conditions with one left exposed to the insects throughout decomposition, while the other was covered with a screen cage to exclude further insect colonization after 3.5 days exposure. Insect samples were collected daily from the center of the maggot mass (or masses) to duplicate the entomological sampling techniques utilized by crime scene technicians and other death investigators. The samples were identified to species and sorted according to developmental stage. The observed sample data time series was then compared to the computer model output.

Creation of a Computer Model

For the carcass exposure study, results were interpreted only for insects in the family Calliphoridae (Diptera), and from this family only two species colonized the remains: *Cochliomyia macellaria* (Fabricius) and *Chrysomya rufifacies* (Macquart). With the covered carcass, all ovipositions ceased once the screen and frame structure was in place proving that the enclosure was an effective barrier to colonization. After the initial oviposition input of 3.5 days, development proceeded normally and all life stages are shown in Figures 10.1a,b. The first instar was recovered on days 3 to 9 for both species, the second instar of *C. rufifacies* was found on days 4 to 10, while the second instar of *C. macellaria* was present in samples until day 14. The third instar of *C. rufifacies* was recovered during the morning

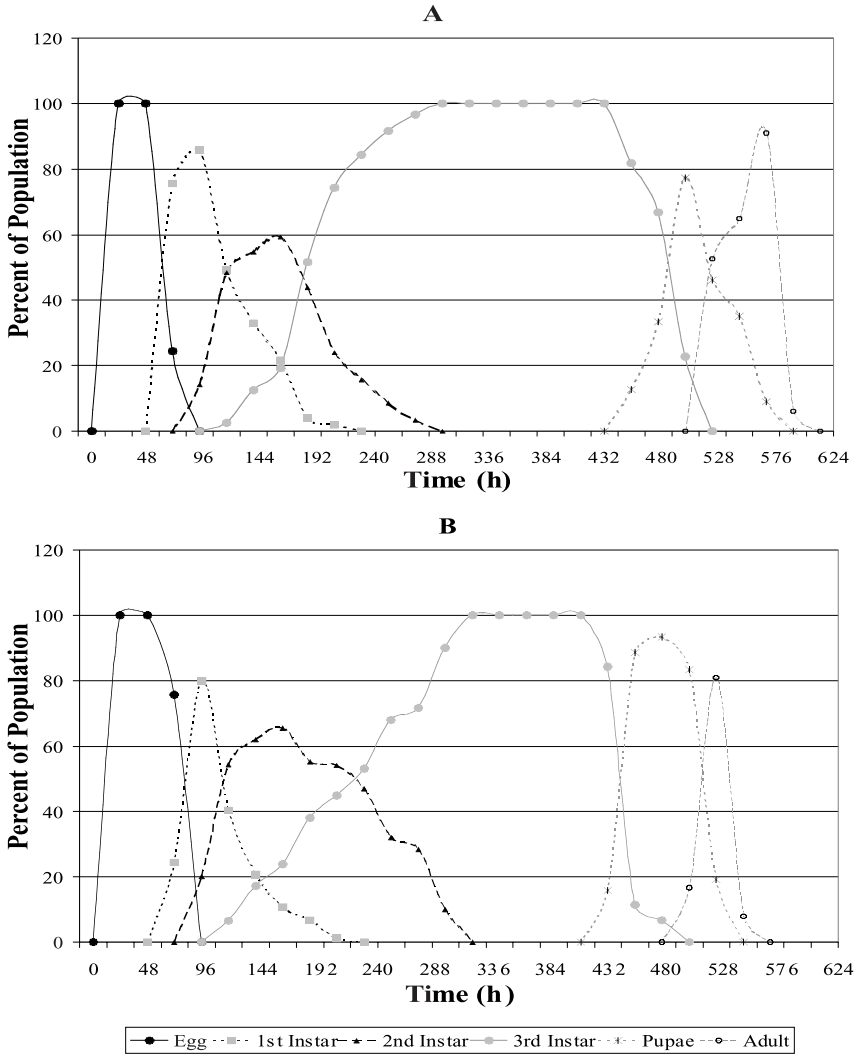

Figure 10.1 Development on *S. scrofa* carcass with oviposition artificially excluded after day 3 (average temperature 18°C). (a) *C. rufifacies*; (b) *C. macellaria*.

hours of experimental day 5, and this stage displayed a duration 24 h longer than that of the *C. macellaria* third instar. Similarly, the pupal stage of *C. rufifacies* also formed 24 h later than the pupae of *C. macellaria*, and adult emergence of *C. rufifacies* occurred 24 h later than for *C. macellaria* with the first adults recovered 22 and 23 days (respectfully) after exposure.

Larval development on the carcass that remained exposed to continual insect colonization was not as easily interpreted as that of the covered carcass. Generally, the residence time of an instar on the carcass was longer, presumably due to longer exposure to oviposition input. With *C. macellaria*, the time in which the first instar was recovered increased by 7 days, and the duration of the second instar was lengthened by 4 days (Figure 10.1b and Figure 10.2b). Onset of the third instar also was lengthened by 4 days, with the start of the pupal stage having an almost equal delay of 3.8 days for both species. This same delay period also held true for the onset of adult emergence for both species.

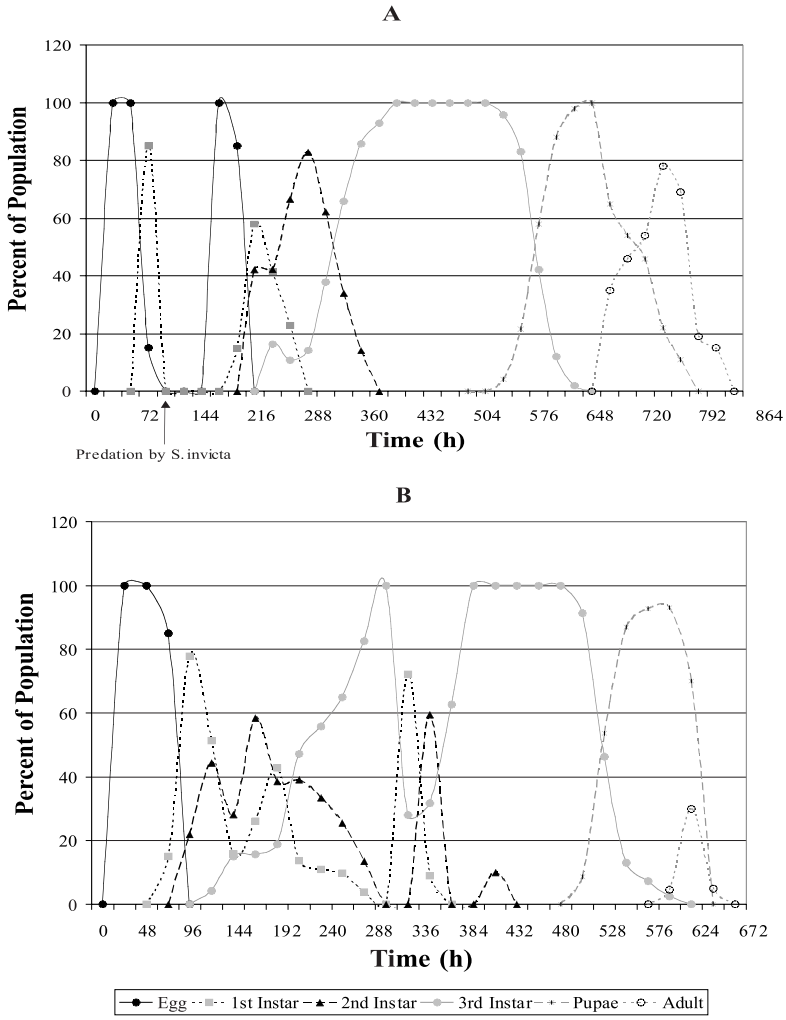

Figure 10.2 Development on *S. scrofa* carcass with normal oviposition (average temperature 18°C). (a) *C. rufifacies*; (b) *C. macellaria*.

On the exposed carcass, *C. rufifacies* was able to develop for a period of 4 days before the carcass population was decimated by *Solenopsis invicta* Buren (red imported fire ant). The ants effectively removed all of the eggs and first instar larvae of *C. rufifacies* during the late morning to mid-afternoon hours of day 4, and the adults of this species did not recolonize the corpse until the late morning hours of day 6 (Figure 10.2a). After recolonization of the carcass (the second oviposition peak in Figure 10.2a), development proceeded much the same as occurred on the covered carcass (Figure 10.1a). However, the ants did not significantly reduce the enormous numbers of *C. macellaria* eggs and first instar larvae that were laid on other areas of the carcass. Therefore, oviposition and initial colonization of *C. macellaria* continued over an extended period of time as eggs were recovered throughout the first 5 days of exposure, and the first instar larvae were found for a total duration of 16 days. Ovipositions, although reduced in number, probably continued on the exposed carcass for a period longer than was actually reported. However,

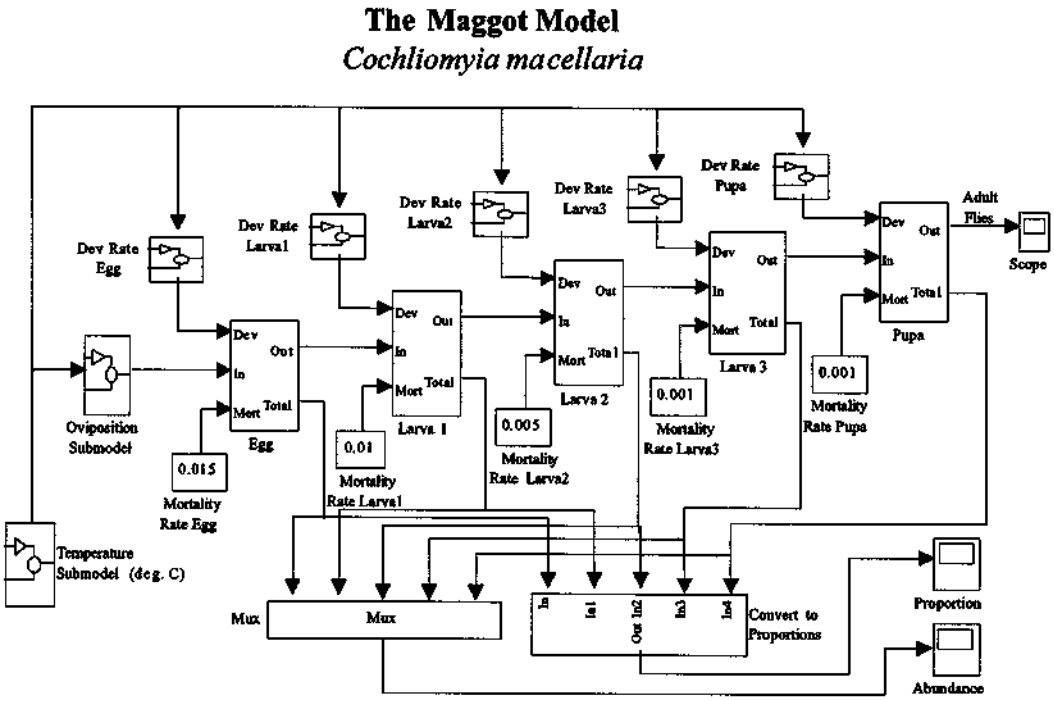

Figure 10.3 Insect development model as an open loop (flow through) MATLAB/Simulink diagram.

the large number of *C. macellaria* maggots made recovery of the relatively few eggs an unlikely event.

The complete maggot model is shown as a Simulink flow diagram (Figure 10.3) where each life stage (egg, first instar, second instar, third instar, pupa, and the adult) consists of a chain of equations like the distributed delay, Equation (10.1). Each of these life stages has an input for the development rate function for that particular stage, an input from the previous life stage, plus an input for the mortality rate. The output (emergence) of each stage is passed to the next, and it is also sent to a multiplexer so that output can be graphed. Two types of graphs are available: one that plots the proportion of the population against time, and the other that plots relative abundance against time. The proportion in each stage is particularly useful for crime scene comparison since it is independent of absolute abundance. The graphical output then can be used for postmortem interval estimation based on entomological evidence through direct inspection.

One of the main complications with which a forensic entomologist must contend when utilizing a computer model for postmortem interval estimation is the effect of temperature on development and, hence, the rate of flow through the substages. However, this can be accounted for by making either the development rate or the time to complete a stage a function of temperature.

The model is driven by input from both the temperature submodel (Figure 10.4) and the oviposition submodel (Figure 10.5). The temperature submodel accounts for ambient temperature and it can be adjusted to fit constant or cyclic conditions. This is accomplished by the use of a constant value that represents the average temperature and a wave function that cycles around the average temperature specified by the user. If the user has empirical

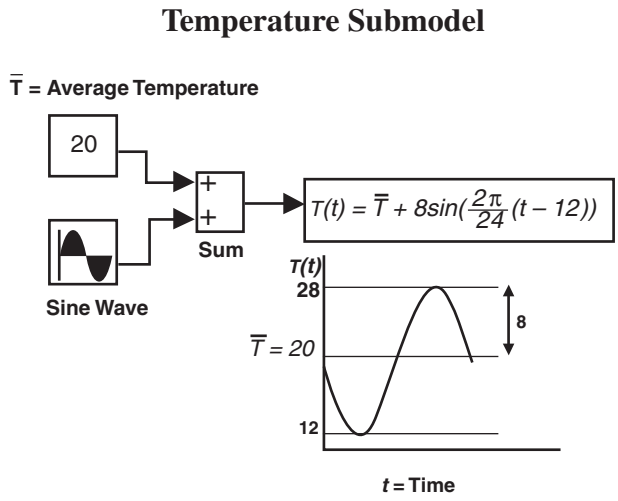

Figure 10.4 Temperature modeled as a sine wave with a 8°C amplitude, around an average temperature of 20°C.

temperature observations, either daily or hourly, they can be entered as data in an ASCII text spreadsheet and imported directly into the model in place of the temperature submodel. The oviposition submodel accounts for the behavior and diurnal activity of adult flies, as well as the added effect of cadaver age and its attractiveness to various fly species. Additionally, the threshold activity temperature can be specified with this submodel so the rate of oviposition can be more accurately predicted. Through the use of a "constant box," a clock function, and a "relational operator," the time at which oviposition would cease (either through normal adult oviposition behavior or by exclusion, such as concealment with a structure or container) can be specified if it is known (Figure 10.5).

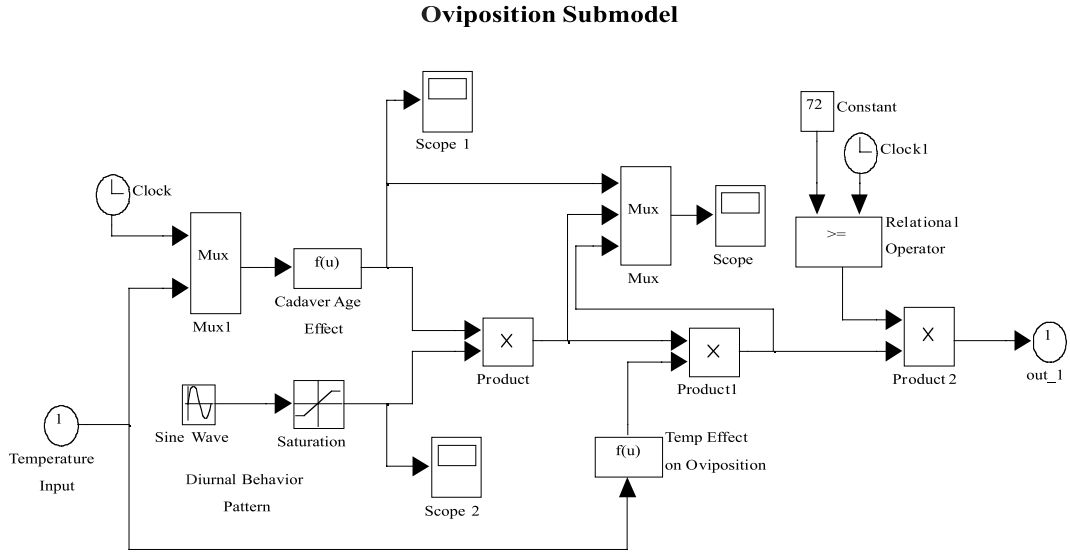

Figure 10.5 The oviposition submodel incorporating the diurnal activity pattern and the cadaver age effect. Note input of temperature from the temperature submodel (Figure 10.4).

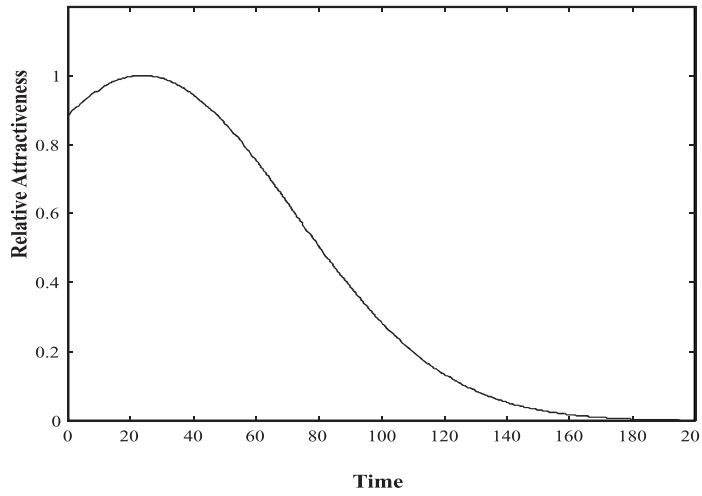

Figure 10.6 Example of the cadaver attractiveness function specified as a curve of normal distribution with a peak at 24 h and a standard deviation of 20 h. This effect is represented as the "cadaver age effect box" in Figure 10.5.

Figure 10.6 demonstrates the cadaver attractiveness function as a curve with a normal distribution ($\mu = 24$ h, $\sigma = 20$ h) plotted against time. The model output shown in Figure 10.7 illustrates the flight behavior of flies that exhibit a diurnal periodicity, which must be accounted for as flies are not active at night. Thus, no oviposition should occur during these hours and the time of cadaver deposition (also the starting time for the model) is an important variable.

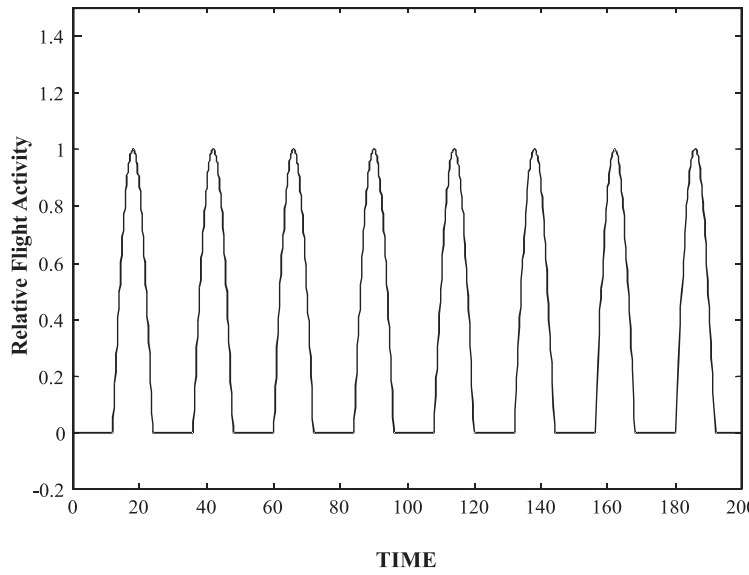

Figure 10.7 Example of the diurnal oviposition pattern with body deposition time at dusk. The first 12 h of this simulation has no fly activity due to the dark period. The "sine wave" and "saturation" boxes in Figure 10.5 produce this output.

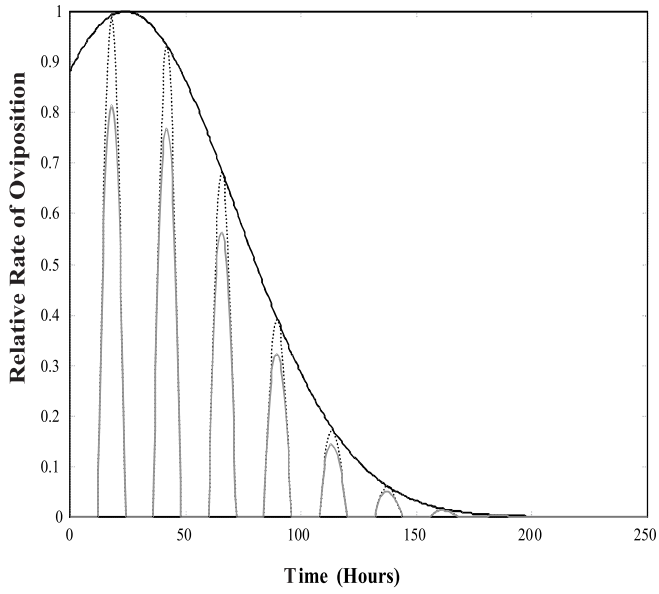

Figure 10.8 Simulation of oviposition using the oviposition submodel (Figures 10.5, 10.6, and 10.7) input to a cadaver deposited at dawn under a 24-h cyclic temperature regime with mean = 25°C, amplitude = 5°C. This output is the product of Figures 10.6 and 10.7.

Figure 10.8 shows the combined effect of cadaver age, ambient temperature, and flight activity on oviposition. The black line represents the cadaver age effect, the dotted line represents the expected oviposition rate at optimum developmental temperatures, and the gray line represents actual ovipositions with a diurnal periodicity. The substage model, or the single development substage (Figure 10.9), is the core of the model as it combines the development rate input, data flow from previous stages, the mortality rate, and passes the combined output to the next substage. This substage model represents Equation (10.2) in the linear cascade process. The output of the oviposition submodel flows through the life

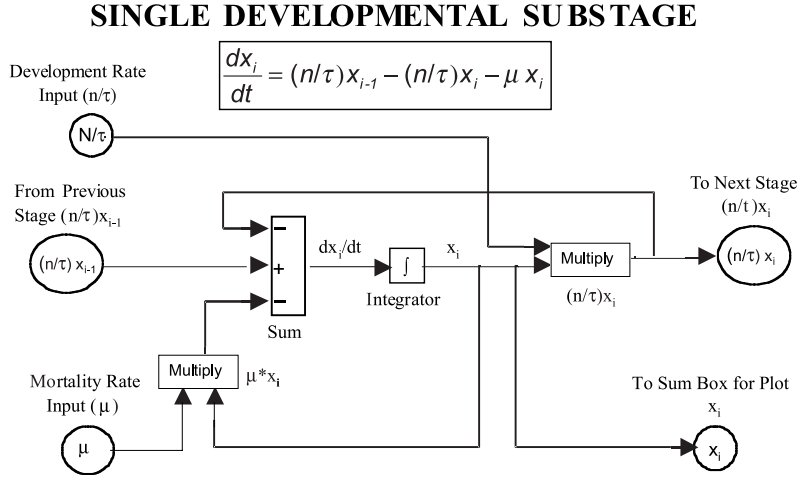

Figure 10.9 Simulink flow diagram of the basic substage model which can be replicated as necessary to duplicate the duration of an insect life stage.

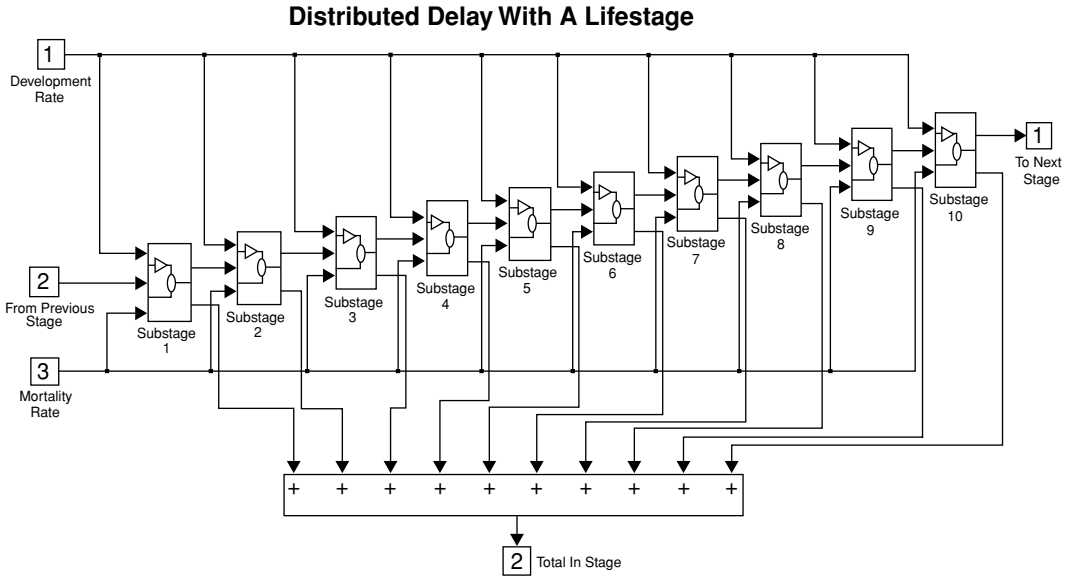

Figure 10.10 Substage flow within a life stage (each substage is a repetition of the diagram in Figure 10.9). This life stage submodel is "grouped" to make the boxes representing egg, instar 1, 2, 3, and pupa in Figure 10.9.

stages of egg, larval instar (1, 2, 3), pupa, and adult. Each of these life stages is configured as a series of single developmental substages linked together forming a "distributed delay" as shown in Figure 10.10. The shape of the simulated developmental curve for each of the insect life stages can be controlled by increasing or decreasing the number of these substages within a particular life stage. The relation between mean (τ) and variance (σ^2) of the emergence curve and n (the number of stages) is represented by Equation (10.3) so n can be easily calculated from the mean and variance of the data.

$$n = \frac{\tau^2}{\sigma^2} \tag{10.3}$$

This mimics the effect of temperature on an insect by regulating the time required to complete a stage, as altering the number of substages thereby changes the mean and standard deviation of the growth curve. For simulation purposes the number of substages were varied for each life stage to match the mean and standard deviation of growth curves obtained through laboratory rearing. This was accomplished through Equation (10.4).

$$\tau = a + \frac{b}{T - c} \tag{10.4}$$

The development rate input for each life stage is controlled through the development rate submodel (Figure 10.11). This submodel is based on actual developmental data obtained under laboratory conditions and must be specified for various species under differing conditions. The settings that must be specified by the user are the (a) minimum

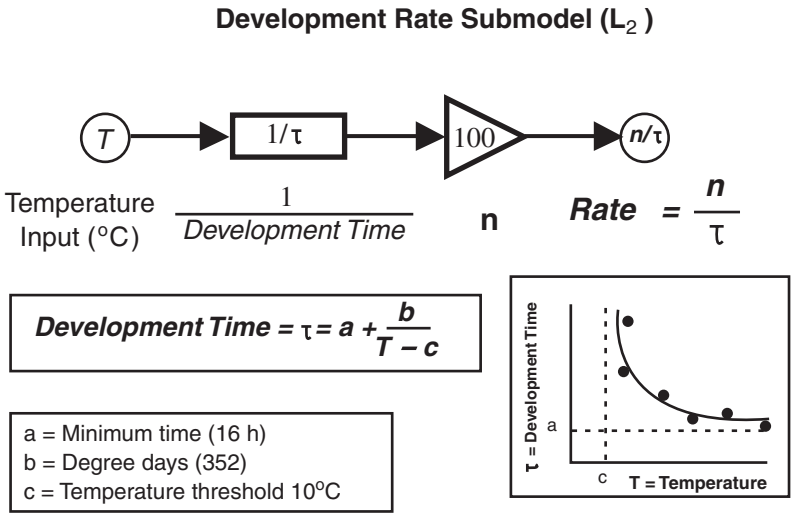

Figure 10.11 Simulink flow diagram of the model that introduces and combines the effect of temperature on insect development.

developmental time, (b) the accumulated degree hours required for development at that temperature, and (c) the minimum threshold developmental temperature.

Simulation output is illustrated in Figures 10.12 to 10.16. The output from the overall model is represented in Figure 10.12 showing the relative abundance of each life stage on a time axis. Figure 10.12 also shows the simulation output for a body deposited at dawn, with average ambient temperatures of 25°C for the developmental period. However, the

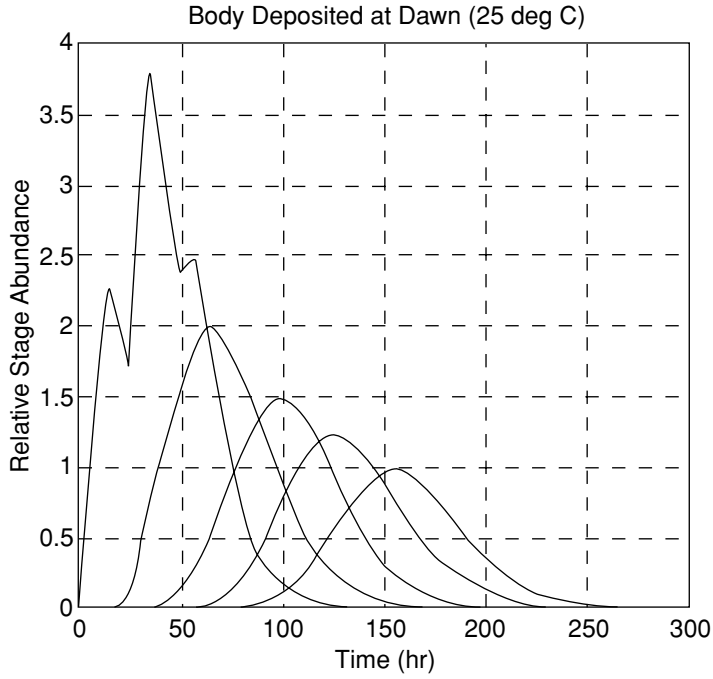

Figure 10.12 Proportion of each life stage over a 300-h simulation period at 25°C.

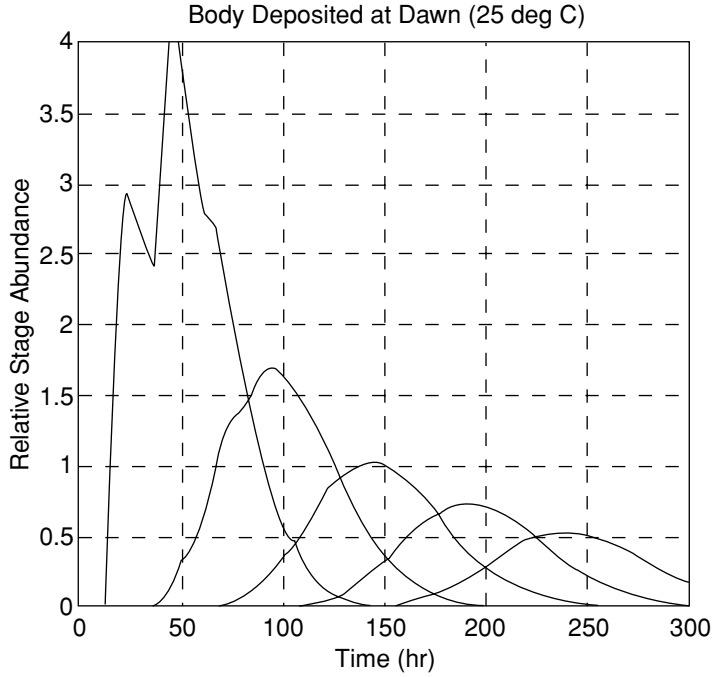

Figure 10.13 Proportion of each life stage over a 300-h simulation period at 12°C.

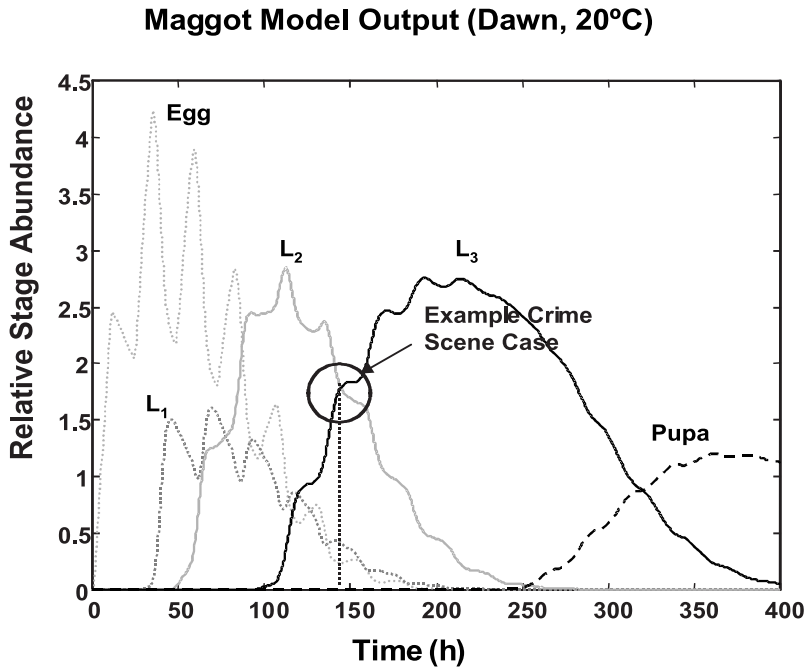

Figure 10.14 Computer simulation output with body assumed to be deposited at dawn.

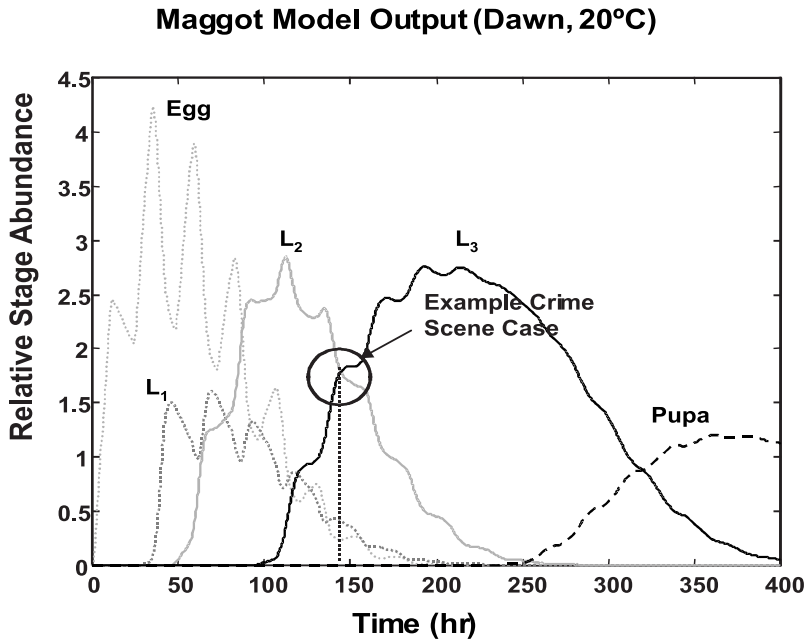

Figure 10.15 Computer simulation output with body assumed to be deposited at dusk.

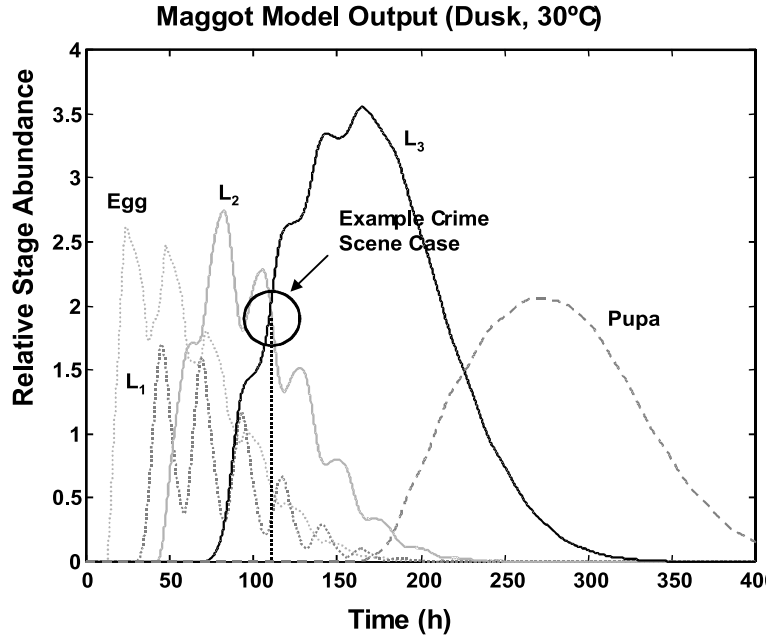

Figure 10.16 Computer simulation output with body assumed to be deposited at dusk.

change of body deposition time can be easily accomplished by a time shift of ±12 h, as in Figure 10.13, where simulation input (the oviposition submodel) was phase-shifted by 12 h to reflect deposition of a body at dusk as opposed to a dawn deposition, with an average ambient temperature of 12°C. Not surprisingly, in simulation trials a shift in the time of first oviposition produced a corresponding shift for all subsequent life stages. Such a shift was still discernable even at the time of adult emergence.

In an actual death investigation (example case), the collected entomological evidence consisted of equal proportions of second and third instar larvae of *Chrysomya rufifacies*. Utilizing the computer model, it was possible to plot the time interval at which equal proportions of second and third instar larvae would occur. Figure 10.14 shows simulation output of relative stage abundance. This example illustrates the development (at 20°C) of *C. rufifacies* on a cadaver assumed to be deposited at dawn. Figure 10.15 demonstrates the same species and conditions, except the body is assumed to have been deposited at dusk. Obviously, a simple time shift, prolonging the estimated PMI, is the only observable difference between the two scenarios. However, if a dusk deposition is still assumed but the ambient temperature is increased to 30°C, then the PMI estimation based on the relative stage abundance is shortened considerably and the alteration of the PMI is easily visualized (Figure 10.16). One of the advantages of a computer model such as this is that many varying scenarios can be simulated in a short period of time, allowing the forensic entomologist to consider and decide on many possibilities.

Such output can allow the forensic entomologist to conduct an analysis utilizing the proportion of various life stages within a sample to estimate the postmortem interval. Such a proportional analysis was difficult to accomplish without the predictive and visualization power of computer software such as MATLAB/Simulink, and the existence of a modeling program based on the actual temperature-dependent development of insect species of forensic importance.

Comparisons of linearity also were conducted, and it was found that of the three species studied, development was essentially linear between 15°C and 35°C. Development deviated from linear with the 10°C and 40°C rearings as complete development was not successful. Mean development time of each life stage for *P. regina* (Meigen), *C. macellaria*, and *C. rufifacies* was plotted and fitted to a hyperbola using TableCurve®(Jandel Scientific) in Figures 10.17 to 10.22.

Figures 10.23 to 10.26 are a comparison of laboratory data with a distributed delay model of the egg stage. Figure 10.23 represents the proportion of unhatched eggs remaining, and Figure 10.24 represents the rate of emergence at 25°C. Figures 10.25 and 10.26 are a comparison of laboratory data and model prediction of egg hatch at 30°C. Figure 10.27 is a comparison of model output and field data for *C. macellaria*. The model output (A) shows the relative proportion of the population with time, and (B) shows data obtained under field conditions for *C. macellaria* developing on a *S. scrofa* carcass. Model output for *C. macellaria* compares closely to field data, with a maximum deviation of approximately 20 h, with the exception of the pupal stage. This discrepancy was due in part to being truncated by field sampling techniques for adult emergence, and by the fact that the model displays the migratory phase of the third instar as a separate output. These two factors probably account for the majority of the variance observed between model and field data for the pupal curve. However, the mean times of the life stages are the same, and it is time that is the most critical value. It is important to note that Vansickle (1977) showed that, in distributed delay systems, as mortality increases the mean and variance decreased,

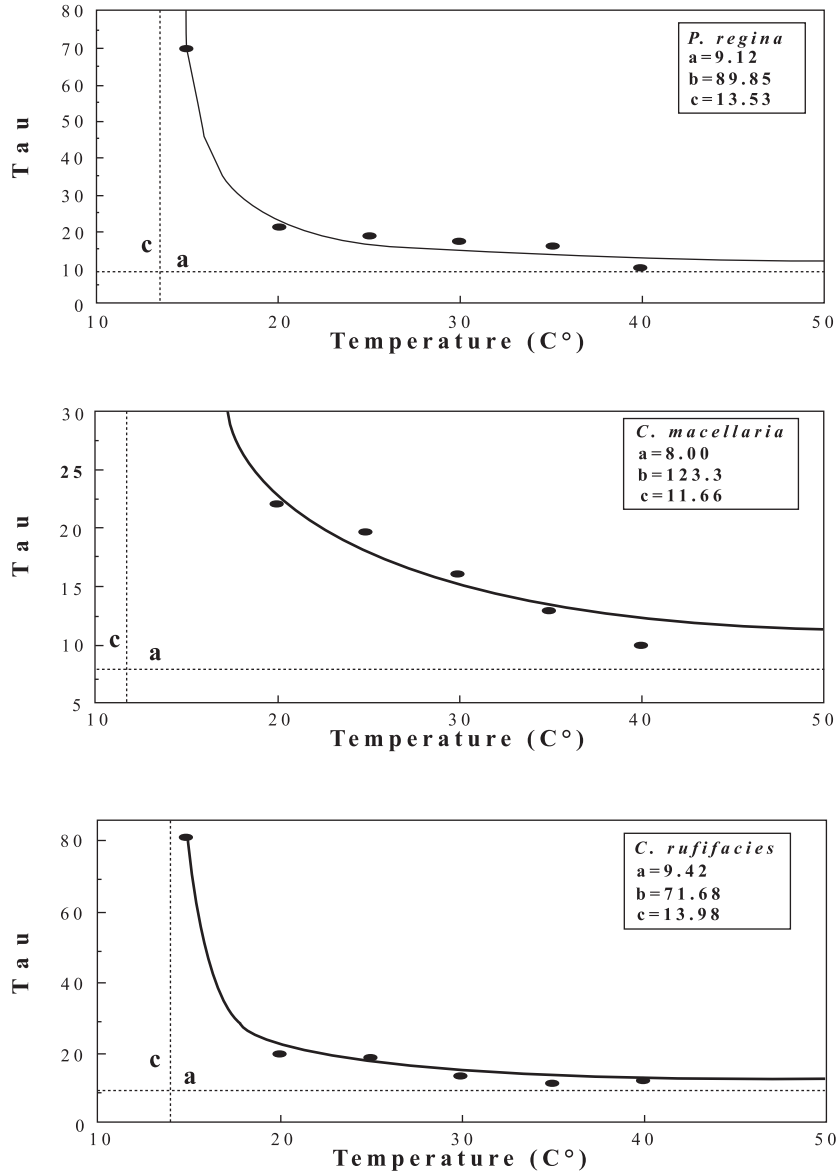

Figure 10.17 Curve fit of laboratory development data of the egg stage for three species of Calliphoridae (for a description of "a," "b," and "c" see Equation (10.4) or Figure 10.11).

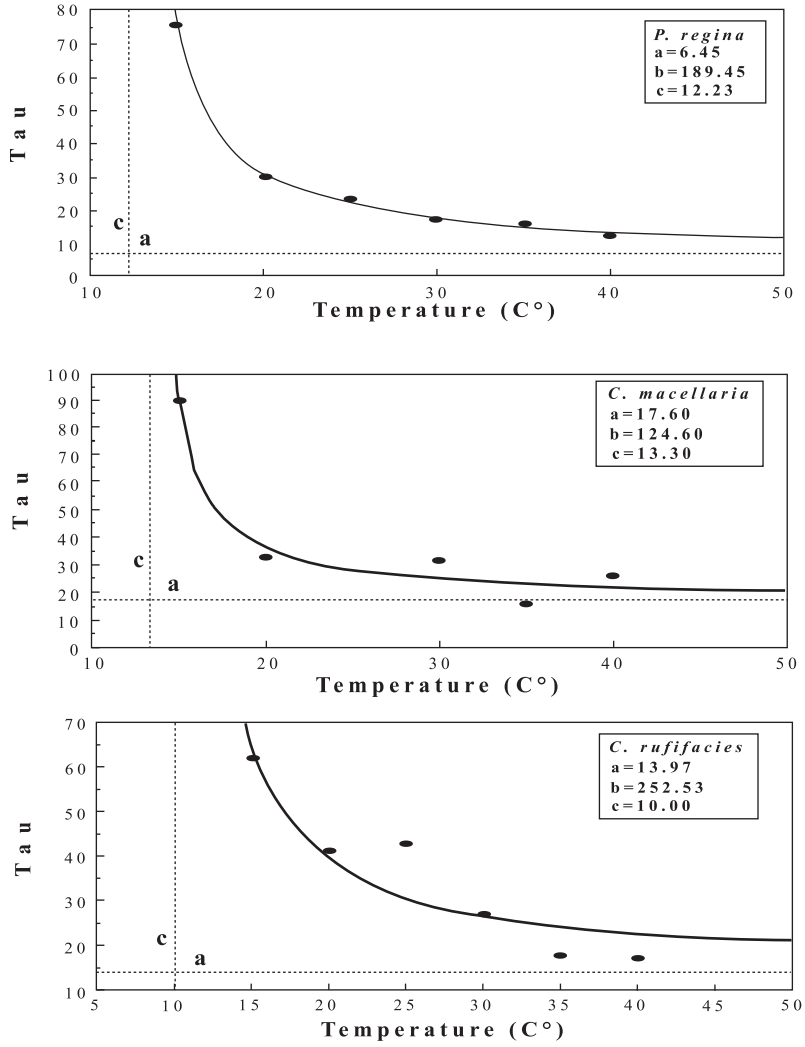

Figure 10.18 Curve fit of laboratory development data of the first instar for three species of Calliphoridae (for description of "a," "b," and "c" see Equation (10.4) or Figure 10.11).

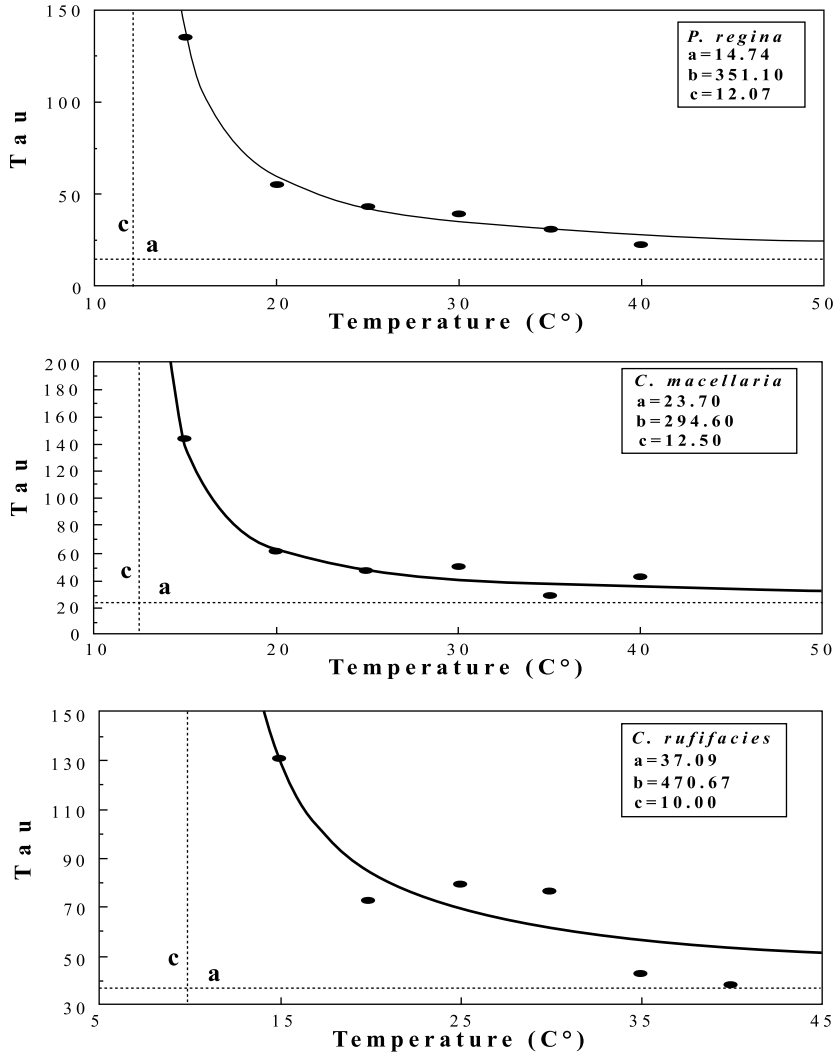

Figure 10.19 Curve fit of laboratory development data of the second instar for three species of Calliphoridae (for description of "a," "b," and "c" see Equation (10.4) or Figure 10.11).

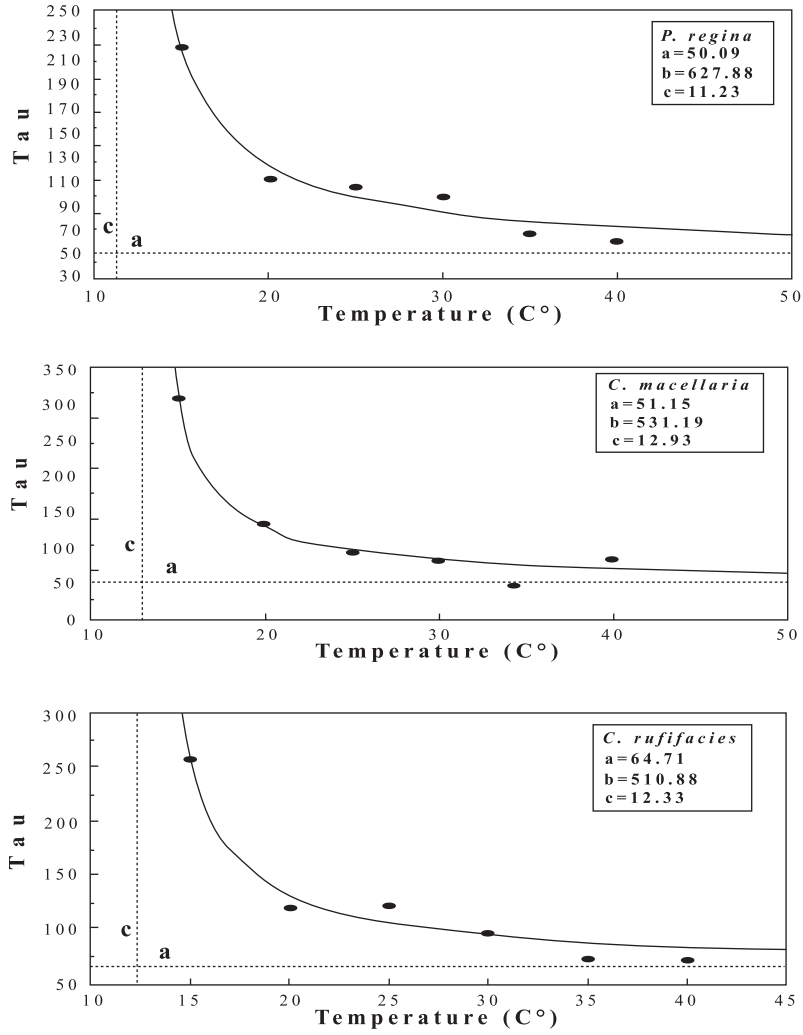

Figure 10.20 Curve fit of laboratory development data of the third instar for three species of Calliphoridae (for description of "a," "b," and "c" see Equation (10.4) or Figure 10.11).

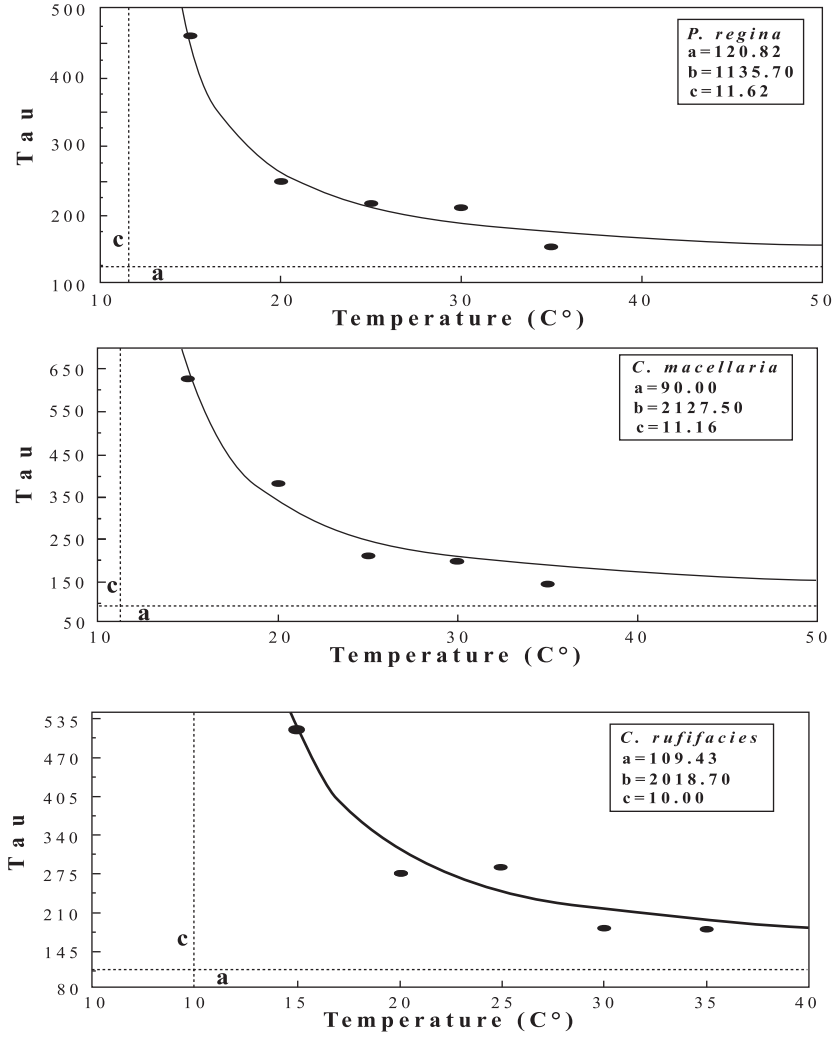

Figure 10.21 Curve fit of laboratory development data of the pupa for three species of Calliphoridae (for description of "a," "b," and "c" see Equation (10.4) or Figure 10.11).

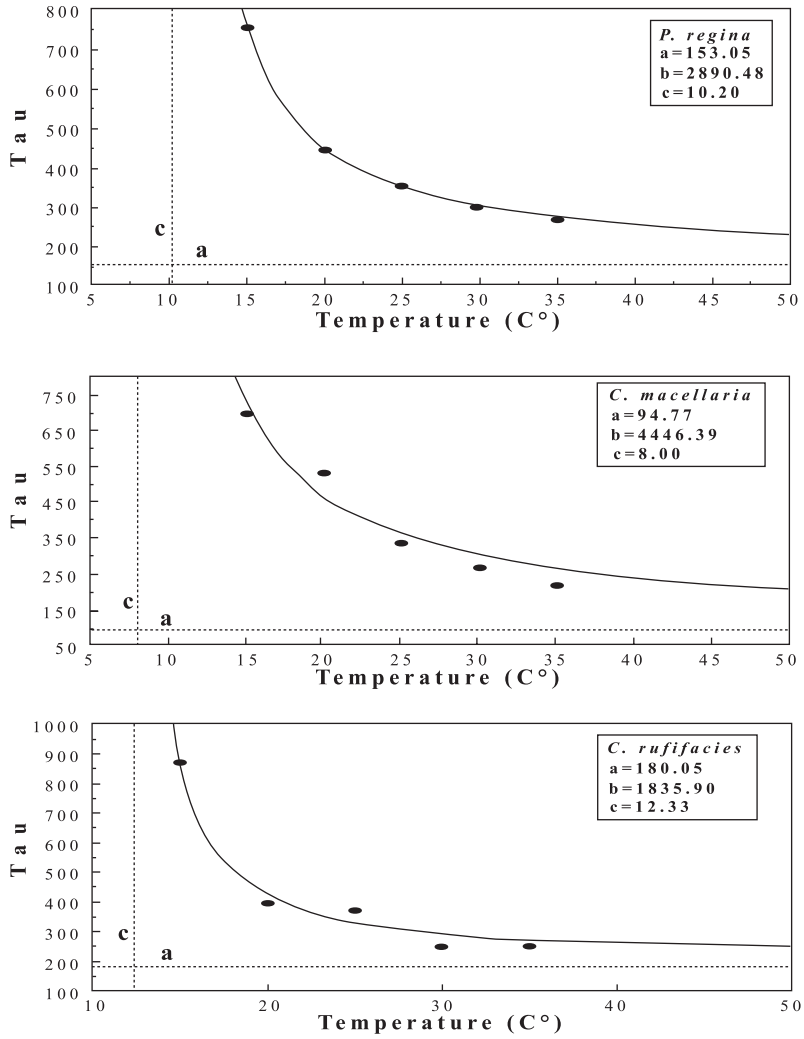

Figure 10.22 Curve fit of laboratory development data of the adult for three species of Calliphoridae (for description of "a," "b," and "c" see Equation (10.4) or Figure 10.11).

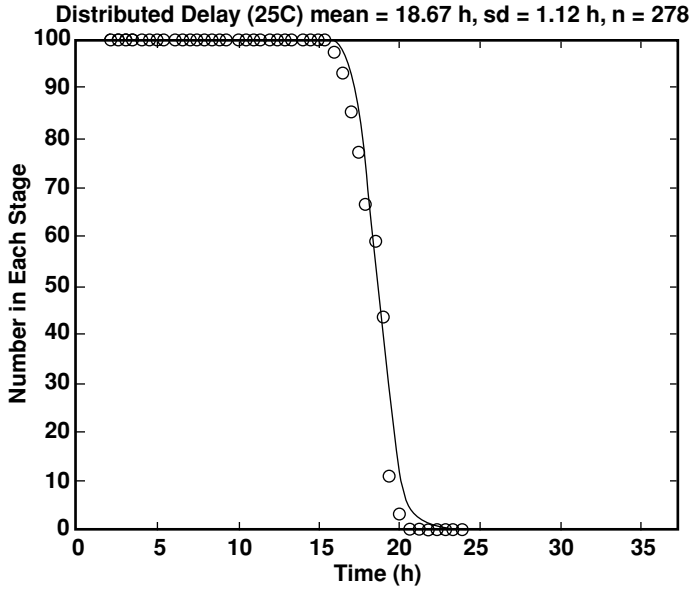

Figure 10.23 Comparison of computer model prediction and laboratory development data for number of eggs remaining unhatched at 25°C. (Line = distributed delay computer model prediction with mean time and SD taken from growth rate curves, and the points = observed laboratory development.)

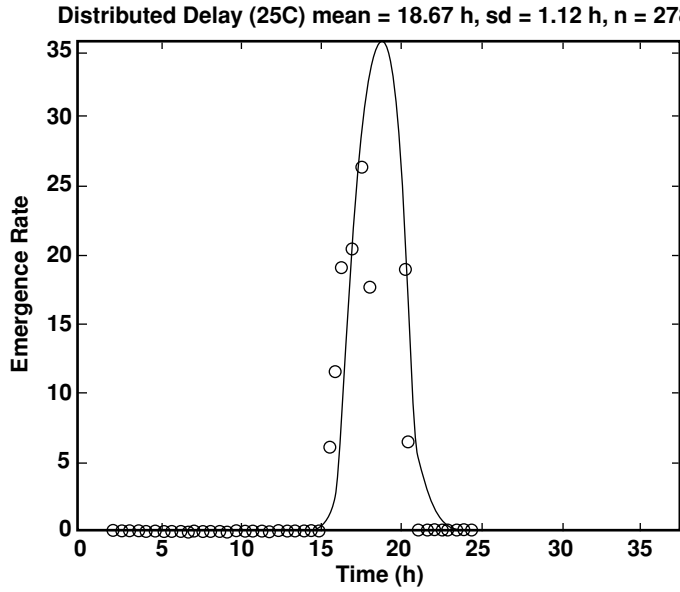

Figure 10.24 Comparison of computer model prediction and laboratory development data for rate of emergence (hatch) at 25°C. (Line = distributed delay computer model prediction with mean time and SD taken from growth rate curves, and the points = observed laboratory development.)

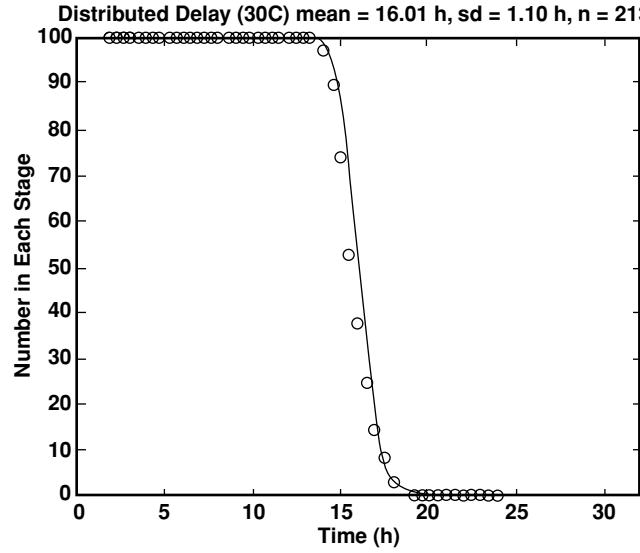

Figure 10.25 Comparison of computer model prediction and laboratory development data for number of eggs remaining unhatched at 30°C. (Line = distributed delay computer model prediction with mean time and SD taken from growth rate curves, and the points = observed laboratory development.)

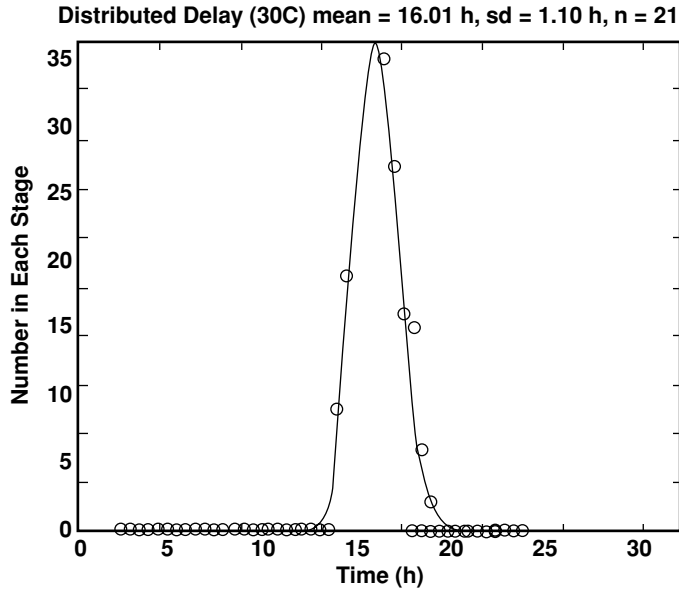

Figure 10.26 Comparison of computer model prediction and laboratory development data for rate of emergence (hatch) 30°C. (Line = distributed delay computer model prediction with mean time and SD taken from growth rate curves, and the points = observed laboratory development.)

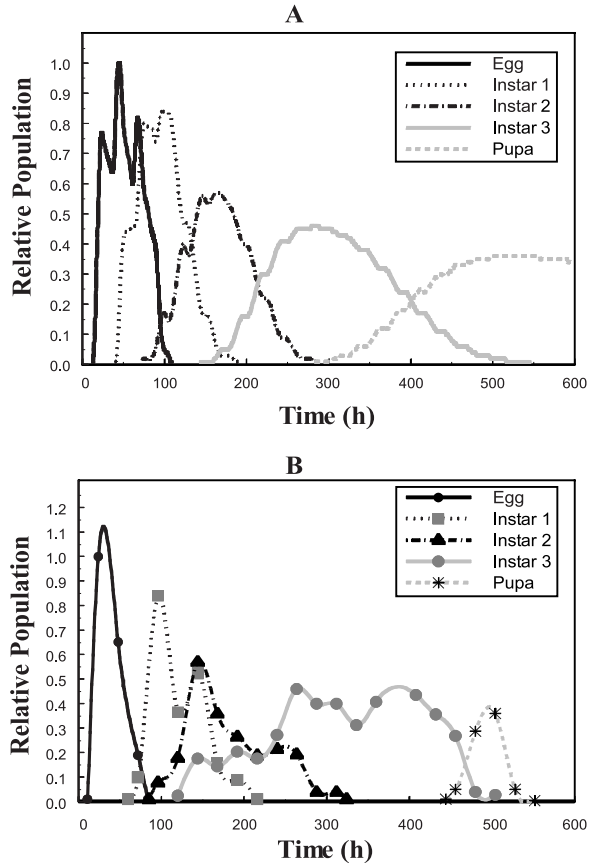

Figure 10.27 Development of immature stages of *C. macellaria*. (A) Computer model prediction, (B) development on *Sus scrofa* carcass in field conditions.

thereby reducing time estimations. With the exception of the predation on *C. rufifacies* by *S. invicta,* the field data and model output agreed well under conditions of low mortality.

This computer simulation model will help to develop increased accuracy and understanding of entomological evidence collected in human death investigations. In future applications a "maggot model" such as this can be developed further so that it can be made available to qualified forensic entomologists on a CD-ROM medium. Such a version would run on IBM-compatible computers and in its final form would effectively operate on common desktop computing equipment. In addition to the accuracy and precision of the actual calculations available, the graphical output of the process of insect development and model diagrams could be helpful in explaining insect development and the conclusions of a forensic entomologist to judges, juries, and attorneys.

Importance of Computer Modeling

The design and construction process of this computer model produced many interesting and unexpected results during the simulation of insect development on human cadavers. The results produced by the MATLAB/Simulink program could have a pronounced effect

on both the methodology and precision of future postmortem interval estimations based on entomological evidence that utilizes this technology.

The output of the computer model emphasizes the importance of knowing the flight behavior of flies when attempting to determine the time of initial oviposition. Additionally, it was discovered that the rate of oviposition was not an important factor in postmortem interval estimation. Not surprisingly, a body deposited at dusk would have a much different input "impulse" than would be expected from a colonization at dawn. If adult flies are not active and do not generally take flight during the night-time hours, a dusk deposition of a body would have an initial window of no insect activity equal to the dark period. Unlike the peaks and troughs of oviposition which become smoothed out and nearly undetectable in subsequent life stages, the initial oviposition *delay* is carried through to all subsequent stages. Thus, the postmortem interval estimation would be prolonged by an interval equal to the dark period unless the investigating entomologist accounted for adult flight behavior. Photoperiod-dependent oviposition is a critical factor in the computer model and of utmost consideration when constructing a PMI estimation.

Preliminary output of the computer model indicates that the peaks and troughs in oviposition rates on a body are not as critically important as once suspected. Such differences in successive ovipositions become negligible and have little significant effect on the emergence shape of subsequent life stages, in particular those of the pupa and adult. In engineering parlance, the insect life stages constitute a "low pass" filter, i.e., a process that passes low frequency input, but not the high frequency of daily ovipositions. This knowledge is beneficial to the forensic entomologist when estimating the postmortem interval as such data are unknown in actual forensic investigations involving human death. Since such data are not collected and impossible to obtain or estimate through larval collections made at the scene, the case of the forensic entomologist can be bolstered by the knowledge that such missing information is not a major hindrance or detrimental to his estimation.

Practical Applications of a Computer Model

In the field of forensic entomology, accurate and current developmental data for the forensically important insects are a paramount concern. It is hoped that the information contained within this work will allow for the further refinement of postmortem interval estimations based on entomological evidence. Detailed documentation of egg hatch, larval growth, and adult emergence for key blow fly species will provide forensic entomologists with current data so that they may confidently bolster their analysis. Additionally, computer models like this should provide invaluable information that can greatly aid forensic entomologists utilizing a variety of methodologies for entomological assessments in death investigations.

Further Studies in Computer Modeling

The biology of most of the forensically important insects is largely unknown. For most of those that have been researched, the data are preliminary and incomplete. Only a small portion of the insects commonly utilized in forensic entomology have their biology adequately detailed in existing literature. For these reasons a great deal of basic biological

research needs to be conducted on the more common species. Laboratory data alone are not sufficient for utilization in human death investigations. Such data need to be compared with data obtained under field conditions so that the two can be used in conjunction to obtain the most accurate postmortem interval estimation possible.

Obtaining accurate field data for forensic insects is difficult because research utilizing human cadavers is currently possible only at a very limited number of facilities. Thus, a domestic pig has been identified as a suitable surrogate and frequently is used for basic research. However, animal-use restrictions involving research are increasing, and the utility of domestic pigs may be only a temporary avenue to obtain data. It is important for the continued success of forensic entomology that field research be not only sustained, but increased.

This work was the first to incorporate the use of computer modeling of insect species life cycles in the field of forensic entomology. Computer simulation is a promising new way to obtain an "independent" analysis by which to estimate the postmortem interval. Such analyses can be compared with the standard techniques utilized by forensic entomologists so that any discrepancies are easily identified and addressed. Computer modeling introduces a new tool into crime scene investigations, which does not require modification of the forensic entomologists' standard techniques, and does not destroy evidence. This methodology deserves further exploration as a practical tool for use in death investigations.

Acknowledgments

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Entomotoxicology: Insects as Toxicological Indicators and the Impact of Drugs and Toxins on Insect Development

11

M. LEE GOFF
WAYNE D. LORD

Contents

Introduction

Careful analyses of the community of insects encountered on a decomposing body, combined with knowledge of insect biology, ecology, and local environmental conditions can often provide valuable forensic insights. These can include the estimation of time since death, movement of the remains after death, indication of antemortem injuries, and the presence of drugs or toxins.

Over the past 2 decades, there has been an apparent increase in the incidence of drug-related deaths reported within the U.S. and other countries. Decedents in such cases are, in many instances, not discovered for a substantial period of time (days or weeks). The resulting state of advanced decomposition and environmental recycling typically encountered in these situations often dictates the employment of various entomological methodologies. The entomological techniques most frequently utilized are based on comprehensive analyses of the insects and other arthropods associated with the remains, their development, and patterns of succession (Goff and Flynn, 1991; Goff and Odom, 1987; Lord et al., 1986).

The accuracy of entomological estimates in deaths involving narcotic intoxication has been subject to debate in recent years as few available studies have explored the effects of drugs contained in decomposing tissues on fly colonization and ovipositional behavior, or on the rates of development of carrion-frequenting insects feeding on such food sources (Goff, 1993). Additionally, relatively few studies have examined the effects of other tissue contaminants, such as toxins or environmental pollutants, on these behaviors and/or the developmental patterns of the insects colonizing such tissues.

In recent years, interest also has focused on the potential use of carrion-frequenting insects as alternative toxicological specimens in situations where traditional toxicological

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sources, such as blood, urine, or solid tissues, are unavailable or not suitable for analysis. The use of anthropophagic fly larvae (maggots) as alternate toxicological specimens is well documented in the entomological and forensic science literature (Miller et al., 1994). Detection of various toxins and controlled substances in insects found on decomposing human remains has contributed to the assessment of both cause and manner of death (Lord, 1990; Goff and Lord, 1994; Nolte et al., 1992). With the development of hair extraction technologies, attention has recently focused on the analysis of chitinized insect remnants that are frequently encountered with mummified and skeletonized remains (Miller et al., 1994). In such cases, the standard toxicological specimens are often absent.

Studies of the use of carrion-feeding arthropods as alternative toxicological specimens and of the impact that tissue toxins and contaminants have on the development of immature insects feeding on these substances currently comprise the major avenues of exploration in the emerging field of entomotoxicology.

Detection of Drugs and Toxins in Carrion-Feeding Insects

As previously mentioned, it is not unusual for human remains to be discovered in a highly decomposed or skeletonized state. Historically, it has been difficult to obtain toxicological information in cases of such advanced decay due largely to a lack of sufficient, analyzable tissue. A variety of arthropods and their cast larval and puparial skins, however, are commonly encountered on putrefied, mummified, and skeletonized remains.

Several recent studies have detailed the detection of toxins and controlled substances in both the insects and chitinized remnants recovered from badly decomposed victims. In these reports, the recovered arthropods have generally been homogenized and, subsequently, processed in a manner similar to other, more traditional tissues and fluids or subjected to extraction techniques developed for the analysis of rigorous tissues such as hair and nails. Analytic procedures have included radioimmunoassay (RIA), gas chromatography (GC), gas chromatography-mass spectrometry (GC/MS), thin-layer chromatography (TLC), or high-performance liquid chromatography/mass spectrometry (HPLC/MS).

Nuorteva and Nuorteva (1982) described the successful recovery of mercury from various species of blow fly larvae (Calliphoridae) reared on fish tissues containing known concentrations of the heavy metal. Mercury was observed to bioaccumulate in the developing larvae as they fed on the contaminated tissues, and the recovered concentrations within the larvae increased with the duration of the feeding period. The accumulation of mercury in the larvae also was observed to be directly related to the presence of mercury in the methylated form. In those larvae reared on tissues in which 94% of the mercury was methylated, a 4.3 times greater concentration was found than in the tissues upon which they had fed. In tissues where a lesser percentage of methylated mercury was present, recovered larval concentrations were only 1.5 times greater.

The mercury ingested by the developing calliphorid larvae was retained through the puparial stage and detectable in the emerging adult flies. Upon reaching adulthood, however, the flies rapidly eliminated the mercury. Two days following emergence, adult flies contained only 50% of the mercuric concentrations detected in the developing larvae. More detailed observations revealed that mercury was excreted into the meconium of the hindgut during the process of pupariation. No adverse effects from the bioaccumulated

mercury were detected in either the adult flies or the developing larvae. In some instances, however, difficulties were observed in pupariation.

The pioneering work of Nuorteva and Nuorteva (1982) clearly demonstrated that substances contained in food sources exploited by carrion-feeding insects could be detected in both immatures and adults, and that their levels could be quantified by toxicological means. As an extension of this study, larvae which had been reared on mercury-laden tissues were fed to adult staphylinid beetles, *Creophilus maxillosus* (L.). Secondary bioaccumulation of mercury also was seen in these predaceous beetles. However, no adverse effects were detected in the adult staphylinids studied. But, Nuorteva and Nuorteva (1982) observed "minimata-like symptoms," consisting primarily of irregularities in motor control in adult tenebrionid beetles, when *Tenebrio molitor* L., fed on fly larvae containing mercury. Subsequently, Schott and Nuorteva (1983) demonstrated decreased levels of activity in the same species of tenebrionid beetles when fed on a diet consisting of dried fly larvae contaminated with a high mercury content. These studies further demonstrated the ability to detect and quantify levels of food-borne contaminants in both adult and immature carrion-feeding insects and the bioaccumulation of these substances within the insects. Additionally, the detection of such substances in predacious beetles feeding on contaminated fly larvae and the resulting development of potentially negative side effects were illustrated.

In a similar manner, Sohal and Lamb (1977; 1979) demonstrated the accumulation of various metals (including copper, iron, and zinc), and calcium in the tissues of adult house flies, *Musca domestica* L. No detrimental effects to the adult flies were associated with the bioaccumulation of these metals. Utsumi (1958) observed that rat carcasses varied in their attractiveness to adult flies depending on the poison causing death. This research, however, did not include any attempt to detect the toxins in the maggots subsequently developing on the rat tissues.

Goff et al. (1997) reported a concentration of 3,4-methylenedioxymethamphetamine in puparial casings of the sarcophagid fly, *Parasarcophaga ruficornis* (Fabricius), which was higher than that detected in the tissues on which the developing larvae of this fly had been feeding. While this observation can be interpreted as yet another example of the phenomenon of dipteran bioaccumulation, the reported deposition of toxins in the cuticle of insects as a method of excretion suggests other potential mechanisms for the accumulation of ingested materials in nonliving insect tissues.

Sadler et al. (1997) failed to observe bioaccumulation of barbiturates in blow fly larvae, *Calliphora vicina* (Rodineau-Desvoidy), reared on an artificial food medium. Additionally, they observed that even closely related chemical compounds appeared to be processed differently by similar cohorts of developing *C. vicina* larvae. They cautioned against the quantitative interpretation of entomotoxicological findings given the current limited knowledge of the ways that drugs and toxins are handled by immature insects. Clearly much more research into the mechanisms and processes by which carrion-feeding insects incorporate ingested drugs and toxins into their living and nonliving tissues is needed before the full forensic potential of entomotoxicology is realized.

Researchers have employed various methods of drug administration in their attempts to accurately duplicate tissue concentrations of drugs and toxins seen in human overdose/poisoning deaths. Sadler et al. (1997), for example, fed developing fly larvae on an artificial food medium spiked with a known concentration of the drug being tested. Other researchers have employed alternative methodologies wherein known quantities of a drug

or toxin are administered orally and by infusion to a live animal model and the resulting vertebrate tissues are then utilized as the insect food source. The latter method allows for the drugs and toxins to be metabolized by the vertebrate host prior to insect ingestion. Goff et al. (1997) have suggested that many drugs, such as cocaine, exert their effects as the mammalian metabolite rather than the parent compound and that live animal models present a scenario more closely related to that seen in actual drug-related deaths. However, further research is needed before a clear understanding of the mechanisms underlying these processes and the optimum research model is elucidated.

An example of the potential forensic application of these types of observations, studies, and data was detailed by Nuorteva (1977). In this case, fly larvae were collected and reared from the decomposing body of an unidentified woman discovered in a rural area of Inkoo, Finland. An analysis for mercury was performed on the emerging adults in an effort to determine the geographic origin of the unidentified victim. The low mercury content of the emerging adult flies indicated that the victim came from an area relatively free of mercury pollution. When the victim's identity was eventually determined, she proved to be a student from the city of Turku, an area relatively free of mercury pollution. In this case, the entomotoxicological analysis allowed police to focus their investigative efforts in a more limited geographic area, thereby enhancing the chances of successful victim identification and case resolution.

Leclercq and Brahy (1985) successfully detected the presence of arsenic through the toxicological analysis of species in the families Piophilidae, Psychodidae, and Muscidae in a case of untimely death in France. Detection of the organophosphate insecticide Malathion in fly larvae was reported by Gunatilake and Goff (1989). In this case, a 58-year-old male with a previous history of suicide attempts was discovered in the crawl space under his mother's home in Honolulu. Adjacent to the victim's body was a bottle of Malathion with approximately 117 ml missing. Toxicological tests (GC) for Malathion were conducted on a variety of the victim's tissues and on two species of fly larvae (Calliphoridae) collected from the remains. Fat tissues from the victim revealed Malathion in a concentration of 17 mg/kg. A combined sample of the two species of fly larvae, *Chrysomya megacephala* (F.) and *Chrysomya rufifacies* (Macquart) revealed Malathion in a concentration of 2050 μg/g. It is significant to note that the age of both species of *Chrysomya* larvae were indicative of a postmortem interval of approximately 5 days. The victim was last reliably seen alive 8 days prior to the discovery of his body. The Malathion in the victim's tissues may have served, in this case, to delay oviposition by adult female *Chrysomya* for a period of several days. Additionally, a far greater diversity of both carrion fly and predatory beetle species would have been expected to have been encountered on remains present for 5 to 8 days in an outdoor Hawaiian habitat. The absence of a well-developed insect community on the remains of the decedent lends support to the notion that the ingested Malathion had a negative influence on carrion insect colonization.

Beyer et al. (1980) detailed the case of a 22-year-old female whose decomposed remains were discovered 14 days following her disappearance. The young woman had a lengthy history of mental illness, including multiple attempts at suicide. An empty bottle from a prescription for phenobarbital tablets filled 2 days prior to her disappearance was found in a purse adjacent to her body. As there were no soft tissues suitable for classic toxicological testing on her almost skeletonized remains, larvae of the calliphorid fly *Cochliomyia macellaria* (F.) found feeding on the remains, were collected and analyzed for drug content.

Phenobarbital was subsequently detected by GC and confirmed by TLC at a concentration of 100 μg/g.

Kintz et al. (1990a; 1990b) have demonstrated further detection of prescription drugs through the analyses of fly larvae feeding upon human remains. In one case, toxicological tests were performed on the remains of a male decedent having a known postmortem interval of 67 days. Liquid chromatography was employed in the analysis of heart, liver, lung, spleen, and kidney tissues as well as calliphorid fly larvae collected from the victim. Results of these analyses revealed the presence of five drugs (triazolam, oxazepam, phenobarbital, alimemazine, and clomipramine) in both the tissues and fly larvae examined. Triazolam was not detected in either the spleen or kidney samples, although the other drugs were present. All five drugs were, however, isolated from the developing fly larvae. In this case, it was not possible to establish any quantitative correlations between the concentrations of the drugs detected in the fly larvae and the human tissues. It is of interest to note, however, that Kintz et al. (1990b) observed fewer endogenous peaks in the chromatograms obtained from the maggot extractions than those from the human tissues. Further evidence of the toxicological potential of insect specimens is provided by Kintz et al. (1990b) in the subsequent successful recovery of bromazepam and levomepromzind from fly larvae obtained from decomposed human remains.

Introna et al. (1990) presents the results of toxicological analyses conducted on fly larvae reared on human liver tissues collected from forty cases in which opiates were detected during routine postmortem examinations. In this study, opiates were effectively identified in the fly larvae through the use of RIA techniques. A significant correlation was reported between the concentrations of opiates observed in the liver tissues and the concentrations detected within the fly larvae tested. While the qualitative drug findings were quite clear, the quantitative relationships between human host and insect consumer concentrations were less dramatic.

Goff et al. (1989; 1991) detail similar qualitative results in studies of known dosages of cocaine and heroin administered to laboratory rabbits. Fly larvae, subsequently fed on the tissues of these animals and analyzed for drug concentrations, demonstrated clear evidence of both drug presence and bioaccumulation. Quantitative relationships between the tested rabbit tissues and the developing fly larvae were suggestive but not definitive. Clearly, far more research concerning the quantitative aspects of entomotoxicology is needed before the full forensic potential of these techniques can be realized.

Nolte et al. (1992) further illustrate the forensic applicability of toxicological information obtained through the analysis of insects collected from badly decomposed human remains. In this instance, the nearly skeletonized body of a 29-year-old intravenous drug user was discovered in a wooded area 5 months after his disappearance. Friends of the victim reported that he had used a substantial quantity of intravenous cocaine immediately prior to wandering away from a rural residence. Associated with his remains were numerous fly larvae and puparia. Skeletal muscle was submitted for toxicological analysis along with samples of the collected insects. Both the victim's tissues and the larval insects tested positive for cocaine and it's major mammalian metabolite benzoylecognine using GC. The empty puparial cases also were subjected to analysis and found to be weakly positive for both the parent drug and the metabolite. The prospect of effectively extracting drugs and/or toxins from the chitin matrix of insect puparia opens new avenues of entomotoxicology, as these and other chitinized insect remnants often remain unaltered in the environment for extremely long periods of time.

With the advent of hair extraction techniques (Baumgartner et al., 1989), further interest in the use of chitinized insect remnants as potential sources of toxicological information emerged. Manhoff et al. (1988) successfully extracted cocaine from fly larvae and beetle fecal material collected from human remains using gas chromatography and mass spectrometry. Miller et al. (1994) isolated both amitriptyline and nortriptyline from empty fly puparia, dermestid beetle exuviae and frass, and mummified human tissues associated with the body of a middle-aged female discovered in her residence more than 2 years following her death. Strong acid and base extraction procedures, originally developed for human hair analyses, were employed to successfully release the drugs from the chitin/protein matrix of the puparia and beetle remnants. Similarly, Goff et al. (1997) were able to demonstrate 3,4-methylenedioxymethamphetamine in both fly larvae and spent puparia reared on infused rabbit tissues.

The potential value of larval and adult carrion-feeding insects and their chitinous remnants as alternative sources of toxicological information has been clearly demonstrated. As with other emerging technologies, however, great care must be taken in the interpretation and utilization of such data, particularly within the forensic arena. Much more research is required before the full potential of this discipline can be recognized.

Impact of Drugs and Toxins on Insect Development

While many of the studies mentioned earlier documented the potential for use of maggots and puparia as alternate specimens for toxicological analyses, few were concerned with the potential effects of these drugs on the development of the insects ingesting them. In providing an estimate of the postmortem interval, particularly within the first 2 to 4 weeks of decomposition, it is assumed that the insects will develop at predictable rates for given environmental conditions. That this might not always be the case was first demonstrated by Goff et al. (1989) in their studies on the effects of cocaine on development of the sarcophagid fly *Boettcherisca peregrina* (Rodineau-Desvoidy). In this study, maggots were reared on tissues from rabbits which had received known dosages of cocaine, corresponding to 0.5, 1.0, and 2.0 times median lethal dosage by weight. Two patterns of development were noted. Control and sublethal dosage colonies developed at approximately the same rate, as indicated by total body length. By contrast, the colonies fed on tissues from the lethal and twice-lethal dosages developed more rapidly. This difference continued until maximum size was attained and the post-feeding portion of the third instar was reached. Due to the increased rate of development during the feeding stages, pupariation occurred first in the lethal and twice-lethal colonies, but the actual duration of the puparial period was the same for all colonies and there were no detectable differences in puparial mortality.

Similar studies were conducted by Goff et al. (1991) for heroin using *B. peregrina* maggots. In these studies, heroin, in tissues as morphine, resulted in more rapid development of maggots and production of larger maggots in all the treated colonies until maximum size was attained. The puparial period was longer in duration for all colonies fed on tissues from the test animals and appeared proportional to the amount of the drug administered. This study demonstrated that an error of up to 29 h could occur if the estimated postmortem interval was based on larval development and 18 to 38 h, if based on the duration of the puparial stage.

In studies on methamphetamine, the situation became more complicated. There were observed increases in rates of development for the sarcophagid *P. ruficornis* for colonies fed on tissues containing lethal and twice-median lethal dosages of methamphetamine, while colonies from the control and half-median lethal dosage animals developed at approximately the same rate. There was increased puparial mortality in the half- and median-lethal dosage colonies and both median-lethal and twice-median lethal dosage colonies failed to produce viable offspring by the second generation. By contrast, for 3,4-methylenedioxymethamphetamine (MDMA), larval and puparial mortality was highest for the control and half-median lethal dosage colonies and lowest for the twice-median lethal dosage colony. Additionally, emerging adults produced viable larvae.

The tricyclic antidepressant amitriptyline was tested in similar manner on *P. ruficornis* (Goff et al., 1993). Here there were no significant differences in rate of development related to the concentrations of the drug administered until the post-feeding third instar. The duration of the post-feeding stage was longer for all colonies fed on the drug and larval mortality was greater in the treated colonies. No significant differences were observed in puparial mortality among the treatment; however, the puparial stage was longer for colonies fed on tissues from the rabbits receiving the median and twice-median lethal dosages.

Recently, Hedouin et al. (1999) have demonstrated the potential underestimation of postmortem interval based on the developmental analysis of necrophagous fly larvae (*Lucilia sericata* Meigen) fed on the tissues of deceased rabbits previously perfused with various concentrations of morphine. In this study, a possible error in the estimation of time since death of up to 24 h was noted if the presence of morphine and its resultant effects on fly development were not considered. During this study, a new experimental model was used to obtain concentrations of drugs in rabbit tissues, which were similar to those encountered in humans who had expired as the result of a drug overdose. This model employed the administration of morphine hydrochloride via ear artery perfusion through a plastic catheter placed into the main ear artery of experimental rabbits. This experimental methodology allowed for a more precise control of both blood and tissue levels of the drug and facilitated more accurate duplication of visceral concentrations similar to those encountered in human cases.

While there have been relatively few applications of these data to cases, Lord (1990) details a case that serves to illustrate the potential significance of these alterations to larval and puparial development. The body of a Caucasian woman, approximately 20 years old, was discovered in a pine woods area northeast of Spokane, WA. The body was physically in the early bloated stage of decomposition and had extensive populations of maggots on the face and upper torso. Maggots were submitted to the entomologist after being refrigerated for 5 days, and reared to the adult stage. Two species were identified from the adults: *Cynomyopsis cadaverina* (Rodineau-Desvoidy) and *Phaenicia sericata* (Meigen). Typically, *P. sericata* oviposits within 24 h following death, while *C. cadaverina* oviposits $1^1/_2$ days following death. Three classes of maggots were present in the corpse, based on size. The first consisted of maggots measuring 6 to 9 mm in length that were consistent with a period of development of approximately 7 days. The second consisted of smaller maggots, consistent with continued oviposition by adult flies. The third consisted of a single maggot measuring 17.7 mm in length and indicative of a developmental period, under prevailing conditions at the scene, of approximately 3 weeks.

Given the other data associated with the case, this period did not seem possible. The possibility that this maggot had migrated from another nearby source was eliminated, as no carrion could be located nearby and the probability of only a single maggot migrating was low. The alternate explanation was that the maggot's growth rate had been accelerated in some manner. It was learned that the victim had a history of cocaine abuse and that she had snorted cocaine shortly before her death. This maggot had most probably developed in a particular pocket in the nasal region containing a significant amount of cocaine.

Conclusions

Insects and other arthropods can prove to be valuable tools in the investigation of homicides, suicides, and other unattended human deaths. In addition to the recognized applications to the estimation of postmortem interval, remains relocation, and assessment of antemortem injury, insects also may serve as reliable alternative specimens for toxicological analyses in the absence of tissues and body fluids normally sampled for such purposes. In cases of badly decomposed and/or skeletionized remains, analyses of collected carrion-feeding insects may provide the most accurate qualitative sources of toxicological information.

While the data reviewed above concerning the potential effects of drugs and toxins on rates of insect development are limited in scope and no adverse impacts on case analyses have been reported to date, it is not unreasonable to assume that such substances, contained in tissues fed upon by carrion insects, have the potential for altering developmental patterns. Any factors mitigating insect development have the potential of affecting subsequent insect-based estimates of postmortem interval. Until more comprehensive studies and appropriate baseline data are available, care must be taken in interpretations of arthropod developmental patterns in cases where drugs or toxins may be a factor. In addition, it becomes essential that forensic entomologists be made aware of any information concerning the presence of these substances in remains.

Entomotoxicology may prove to be another valuable tool in the forensic science arsenal. More detailed and comprehensive research is required, however, before the full potential of this emerging discipline can be recognized.

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DNA Techniques for Forensic Entomology

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Introduction and Brief Review of Forensic DNA Typing

At a time when many aspects of forensic science are dominated by recent advances in the field of molecular biology, it is no surprise that DNA technology should also become a tool of the forensic entomologist. At present, efforts to develop these tools are still mostly at the research stage. However, they have the potential to move very quickly into widespread use by those who analyze insect evidence in forensic investigations.

Since 1985, DNA typing of biological material has become one of the most powerful tools for personal identification in forensic medicine and in criminal investigation (Benecke, 1997b). The advantages of using DNA are that it provides a huge amount of diagnostic information compared to some older techniques (such as blood-group typing), it is present in all biological tissues, and it is much more resistant to environmental degradation than most other biological molecules (e.g., proteins). The entire DNA content of an organism is termed its *genome*, and techniques that extract all regions of DNA from a biological sample are said to yield *genomic DNA*.

A region of DNA that is used for identification purposes is often referred to as a *marker* or a *locus* (pl. *loci*). It displays one of two general types of variation between individuals (or species). A locus may vary between individuals in its length or in its sequence (the arrangement of paired bases that make up the DNA molecule). These two types of variation

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are measured using different techniques (see below). The variants at a particular locus are called *alleles,* and this variation is responsible for the differences observed between individuals. A general term for a genetic difference observed between individuals is a *polymorphism*. The allele, or combination of alleles at however many loci were examined, determined for a biological sample constitutes its DNA *type*.

In most cases the purpose of a forensic DNA test is to investigate the possibility that two or more biological samples originated from the same individual (or species). Identity between the DNA types of two samples, for example a hair found at a crime scene and a blood sample taken from a suspect, can be highly incriminating. Conversely, a mismatch between samples, such as a semen stain from a rape victim and a blood sample from a suspect, can be highly exculpatory. Interpretation of a match depends very much on how rare the DNA type is. If the type is common, then a match between evidence and suspect could easily occur even if the evidence came from someone other than the suspect (i.e., a random match). If the type is extremely rare, then it would be very unlikely for the evidence to match the suspect if it had come from someone else. It is often the job of a forensic scientist to estimate this *random match probability* of a DNA type in order to help determine the weight to be given to forensic genetic evidence during a trial. However, it is up to the judge or jury, and not the forensic scientist, to determine guilt or innocence.

A locus that is less than about 1000 base pairs (bp) in length can be easily amplified (many copies made) using a method known as the polymerase chain reaction (PCR) (for more details on PCR procedures see (Newton and Graham, 1997)). A key ingredient(s) in PCR is the primer(s). This is a short piece of DNA that anneals to the sample DNA, thereby creating a location where the "copying" can begin. *Specific primers* are selected by the investigator to anneal at known locations on the sample DNA. Thus, the resulting amplified *PCR product* will be from a known locus. In contrast, *nonspecific* primers anneal somewhere on the sample DNA, but the investigator has no idea where. Despite this fact, the resulting PCR product can still be used for some analyses even though one does not know what has been amplified.

The major advantages of PCR-based techniques are that they are faster (requiring hours or days rather than weeks) and require much less sample DNA (see section on minisatellites) than methods without PCR (Hillis et al., 1996). Another benefit is that loci for PCR analysis have been developed that are less than about 350 bp in length, allowing the use of sample DNA that is very degraded and broken into short pieces.

Types of Loci

The science of forensic DNA typing is notable for its wealth of specialized jargon and acronyms, and this can be very confusing for the beginner. In order to clarify the manner in which forensic entomologists have adapted existing DNA methods for their own purposes, the following sections will describe the common genetic procedures and terms used in identity testing.

Human Leukocyte (or Lymphocyte) Antigen (HLA) DQα

This and some similar methods are PCR-based tests in which the loci show sequence variation (Helmuth et al., 1991). The PCR product is applied to a nylon strip with attached

probes designed to bind each of the possible alleles. A particular allele is detected by the location on the strip where binding takes place.

Satellite DNA

Satellite DNA is composed of a particular sequence repeated many times. The alleles differ from each other in the number of repeating units ("repeats"), and these alternate "head to tail" in what is called a *tandem* arrangement. Such loci also are called *variable number tandem* repeats (VNTRs). The large number of alleles shown by VNTR loci, and the fact that well established population genetics and statistical theory may be used to calculate the probability that two individuals selected at random will have the same VNTR type (Evett and Weir, 1998), has led to the great technical advances that have recently been achieved in identifying the individual source of a biological sample.

Minisatellites

Minisatellites have a repetitive core in which the repeat is greater than ~15 bp in length and can be repeated up to 10,000 times. Classical "DNA fingerprinting" or *restriction fragment length polymorphism* (RFLP) is performed by detecting very long minisatellite loci, up to 10 kilobase pairs (kb) in length (Jeffreys et al., 1985) (Figure 12.1). DNA from the sample is cut on either side of the locus with *restriction enzymes*: molecules that recognize a specific short DNA sequence and cleave the double strand. The resulting DNA fragments are separated according to length in a polyacrylamide gel by the forces of an electric field. This is commonly termed *gel electrophoresis* and it operates on the principle that during a given length of time a short molecule will migrate farther through the gel than will a long molecule. To detect the fragments that can vary in length between individuals, species, or

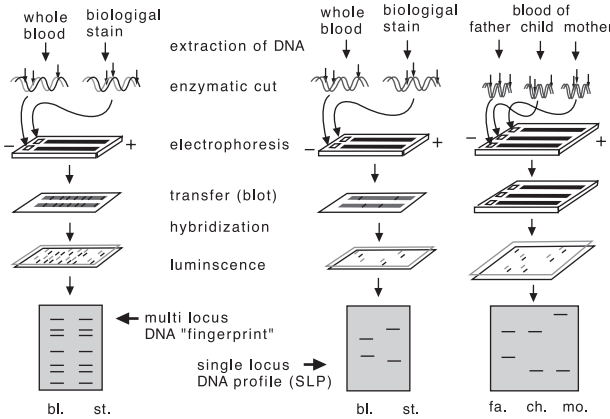

Figure 12.1 Principle of RFLP typing. Genomic DNA is cut by use of restriction enzymes, separated by length in an electric field, blotted to a membrane, hybridized against fluorescent or radioactive oligonucleotide probes, and made visible on an x-ray film. The left column shows the use of a multilocus probe, which will produce multiple bands within a lane. The right columns show the use of single locus probes, which result in one or two bands per lane. This is because a locus is represented by two alleles, which may be the same or different lengths. Two biological samples that originated from the same individual will show the same alleles, as shown in the left column but not the center column. A child inherits one allele from each parent. (Adapted from Benecke, M. 1997b. *Naturwissenschaften,* 84:181–188.)

populations, the DNA is transferred from the gel to a nylon membrane (a Southern blot). Probes, molecules that bind specifically to the DNA of a particular locus (and which, unlike the sample DNA, can be detected because they contain fluorescent or radioactive molecules), are then used to detect the location and, therefore, the length of the allele.

The great range in lengths of minisatellites used for RFLP makes them the most variable of forensic loci (and, therefore, the most discriminating) because so many different alleles are possible. Unfortunately, their length also means that they are destroyed when DNA is broken up by ultraviolet (UV) light, bacteria, etc., as often happens to forensic specimens. Also, because it is only the original sample DNA that is detected, a relatively large amount (at least 5 to 10 μg) must be obtained. This means that the equivalent of up to 400 μl of a 2-day-old dried blood stain on cotton, or 200 μl of dried blood on paper, glass, or wood are necessary for just one successful RFLP test. About 50 μl of fresh ejaculate contain enough genomic DNA for one RFLP test, but postcoital swabs, a single hair, or a small drop of dried saliva will usually not allow RFLP typing due to the low amounts of DNA found therein. One exception to this limitation is minisatellite locus D1S80 which has a repeat core only ~220 to 650 bp in length and which can be amplified by PCR prior to electrophoresis. This variation on VNTR analysis is sometimes called *amplified fragment length polymorphism* (AMPFLP).

Microsatellites

Microsatellites, also called *short tandem repeats* (STRs), have repeating units < 7 bp and a total length of 400 bp or less. Consequently, they can be amplified and analyzed even from very small and degraded samples. The ability to conduct PCR on a single STR system locus requires only 50 pg of template DNA, the equivalent of around five cells. In rare cases, even a single cell can be DNA typed. Many forensic laboratories now simultaneously amplify many STR loci in the same reaction, a process called *multiplexing*. In practice, at least 100 diploid cells with nuclei must be recovered in order to succeed in obtaining a multiplex DNA type.

The use of STRs began to gain acceptance and widespread use in 1992 (Benecke, 1997b) and have since become the method of choice for identifying individual humans. The length of STR alleles is measured using electrophoresis in a polyacrylamide gel or capillary tube (Figure 12.2). The code names commonly used for STR loci reflect their location. For example, HUMTH01 is from an intron within the human tyrosine hydroxylase gene.

Randomly Amplified Polymorphic DNA

Most DNA typing applications were developed for the specific detection of human DNA and, therefore, only a few VNTRs of invertebrate DNA are known. This limitation can be overcome with *randomly amplified polymorphic DNA* (RAPD), a technique that can be used on virtually any organism. RAPDs use nonspecific primers that can amplify many regions of a sample DNA at once. The resulting PCR products are separated by electrophoresis, and a "band" or "peak" of a particular length can be considered a locus even though the investigator doesn't know what portion of the sample DNA it represents (Figure 12.2). RAPDs allow the amplification of up to 100 loci in one PCR. The high number of amplified RAPD loci can make it difficult to sort out informative PCR polymorphisms from noninformative ones. Therefore, to detect and analyze the highest possible number of RAPD PCR products (and their length), a semiautomated electrophoresis unit that is directly coupled with a fragment size analysis software should be used (Benecke, 1998;

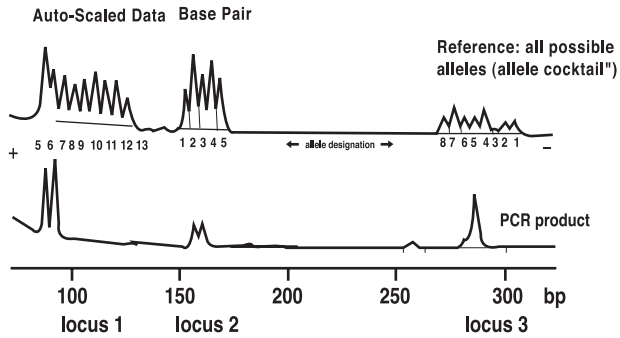

Figure 12.2 Principle of STR typing. Here, three STR loci were amplified and separated together in one run. DNA fragments are separated by size by electrophoresis as in Figure 12.1. In this case the analytical instrument has recorded the intensity of fluorescence of labeled DNA as a line. A peak corresponds to a band on the electrophoresis gel. An allele mixture (all possible alleles, "cocktail," top row) is compared to the alleles actually found in a specimen (bottom row). Once the alleles of an unknown sample are determined, they can be compared to other specimens and stored in databases. (Adapted from Benecke, M. 1997b. *Naturwissenschaften,* 84:181–188.)

Moscetti et al., 1995). Although there are several reports that RAPDs are highly sensitive to small changes in experimental conditions (Albornoz and Dominguez, 1998), RAPD results can be exactly reproduced if stringent laboratory conditions are maintained (Benecke, 1998).

Mitochondrial DNA

All of the previously described techniques utilize nuclear DNA (nuDNA), which comes from the cell's nucleus. Another source of DNA in a cell is the mitochondrion (*pl.* mitochondria). Mitochondria are the cellular organelles that are the site for the production of adenosine triphosphate (ATP), the molecule that provides the energy needed for many metabolic processes. Several lines of evidence support the theory that all mitochondria are descended from a free-living bacterium that formed a symbiotic relationship with a eukaryotic (nucleus-containing) cell in the distant past (Gray et al., 1999).

Although mitochondrial DNA (mtDNA) analysis is a relatively new aspect of forensic science (see reviews by Butler and Levin, 1998; Holland and Parsons, 1999), the techniques now used by forensic scientists are already a routine part of fields such as medicine (Wallace, 1999) and systematics (Hillis et al., 1996). Forensic scientists typically turn to mtDNA for: (1) identification of an individual when the recovered specimen contains too little useful DNA for nuDNA analysis (e.g., a hair shaft or an old bone), (2) identification of remains using a maternal relative as a reference (see discussion of inheritance patterns below), and (3) identification of species.

Like a cell nucleus, a mitochondrion contains DNA that is copied and passed down through the generations. Mitochondrial DNA contains the genes for some of the protein and ribonucleic acid (RNA) molecules needed for mitochondrial function. Mitochondrial DNA differs from nuclear DNA (nuDNA) in several ways that determine the relative strengths and weaknesses of each as a forensic DNA marker. Although there are exceptions to almost all of the generalizations that follow, they are not likely to be encountered by a forensic scientist.

Nuclear DNA is diploid. There are two copies of each locus, one inherited from the mother and one from the father. Recombination, or the "shuffling" of nuclear loci between chromosomes during gamete formation, makes it very difficult to trace genealogical relationships across more than one generation. Mitochondrial DNA is haploid. There is usually only one version (sequence) in an organism that can be detected, and inheritance is strictly maternal. Because there is no genetic recombination, an organism has the same mtDNA *haplotype* as other members of its maternal line, e.g., its mother, its great grandmother, or its siblings.

The total amount of genetic information in mtDNA is much smaller than that of nuDNA. The typical animal (including human) mtDNA molecule is between 15 and 20 kb in length, while, for example, human nuDNA contains about 3 billion bp (Avise, 1994). Thus, a typing system based on nuDNA has the potential to be much more discriminating than one based on mtDNA. With the exception of identical twins, each human has unique nuDNA, while this may not be the case for mtDNA. On the other hand, while there are only two copies of each nuDNA molecule per cell, there are often hundreds or thousands of copies of each mtDNA molecule (Holland and Parsons, 1999). This is because each mitochondrion has several copies, and there are many mitochondria in a cell. This greater abundance of mtDNA in tissues means that mtDNA can often be extracted and analyzed from very small, degraded, or otherwise poor sources of DNA that are not suitable for nuDNA analysis (Holland and Parsons, 1999).

Animal mtDNA is a circular molecule and has very little DNA that does not contain the code for making a protein or RNA molecule (Avise, 1994). The one major "noncoding" region in vertebrates is called the *control region* (because it contains one site where DNA replication originates) or *D-loop* (because in electron micrographs the two DNA strands at this location are found to be "displaced" into a loop as part of the process of replication (Holland and Parsons, 1999). Invertebrates have a similar noncoding region usually called *A+T-rich* because of the high proportion of adenine and thymine bases (Zhang and Hewitt, 1997). The A+T-rich region is also thought to include the origin site for replication, although this function is less understood compared to the vertebrate D-loop.

In most cases mtDNA analysis involves the comparison of the mtDNA sequences of two or more specimens. The procedure for determining the sequence usually involves some version of the Sanger reaction (Hillis et al., 1996). Investigators do not typically sequence the entire mtDNA molecule, but instead they examine a region that shows variation that is appropriate for their purposes. D-loop sequences are used to distinguish individual humans (Holland et al., 1995) and also may be used to separate very closely related species, while protein-coding genes are most often used to recognize species. Although there is little evidence that any of the protein-coding genes are (for a given number of base pairs) a more effective marker than any of the others (Caterino et al., 2000), the need to compare new results with previous work has led to a concentration of studies on just a few regions. For vertebrate species, this is the cytochrome b gene (Cyt b) (Johns and Avise, 1998), whose forensic utility has been clearly recognized (Barallon, 1998; Zehner et al., 1998). For insects it is most common to sequence some or all of cytochrome oxidase subunits one and two (COI+II), although this is not the overwhelming choice that Cyt b is for those who study vertebrates (Caterino et al., 2000; Simon et al., 1994). Once sequence data are obtained from an unknown specimen, they can be compared to a huge number of identified sequences by performing a BLAST search of the GenBank database.

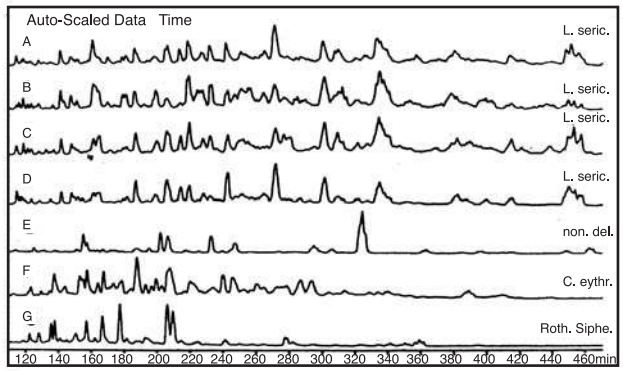

Figure 12.3 RAPD pattern (peak view) from a case in which the question was asked if a pupa found outside of a body bag could be connected to maggots found inside the body bag. (**A–D**) four individual *Lucilia sericata* (Meigen) maggots from the inside of the body bag, (**E**) pupa found near the corpse, (**F**) *Calliphora erythrocephala* (Meigen) (= *C. vicina* Robineau-Desvoidy) from another case, and (**G**) Carrion beetle (*Oiceoptoma thoracicium*) from another case. Above a certain threshold, the peaks of (**A–D**) show a high similarity. Due to its different DNA profile, the pupa is unlikely to belong to the same species as (**A–D**). DNA profiles of (**F**) + (**G**) differ from all others. (Modified from Benecke, M., *Foren. Sci. Int.,* 98:157–168.)

Forensic Entomology Applications

There are few published reports on the use of DNA techniques by forensic entomologists. However, there are several ongoing research programs that will soon expand the available applications. Most efforts have been directed at improving our ability to identify the insect specimens. It is also possible to identify the gut contents of blood or carrion feeding arthropods and, thereby, associate an insect with a living or dead human even when contact between the two is not observed.

Nuclear DNA

As previously described, nuDNA was used earlier than mtDNA for forensic genetic testing, and this also has been the situation for forensic entomological applications. Apart from numerous applications for species identification (see overview in Benecke, 1998), Replogle, et al. (1994) demonstrated that human DNA from dried crab louse, *Pthirus pubis* (L.), feces could be successfully typed using AMPFLP. The excreta were obtained from adult crab lice that fed on human volunteers, and the alleles of the human specific loci D1S80 and HUMTH01 in the insect feces matched those of the volunteers. Victims of sexual assault may acquire lice that have fed on their attacker, and it is now possible to link louse and human host in such situations. Benecke (1998) used RAPD profiles to identify a puparium collected during a death investigation (Figure 12.3). In this case investigators needed a PMI estimate right away, and the puparium could not be identified by conventional means.

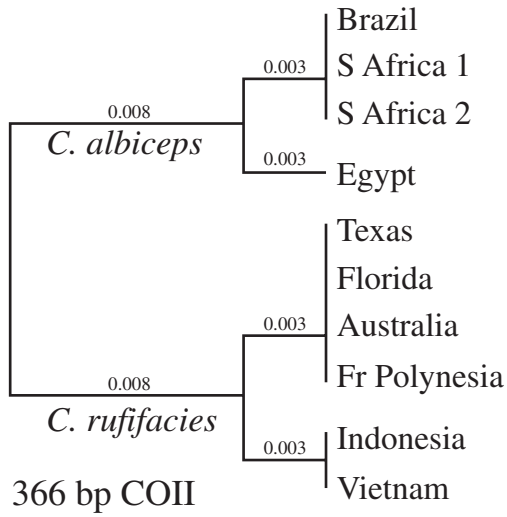

Figure 12.5 UPGMA phylogram (Hillis et al., 1996) of *Chrysomya* blow fly specimens described in Wells and Sperling (1999). Numbers on each branch indicate genetic distance, i.e., there is 1.1% sequence divergence within this part of the mtDNA COII gene between *C. albiceps* (Wiedemann) and *C. rufifacies* (Macquart) from a variety of geographic locations. These two species are difficult and sometimes impossible to distinguish for all life stages using morphological criteria.

Mitochondrial DNA

One of the potentially greatest benefits mtDNA offers the forensic entomologist is in species determination. The eggs or larvae of many forensically important dipteran species are particularly difficult to distinguish morphologically (Benecke and Seifert, 1999; Greenberg and Singh, 1995; Liu and Greenberg, 1989), and an incorrect or uncertain identification can seriously harm or impede an investigation. This is because adult arrival times, egg duration, and larval growth rates can vary dramatically between species. Proper species identification is usually an essential first step in the use of entomological evidence in a legal investigation.

Fortunately, the chance of an improper identification on morphologically similar species is likely to be diminished in the very near future. The protocols for analyzing calliphorid mtDNA have been extensively demonstrated from experiments done on species of veterinary importance (Azeredo-Espin and Madeira, 1996; Gleeson and Sarre, 1997; Narang and Degrugillier, 1995; Roehrdanz and Johnson, 1996; Stevens and Wall, 1997; Taylor et al., 1996; Valle and Azeredo-Espin, 1995). In terms of both size and structure, the mtDNA of a calliphorid fly closely resembles that of *Drosophila yakuba* (Burla) (Diptera: Drosophilidae), the first fly species for which the entire mtDNA sequence was described (Clary and Wolstenholme, 1985). Therefore, there is a great deal of basic biological information available concerning fly mtDNA, which makes it easier to design PCR primers and to interpret the results of any study on a new fly species.

Sperling et al. (1994) were the first to demonstrate how mtDNA sequence data from (easy to identify) adult specimens of forensically important flies could be used to identify immature forms of the same species (Figure 12.4*). Using the same techniques, Wells and

* See color section insert following page 78.

Sperling (1999) found that calliphorid species that can be difficult to separate taxonomically even in the adult stage have distinctly different mtDNA (Figure 12.5).

This author (J. D. Wells), in collaboration with Felix Sperling, has now obtained COI+II sequence data for more than 20 species of carrion flies found in North America, and these data will soon be publicly available. In almost all cases, closely related species can be separated using a short (~300 bp) region such as can be obtained from even very degraded DNA (see Figure 12.4 in color insert). Because there is often some intraspecific variation in mtDNA haplotypes, two samples from the same species may not match exactly. Only experience with the taxonomic group in question will allow an investigator to know if the differences observed between two samples fall within the range of normal variation for that species. Even if identification is uncertain, phylogenetic analysis can be used to reveal the specimen's closest relative(s) and narrow the choice of species to which it belongs.

Sequence data also can be used to design a restriction fragment length polymorphism test for PCR product (PCR-RFLP) (Sperling et al., 1994) (Figure 12.6). This is a faster and less expensive method than DNA sequencing. However, we believe that large samples will be needed to validate PCR-RFLP for this purpose. A single point mutation can eliminate

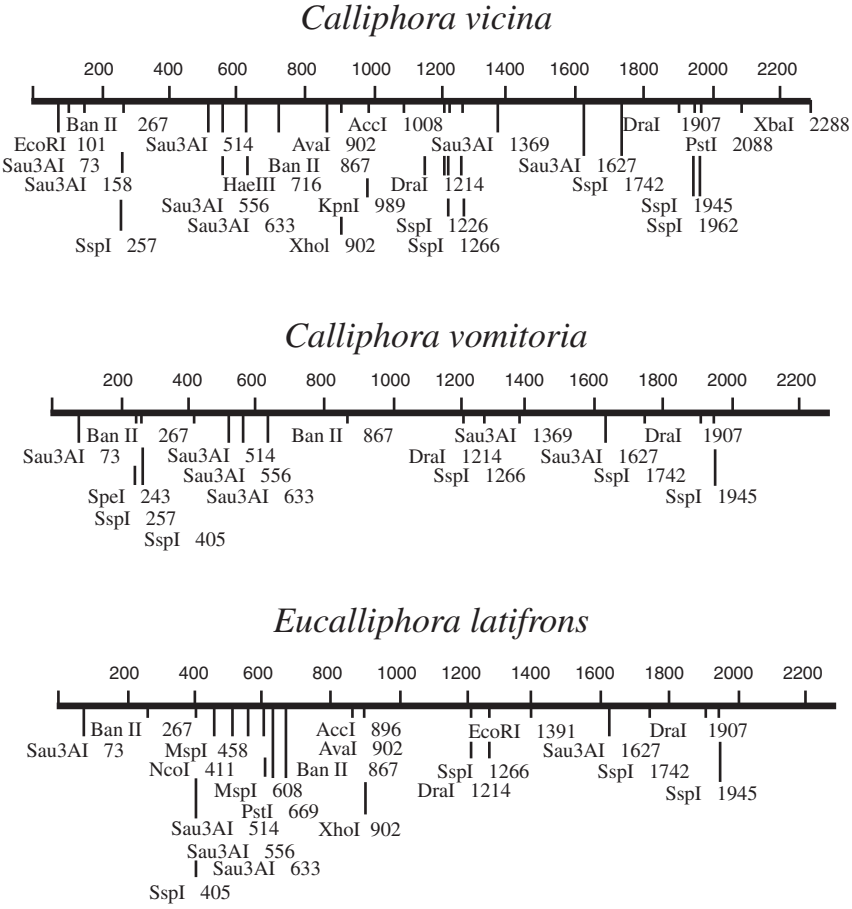

Figure 12.6 Restriction site maps for the 2.3 kb COI+II region of mtDNA for three closely related blow flies. Sequence data were searched using the computer program GeneJockeyII (Taylor, 1993) for sites that will be cleaved by a particular restriction enzyme.

a restriction site, so a reliable PCR-RFLP test must utilize restriction sites that are fixed or nearly fixed for a given species.

As described in the previous section, insect specimens can be a source of noninsect DNA, that of the organism upon which they have fed. Lord et al. (1998) typed human mtDNA from the gut contents of a crab louse. Similar results using the gut contents of blow fly maggots have been produced (Introna et al., 1999). Such analyses may prove to be crucial evidence in the creation of victim/suspect associations. Investigators that find maggots but no body, now have the potential to identify the insect's last meal. There also are occasions, particularly if the scene has been disturbed, where both maggots and a corpse are present but not in physical contact. DNA analysis of maggot gut contents provides an independent means for associating larva and victim. This ability also may prove invaluable in cases of multiple homicides or mass burials.

Comments

Just as with other aspects of a forensic entomological investigation, it is difficult to predict in advance which DNA methods will be most useful for investigators. Instead we need to develop and evaluate as many techniques as possible, and to make such information widely available. In addition to the methods described, new techniques are constantly appearing in one professional field and quickly spreading to others.

It is essential that DNA samples be obtained from insects likely to be encountered during forensic investigations all over the world. We strongly encourage all practicing forensic entomologists to expand their normal specimen collection techniques (Benecke, 1997a) to include freezing or preserving in 95% ethanol, and to either undertake molecular genetic studies themselves or to make this material available to others willing to characterize the DNA for identification purposes.

Acknowledgments

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Entomological Alteration of Bloodstain Evidence

13

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Contents

Introduction

The examination and identification of physical evidence is a key part of most criminal investigations. To ensure that the value and integrity of this evidence is maintained, it is essential that the proper steps be taken in its documentation, collection, and preservation. Physical evidence is defined as any item that may be identified as having been associated or involved with a crime scene. One such item that is present at most violent crime scenes is blood evidence. The correct analysis of bloodstains left behind at a scene or on an item of evidence is in many cases the determining factor between guilt and innocence.

The proper interpretation and analysis of physical evidence is a combination of formal training within a particular forensic discipline, experience working within the chosen field, and a good dose of common sense. This statement is especially true for the analysis of bloodstains. This chapter will address how bloodstain patterns may appear at a crime scene, and explore some of their possible interpretations, discuss the manner in which they are examined and/or analyzed, and identify the entomological factors that can lead to their alteration and subsequent misinterpretation.

The science of forensic entomology is devoted to the study of insects serving in the capacity as evidence in legal, medical, or civil proceedings. In many cases, this entomological evidence is recovered in the form of living or preserved insects that have colonized human remains. Under such circumstances the arthropod fauna can be of great importance to investigators in both the calculation of a postmortem interval estimation, and as indicators of events such as body transportation after death. The advanced technological procedures that are available in a modern crime laboratory even permit insects and insect fragments to be analyzed when tissue from the deceased is unavailable. The results of such

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toxicological analyses can determine if drugs such as cocaine were in the victim's body at the time of death, and can even serve in the recovery of DNA evidence.

Insects also may make the analysis and interpretation of the events occurring during the commission of a crime more difficult. The removal of flesh and soft tissue from bodies by flies and beetles, and the alteration of sites of trauma are some of the most obvious alterations of evidence. The consumption of skin and/or hair by ant and cockroach feeding can cause postmortem artifacts as well on a cadaver that might mislead investigators. These artifacts may resemble burns or chemical scarring and are usually readily apparent to the investigator. One form of evidential alteration that is not usually considered and often overlooked is that the insects present at a crime scene can alter bloodstain patterns and can readily transport blood to new locations.

The analysis of bloodstain evidence, and the interpretation of bloodstain patterns, has evolved into a forensic discipline of its own. Unfortunately, relatively few investigators receive more than a minimum of training in this specialized field. Such training is often limited to the protocol for the recovery of samples used for chemical analyses and presumptive tests. However, this is slowly changing as the importance of bloodstain analysis is becoming widely recognized and respected. To a bloodstain pattern expert, the size, sequence, and array of blood drops, which may be overlooked by an untrained individual, can hold a wealth of information. The recognition of insect-produced artifacts and their distinction from unaltered blood present at a crime scene is essential for the correct interpretation of bloodstain evidence.

Any insect or arthropod may leave bloody tracks of a diminutive nature simply by walking through wet blood and tracking it onto a nearby surface. In the case of flies, artifacts in the form of regurgitated or defecated blood may be deposited as well. Fleas also can be responsible for defecating partially digested blood that originated from the victim or others who were present at the scene. Characteristics such as size, shape, and pattern are used by bloodstain analysts to distinguish insect artifacts from legitimate or unaltered blood spatter and will be discussed in the following sections.

It is important to understand the key information to be gained in the analysis of this type of physical evidence. This information can include what did (or did not) take place, and answer the question of who may have been involved in these actions. It is beyond the scope of this chapter to address all of what bloodstain patterns can provide in the way of information in a criminal investigation. However, it is hoped that this chapter will be a tool to assist in the interpretation of blood when it is present at a crime scene, especially in death investigations where decomposition has occurred and insects are present. This information will be presented as follows: a brief history of bloodstain analysis, basic terminology, techniques, entomological artifacts, and a practical approach to the use of this information. This chapter will conclude with a case review on how bloodstain evidence was documented and analyzed, and how entomological evidence influenced the analysis of bloodstains in an actual death investigation.

> Violent crimes usually result in bloodshed. When liquid blood is acted upon by physical forces, patterns will be deposited on the various items at the crime scene as well as the clothing of the individuals present during the activity. These patterns can yield valuable information concerning the events which lead to their creation. The information can then be used for reconstruction of the incident, as well as to evaluate the statements of the witnesses and the crime participants. Bloodstain pattern evidence can be of value in the investigation of any violent crime in which bloodshed occurs. (Wolson and Johnson, 1994)

History of Bloodstain Analysis

Bloodstain interpretation and analysis is an important tool that may be used by crime scene investigators to explain what actions took place during the act of a crime. It is also just as important in the establishment of what could not have happened at any particular crime scene. However, in order to gain the maximum benefit of blood evidence, the accurate recreation of a crime scene is a basic requirement. This allows those trained in this forensic discipline to answer many of the questions that must be addressed when processing a crime scene.

Although this discipline is considered by many to be "new" science, its use dates back to the mid-1800s. In fact, even nonscientific references may be found in 11th century European law with the term "red handed" being used as a way to identify a criminal who had blood on his hand. More popular literary references are those of Shakespeare in *Macbeth* and Sir Arthur Conan Doyle in *A Study in Scarlet*. In both stories blood was used as evidence in a crime.

A notable early scientific contributor to this field was Dr. Eduard Piotrowski of the University of Vienna. During the late 1800s, he recognized bloodstain patterns and their causes by analyzing them in laboratory settings. Another of the earlier known studies was by Dr. Paul Jeserich, a chemist who lived in Berlin. He examined the bloodstain patterns that were present at homicide scenes just after the turn of the 20th century. More recently, Dr. Vincent Balthazard researched bloodstains in the 1930s. His contribution of the impact angle formula to determine the point of origin is perhaps the most significant in the field of blood pattern analysis. Other notable pioneers in the study of bloodstain evidence are Ernest Ziemke and Dr. W. F. Hesselink.

> In 1856, J. B. Lassaigne wrote *Nueu Untersuchungen zur Erkennung von Blutflecken auf Eisen und Stahl (Examination to Differentiate Bloodspots from Iron and Steel)*. In the latter section of his paper, Lassaigne discusses marks that appeared similar to bloodstains, but were caused by insects. Although Lassaigne made it clear he believed that the "crushing" of dead flies had created such stains, he only implied having found such stains at various scenes. If he directly associated their presence with the presence of flies, it was not recorded or stated. Based on his descriptions, these stains seem similar to what we might refer to as "flyspecks." Unfortunately, the translation and overall description of what Lassaigne actually found cannot satisfy the issue. Had Lassaigne indeed found flyspecks and accounted for fly activity in his investigations? Was he simply establishing a causal connection without considering the regurgitation of blood by flies at a bloodstained scene? Whatever the case, Lassaigne's observations established his attention to detail and concern for differentiating such stains from normal bloodstains (Bevel and Gardner, 1997).

One of the first known cases of bloodstain pattern interpretation in the U.S. was during the Dr. Sam Sheppard homicide case in 1955. In the 1966 retrial, Dr. Paul Kirk, a professor in Berkeley, CA, testified for the defense on several critical points. During the original trial, the prosecution presented information that the suspect holding the murder weapon, dripping with the victim's blood, made the blood trail at the scene. Dr. Kirk testified that the trail could not have been made in this manner, but rather from an open wound. Since there were no such injuries to Dr. Sheppard, the wound had to be present on the suspect. Dr. Kirk's testimony also negated other points concerning bloodstain evidence on Dr. Sheppard's watch and in the victim's bedroom for the same reason. However, certain aspects of this case are again being presented in court, and many bloodstain experts currently

disagree with the original findings of Dr. Kirk. In addition to his pioneering work on this case, Dr. Kirk authored *Crime Investigations*, in which bloodstain pattern interpretation is addressed. Since that time, numerous articles on this discipline have been published that explain the science and how conclusions can be made from blood evidence with a reasonable degree of scientific certainty.

In 1971, *Flight Characteristics and Stain Patterns of Human Blood* was published as the result of the research and studies of Dr. Herbert MacDonell and Lorraine Bialousz. Many researchers in this field have taken the groundwork established by MacDonell and Bialousz and incorporated it into their respective studies, publications, and teachings.

Questions to be answered by the examination of evidence at a crime scene will fit into two basic categories: "what is fact" and "what can be determined within a degree of scientific certainty." Determining factual information is usually the easier of the two equations, but it is the portion that is most often overlooked. Such things as "there lays a dead body" or the fact that "blood is present throughout the scene" are available to us immediately as we enter a scene and are indisputable. In a majority of cases, but not always, the "fact" is not questioned. Therefore, the more difficult aspect of the examination of physical evidence is the second half of the equation. This requires the investigator to answer the what, when, why, and where of all the circumstances surrounding the investigation. With bloodstain patterns, these are exactly the questions that are brought forth as the analysis of the evidence begins. Once "fact" is established, it then becomes a question of how to determine the answers to the what, when, why, and where. There are many aspects involved with the various avenues of reaching these conclusions, but as with all forensic scientific findings, they should only be based on accepted and proven practices.

With all facets of forensic sciences, there is a protocol and sequence of questions and deductions that must take place to eliminate certain possibilities and to reach a point of identification and/or threshold degree of scientific certainty. In many of the cases involving bloodstain patterns, this degree of scientific certainty is the best standard for case analysis. Also, a certain percentage of deductions made from the review of bloodstains will be scenarios of what could not have happened. This protocol of questions and deductions can be a long, detailed, and arduous process. At other times, it is a split-second thought that has become second nature from years of experience.

Before a technique becomes an accepted protocol, it should be used consistently to ensure proper review of the evidence. No matter how time consuming or detailed the process may be, it is important that proper protocol be practiced and followed without error, and such protocol should be both consistent in its application and in its simplest form before being utilized to derive any conclusions. It is beyond the scope of this chapter to provide a complete crime scene protocol. It does not include all that could, or should, be done with bloodstain evidence recovered from a crime scene. What this protocol will cover is general steps in crime scene documentation, the examination and analysis of the blood as it pertains to the pattern interpretation, and the entomological factors that may lead to its alteration.

It must be understood that each investigation and crime scene is unique. There can never be an exact order, or series of steps, in any protocol that can be universally applied to the processing of all crime scenes. There are many extenuating circumstances that will affect the decisions and priorities of the investigation. With that said, there are some guidelines that can be used to establish a crime scene protocol that will ensure that bloodstain evidence is handled properly.

Protocol and Techniques

The key to any investigation is communication between all of the parties involved. No individual or unit can work successfully within a communication void. Very little can be accomplished and much can be destroyed if the information from the investigative team and the crime scene unit is not shared. Additionally, an outline of crime scene processes must take place to ensure evidence is documented properly, and that evidence is not destroyed or rendered useless.

Listed are five steps that provide a general outline of actions to be taken at a crime scene as it relates to the proper documentation, collection, and preservation of physical evidence.

1. Initial visual inspection, notes, and information gathering
2. Photography and videography
3. Scene sketch and measurements
4. Scene search and collection of evidence
5. On-scene processing (latent prints, bloodstain patterns, casting, etc.)

Within these five areas, the process of bloodstain pattern interpretation has its own guidelines to be used to determine the value of bloodstain pattern evidence. However, this is only in the area of bloodstain pattern interpretation and does not include the processing of an entire scene or the preservation of blood for serological examination.

Utilization of the team approach to processing a scene with bloodstain evidence is preferable. If a team approach is not possible, the procedures will remain the same, but a team allows the various tasks to be done in a more timely manner. Also, it is better to have another set of eyes and ears, and another investigative mind to help eliminate the chance of missing critical evidence.

Bloodstain analysis begins with a visual examination of the overall scene. As obvious as this seems, the importance of a thorough visual examination is often overlooked. This step will determine all subsequent processes within the scene, and will provide the primary direction of the investigation. It is within this step that fact vs. the unknown may begin to be established. It must be noted and emphasized that conclusions should not be drawn at this time, although in many cases this information is the foundation for the final interpretation.

Written notes are crucial and such documentation will be an ongoing process during the entire investigation. This information can come from personal observations, others on the investigative team, victim(s), witness(es), and sometimes, even the suspect(s). In addition to the basic crime scene notes, the first observations required for the proper analysis of bloodstain evidence should include environmental conditions, identification of the areas where there is apparent bloodshed, and a record of the blood's condition (i.e., wet or dry, color, consistency, etc.).

The phrase "apparent bloodshed" is often used in this area of forensic science. Determining whether a substance is blood or not will depend on several factors: the level of the analyst's training and experience, the appearance of the substance, and the presence or known presence of a blood source. A blood source may be a person who has been injured, a person who has blood on them, or a bloody object. If there is any question that the substance is blood, a presumptive blood test should be performed. This may be done at

different times during the analysis. The scene and its components dictate when this will be done. If the test result is negative, the remainder of the analytical process is eliminated and all such information should be included in the scene notes. Eventually, all of the bloodstains analyzed will be collected and examined for various properties including a determination as to whether it is, or is not, human blood.

Other ongoing documentation will be photography, videography (when applicable), and sketches. Proper lighting and light angles are important in scene photography, as the photographs may be used for further analysis and scene reconstruction. Sketches also are useful for visual interpretation of blood evidence notes without the distractions of non-evidentiary objects that may be present in the photographs.

The first step in the actual analysis is to determine, if possible, the bloodstain pattern(s). In order to accomplish this, it is necessary to be familiar with the terminology used in this discipline. Some of the most important and basic terminology used to define bloodstain patterns is included in this chapter. The following are some of the most common pattern definitions, while more complete lists may be found in Bevel and Gardner (1997) and MacDonell (1993).

- **Cast-off pattern:** the pattern created when blood is flung from an object in motion.
- **Drip pattern:** the pattern created when blood drips into liquid blood.
- **Impact pattern:** the pattern created when blood put into motion by force to a bloody object strikes another surface.
- **Passive drop or flow pattern:** the pattern created when the force of gravity alone acts upon blood.
- **Projected blood pattern:** the pattern created when blood under pressure and in volume lands on another surface.
- **Transfer pattern:** the pattern created when a wet bloody object comes in contact with another surface.
- **Wipe pattern:** the pattern created when a nonbloodied object moves through wet blood and changes the appearance of the blood.

It is important to note that a single drop of blood does not identify the pattern. The entire area of bloodstains must be evaluated. However, a single drop must not be overlooked, as it could be an important piece of bloodstain evidence. The overall pattern may indicate what happened where, how, and when; but a single drop may provide the "who" part of the "who, what, when, where, and how" equation.

In 1995, the Tallahassee Police Department investigated a homicide in which an unknown suspect attacked a local college student in her apartment. During the stabbing assault, there was a violent struggle that began in the rear bedroom and moved throughout the apartment. It ended at the front doorway where the victim was eventually found. The scene was covered in blood with a multitude of patterns showing the movement and stabbing strikes (Figures 13.1 to 13.3*).

From this entire scene, it was not any of these patterns that specifically identified the attacker, but rather one lone drop of blood on the bathroom floor (Figure 13.4*). This bloodstain was separate from all other patterns, and it was not consistent with the attack. The suspect, who was picked up later the same day, had minor wounds on his hands

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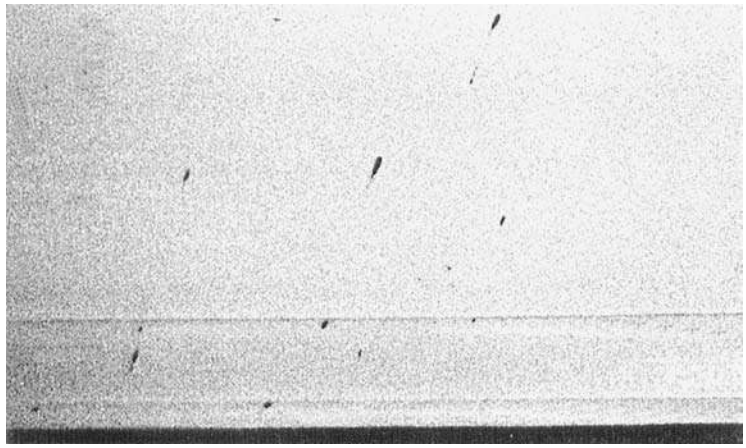

Figure 13.6 Cast-off pattern on wall at the scene of the stabbing. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

(Figure 13.5*). He had apparently transferred this one blood drop as he traveled through the hall adjacent to the bathroom. A blood test confirmed that this drop matched that of the suspect. In this case it was the observation of where the blood did *not* come from, rather than where it did, that made it stand out and eventually defined its importance.

It is not always possible to accurately identify a pattern due to conditions and circumstances too numerous to list here. An analyst examines all aspects of the blood evidence available in order to make a pattern determination. The results of these examinations should be included in the scene notes and reports, even if a pattern cannot be identified.

Since each pattern at a scene reveals certain unique information, a brief discussion of each of the previously listed patterns is warranted. The cast-off pattern when made by the instrument of impact attack will tell the minimum number of blows struck. The first blow will draw blood that adheres to the instrument, and the subsequent blows will create the pattern. The blood drops in this pattern will generally be linear in appearance (Figure 13.6). A drip pattern may indicate the location of a blood source that did not move for a period of time (although the time frame is difficult or impossible to determine), and a pool of blood with satellite blood drops characterizes this pattern (Figure 13.7).

There are three types of impact patterns characteristic of high-, medium-, and low-velocity impact. Each is typically defined by the diameter size of the majority of stain drops, although stains of all sizes may be present. There are certain, but not always inclusive, actions that may be associated with each type. The representative drops within a bloodstain pattern will define the pattern. However, drops of various velocities also may be found within the same pattern. High impact stains have the smallest diameter (0.1 mm or less) and are mist-like in appearance. Such patterns are produced when blood is exposed to a force of 100 ft/sec or greater. However, bloodstains from high-velocity impacts also are produced in the medium-velocity size range. High-velocity impacts are typically associated with gunshot wounds, and sneezing and coughing of fluid that contains blood (Figures 13.8 and 13.9*). Insects present at the crime scene can also create droplet-sized stains such as these. Often the mechanical transmission of blood from a pooled source to a blood-free surface is accomplished by the tarsi (feet) of flies or the fecal material of fleas. Roaches

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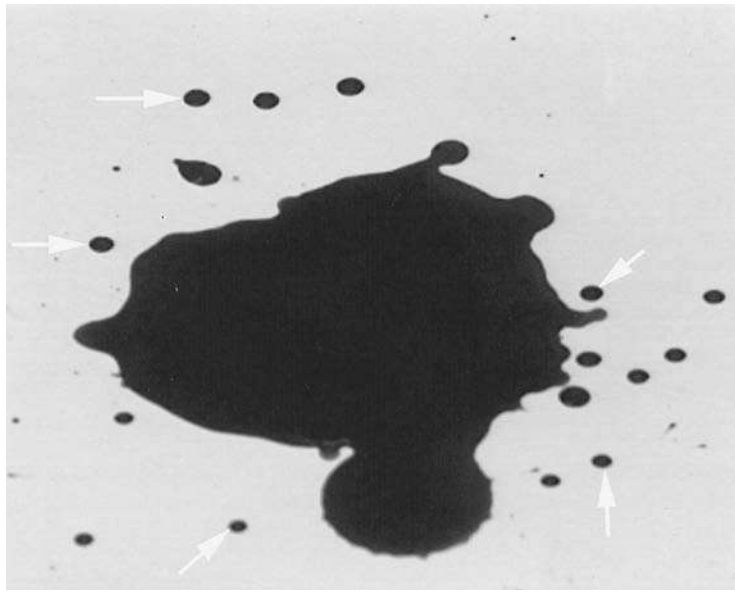


Figure 13.7 A typical blood pool and its satellite drops. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

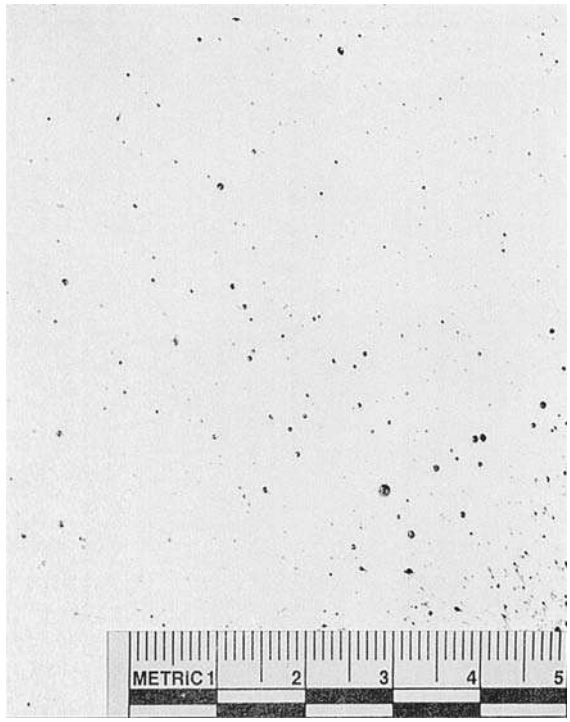


Figure 13.8 A high-velocity impact pattern showing mist-like droplet spray. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

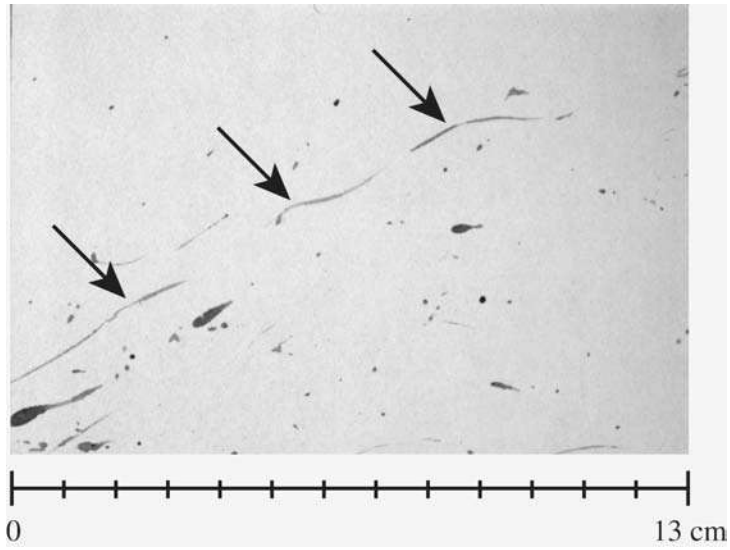


Figure 13.10 Transfer pattern produced by adult roach, *Periplaneta americana,* running through pooled blood. Note the elongated trail produced by the roach dragging its abdomen. (Photo courtesy of James L. Castner.)

also can produce apparent blood droplets by their blood-contaminated tarsi, but the droplet size is larger than that produced by fly tarsi.

In the case of roaches, such artifacts are usually easily distinguished as the overall droplet pattern reveals a series of "tracks" that often have a center drag mark or "smear" caused by the roach dragging its undersurface or the abdomen tip as the insect walks (Figures 13.10 and 13.11). It is this intermittent smear that first draws attention, and the

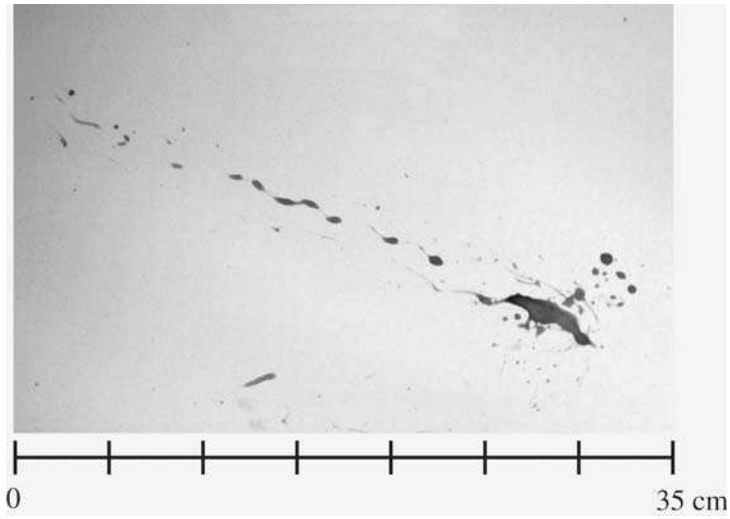


Figure 13.11 Transfer pattern produced by adult roach, *Periplaneta americana*, slowly walking through pooled blood. This pattern was produced by a larger roach dragging its abdomen and is more robust and readily apparent than in Figure 13.10. (Photo courtesy of James L. Castner.)

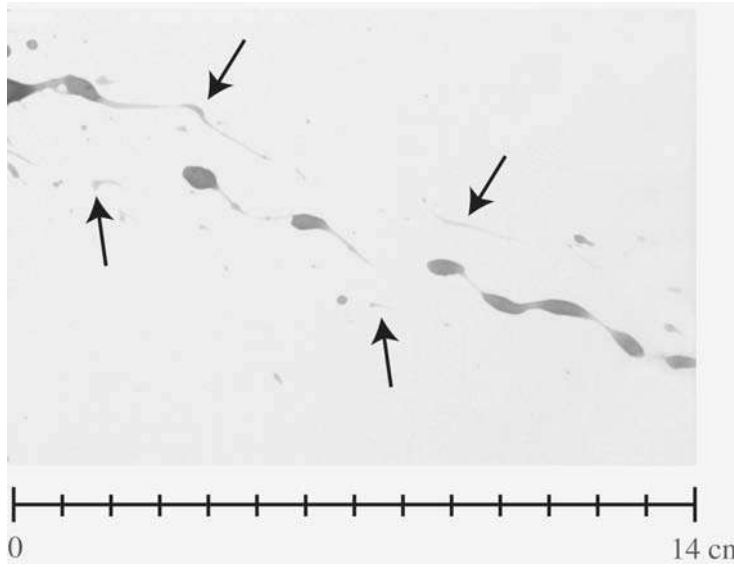


Figure 13.12 Enlargement of Figure 13.11, showing the drag pattern produced by the roach's abdomen and tarsal imprints. (Photo courtesy of James L. Castner.)

imprints made by the roach's tarsi are usually apparent upon closer inspection (Figures 13.12 and 13.13). In many cases, such details are best viewed with a hand lens. With flies, such an event is not as easily distinguished since the pattern produced is more isolated, smaller, and not as uniform. Flies with blood-soaked tarsi may alight on any surface: vertical, horizontal, or inverted. Once on the surface, the fly may not walk, or not all of the legs may be contaminated with blood, thus producing no discernible tracks or repeat-

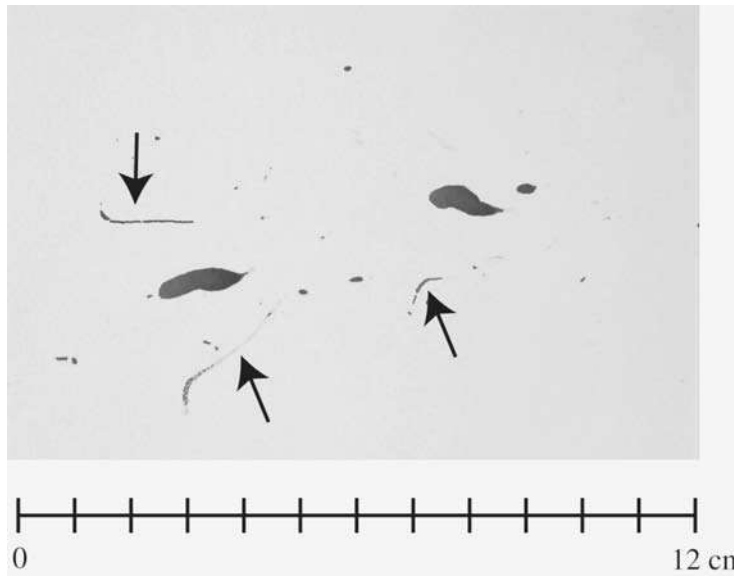


Figure 13.13 Bloodstain artifacts produced by a roach moving through pooled blood. Note imprints of abdomen and distinct tarsal tracks (indicated by black arrows). (Photo courtesy of James L. Castner.)

Figure 13.14 All of an insect's legs are not always in contact with the surface. Insects groom frequently, and do so by rubbing the body with their legs. This behavior often produces irregular artifacts in bloodstain evidence. Here, *Sarcophaga bullata* (Parker) cleans its compound eyes, leaving only four legs in contact with the surface. (Photo courtesy of James L. Castner.)

able patterns. Additionally, the fly may not alight with all six tarsi in contact with the surface. Flies often rest on only the hind two pairs of legs using the first pair to clean the eyes and head (Figure 13.14), or they may rest on the first two pairs using the hind pair to clean the abdomen. Thus, the typical "six-point" track of an insect is often not discernible. When a fly with blood-contaminated tarsi walks on a surface, a repeatable pattern can be produced, but no drag mark or smear occurs since the adult fly does not drag its abdomen as it walks. Therefore, such insect-produced blood droplets may be difficult to distinguish from high velocity droplets produced during the commission of the crime.

Many of these droplets created by insects will be found in patterns of three pairs, or readily distinguishable pairs of two. Often these tracks will be visible as a linear pattern of three pairs of droplets with each row separated by 0.5 to 1.75 cm with flies and 2.0 to 3.0 cm with roaches. Such tracks will only span a short distance due to the small amounts of blood that typically cling to the small tarsi of flies, and due to the fact that flies do not usually walk far before once again taking flight. These patterns most commonly occur on vertical surfaces (such as light-colored walls), and on ceilings because of the behavioral propensity of flies for landing on these surfaces.

Fleas also may produce artifacts that can be confused with high-velocity blood spatter. Fleas are commonly found in residences and can form enormous populations in a very short period of time. Fleas feed on the blood of living mammals (both human and animal) and pass large quantities of their undigested bloodmeal in their feces. The fecal material of fleas is at first a semiliquid, which dries into a powder if it is not passed onto an absorptive surface. This fecal material will appear as small specks that are mist-like in appearance, and will test positive for human or animal blood (depending on the source) if a presumptive test is conducted at the scene. Standard laboratory testing can be accomplished on human blood recovered from the feces of fleas, and such evidence can be used to link a suspect to the victim, crime scene, or a particular and relatively confined area. A suspect would not have to linger long before fleas present at the scene would begin to feed and pass human blood onto substrates at the scene. The closely spaced and small "droplets" of flea fecal material often have the appearance of a fine spray coating over a surface. This "spray" is found predominately on white or light colored surfaces (not only because it is more apparent, but also because fleas are attracted to lighter colors), and is typically confined at a height below 24 in. The most common areas to search for this evidence is along

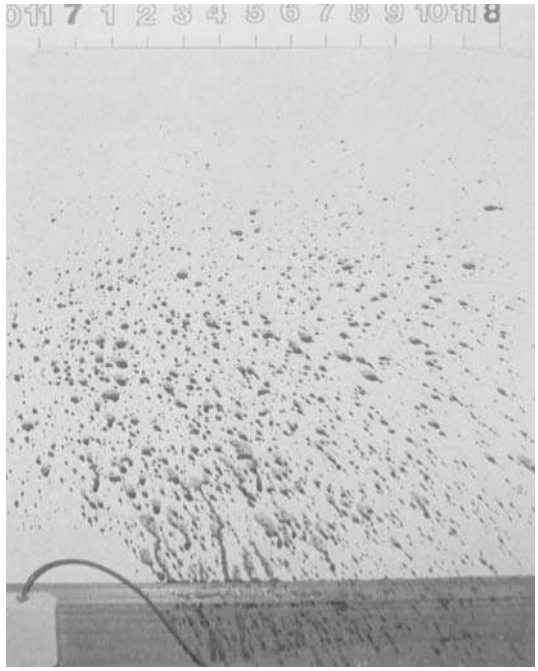
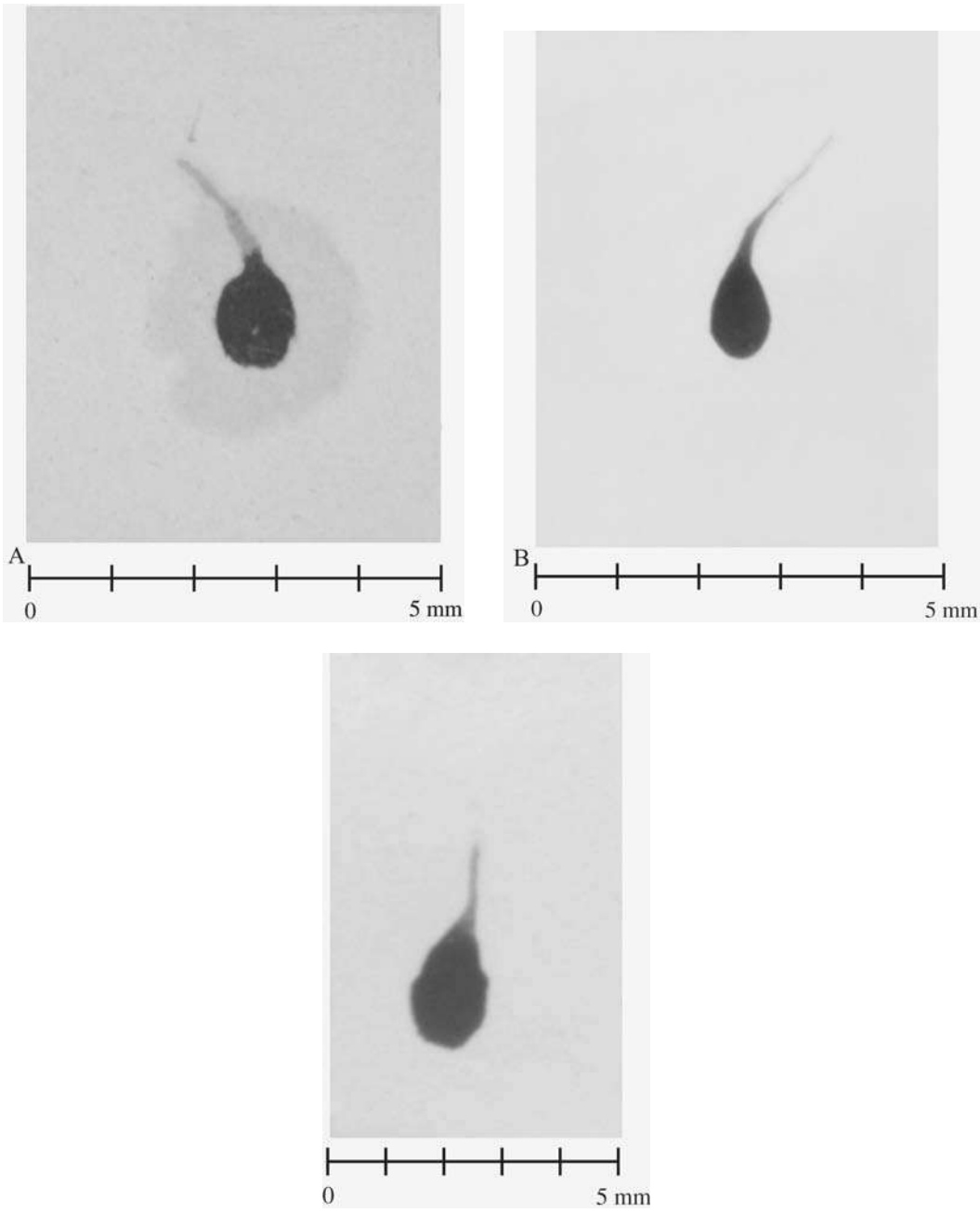


Figure 13.16 A low-velocity impact pattern (with other patterns) on a wall and baseboard. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

baseboards and floor trim. They also are common on carpets and rugs, but may be difficult to detect with the unaided eye on many fabrics, depending on fabric color and texture.

Medium-velocity impact stains range in size from 1 to 4 mm in diameter (Figure 13.15*) and are produced by a force other than gravity of 25 to 100 ft/sec. They are most commonly associated with blunt trauma injuries. Many artifacts produced by the presence of insects overlap with this droplet size class as well as the low velocity impact size class. Low velocity impact stains have the largest diameter of 4 mm and larger, and are formed when blood is exposed to a force of approximately 5 feet per second or less. These are usually associated with gravitational force and may form part of another pattern as illustrated in Figure 13.16. Within the medium-velocity-size class exists certain patterns that can be immediately recognizable as produced by (or associated with the presence of) flies. Flyspecks are the term entomologists give to the deposited liquid fecal material of flies. This material contains large volumes of partially digested and undigested blood if the adult flies have recently fed on free blood or a body at a crime scene. Thus, a fly feeding on human blood will pass a large amount of partially digested blood that will test positive as human if a presumptive test is conducted at the scene. Blood contained within these droplets can be collected and used in all standard laboratory molecular and blood serology tests. Drops such as these can usually be distinguished from those created during the commission of a crime by their overall shape. Flyspecks are a swipe pattern that typically exhibits a "comma" shape, with the tail of the drop trailing to the left, right, or straight, of center depending on the movement of the fly abdomen (Figures 13.17a,b,c). The fly touching the tip of the abdomen to the surface as it defecates and walks about produces

* See color section insert following page 78.



Figures 13.17 Characteristic blood imprints of fly fecal spots, or "flyspecks," with tail curved to the left (**a**), right (**b**), or center (**c**). Swipe patterns such as these can be easily recognizable to the trained analyst. (Photos courtesy of James L. Castner.)

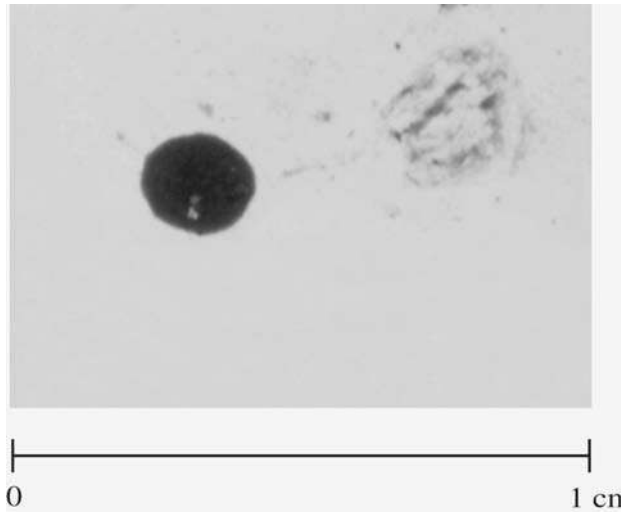


Figure 13.18 Typical symmetrical bloodstain produced by the regurgitation of blood by an adult fly. This regurgitate spot was deposited on a vertical plaster surface by *Phormia regina* (Meigen). (Photo courtesy of James L. Castner.)

this type of bloodstain artifact. It has the same basic characteristics as many other swipe patterns, and can be easily recognized by the trained analyst.

Flies also will produce medium to large droplets due to their natural feeding behavior and digestive habits. As an adult fly digests its liquefied meal, it frequently regurgitates its gut contents. This regurgitation accumulates as a medium to large droplet at the tip of its sponging mouthparts. Often this drop either falls to the surface or it is accidentally touched to a surface. Upon contact with a surface, the drop usually adheres or is absorbed and is only partially reconsumed by the fly. This drop is usually symmetrical and has circular borders (Figure 13.18). If the regurgitate is touched to a nonabsorptive vertical surface, it may produce a tail descending straight down the midline and lower edge of the droplet, but this is not always the case due to the viscous nature of the regurgitate.

When an impact pattern is created, the blood drops generally form a radiating pattern with the highest concentration in the center, but seldom in a complete circle. From this pattern, the analyst may calculate the impact angle, which is define as the internal angle at which blood impacts a surface, and use the results to determine the point of origin of the blood drops. The mathematical formula utilized to calculate the impact angle is relatively simple. First, select representative and well-formed stains within the pattern. Of these stains, each one will be calculated individually. Measure the width of the stain and divide by the length of the stain. Using any scientific calculator, simply compute the arc sine of the resultant value (angle of impact = arc sine W/L). The result will be the impact angle, and these measurements can then be utilized to calculate the point of origin. The results are used to form a three-dimensional view that provides a range of height and distance of the blood source from the impact surface. Some of the steps used to determine this are depicted in Figures 13.19 and 13.20.

The passive pattern may be used to show movement. A trail of passive drops will indicate the blood source's direction of travel, or the flow may show movement of a body after death. The most common instance occurs when the subject bleeds from the nose or

Figure 13.19 Blood drops being charted to determine the point of convergence. This step is an integral component of determining the point of origin. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

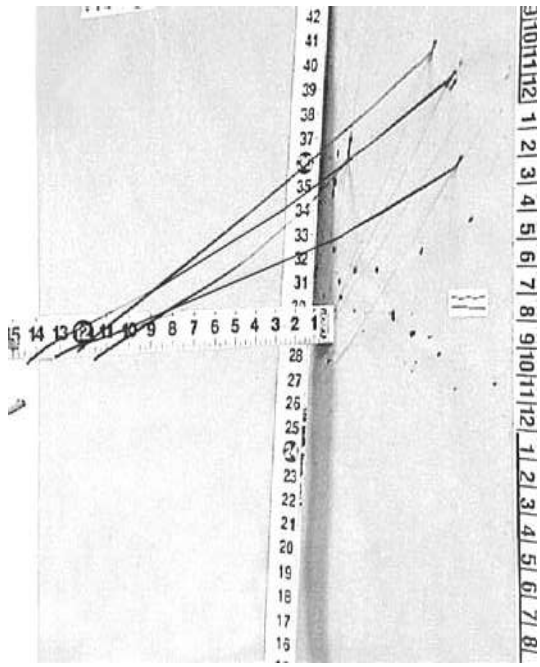


Figure 13.20 The three-dimensional view revealing the height and distance ranges of the blood source from the impact surface. This is the final step in determining the point of origin to reveal the height and distance ranges. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

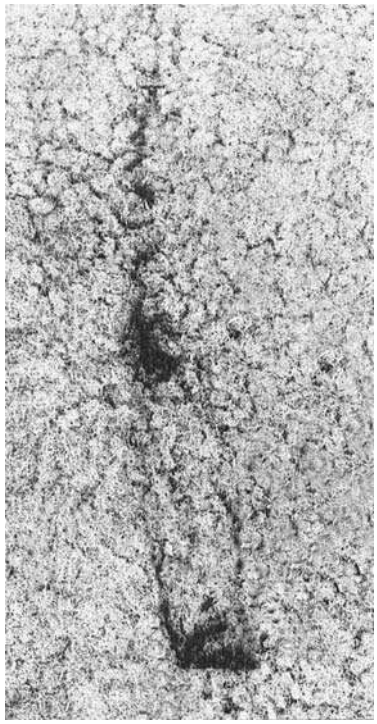


Figure 13.23 Transfer of bloody axe head on carpet in assault case. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

Figure 13.24 Axe head that created the transfer in Figure 13.23. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

mouth as seen in Figure 13.21*. The blood will flow from the orifice to the lowest point, independent of the body position.

Projected blood patterns are commonly associated with arterial spurting or gushing. Such patterns also may be created by the vomiting of blood, and are not usually duplicated through insect activity. Movement of a person or object through pooled blood may cause the blood to "project," which is characterized by a large volume of blood with spines. On a vertical surface, it appears as a large upside down drop with a drip. This phenomenon is illustrated in Figure 13.22*, which is from a shooting case. Larger insects (such as roaches) walking through areas of pooled blood will alter the projected pattern, and typically produce pronounced tracks. Therefore, it is important to note what types of insects were observed at the crime scene and to collect the insects properly. Chapter 3 addresses the proper insect collection protocol at a crime scene.

A transfer pattern may be a smudge or a swipe that could show movement. A flat bloody hand swiped across a surface will leave its form and show the direction of travel of the hand. The transfer may identify an object such as a weapon, shoe, or fingerprint and the blood may be in the form of an outline, or a partial or solid pattern. In some cases it is possible to match the object or print when the original is obtained (Figures 13.23 and 13.24).

Wipe patterns may be used to establish a time line of events when it can be determined what caused the blood at a particular location. Such bloodstains and patterns obviously

* See color section insert following page 78.

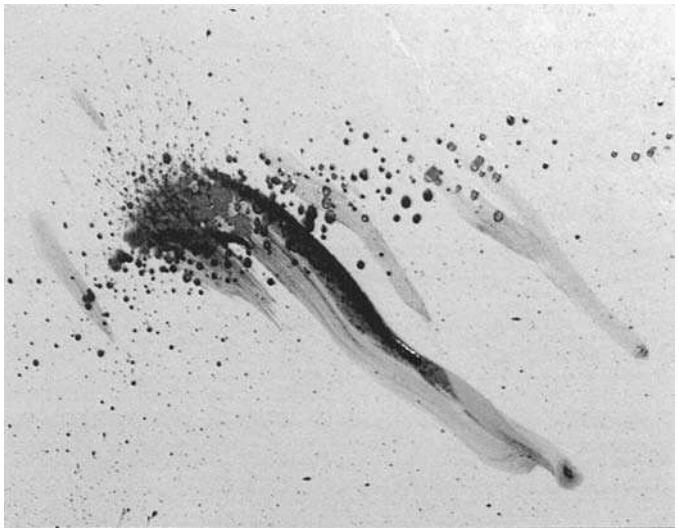


Figure 13.25 A wipe pattern created when fingers moved through the blood. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

will be altered if they interact with another object (Figure 13.25). Another condition that significantly changes the appearance of bloodstains is a void. This is a gap in an otherwise continuous pattern, and it is observable in Figure 13.21*. Such patterns sometimes show an identifiable outline of the object that intervened between the blood source and the impact surface. However, pattern identity alone does not provide enough information to reconstruct the scene and incident. Patterns illustrate the actions that took place, and it is necessary to define all of the other bloodstain evidence. There are always general topics to consider, but each scene and its components will dictate which topics need more detailed deliberations.

The analyst considers if and how the observable patterns are connected. This could be through contact, movement, or travel. These examples are detailed below. In Figure 13.26, a cast-off pattern associated with an impact pattern is indicative of blunt trauma. The fabric transfer pattern such as the one in Figure 13.27, found on several different surfaces, shows movement within the scene. The blood trail with a dripped pattern in Figure 13.28* points to a blood source moving and stopping at various intervals.

It is important to establish how many persons could be a blood source. The presence, or known presence, of a person or persons at a scene with bleeding injuries indicates the minimum but does not eliminate an unknown presence. An indicator of such an unknown may be possible from the bloodstain evidence at the scene. A trail of arterial spurts leading away from a body indicates another injured person, although there's a good possibility that a second body will be found at the end of the trail. Such circumstances make it imperative that the collection of blood samples includes every area that could have come from an unknown source. Only a specific blood test will confirm or refute the presence of an unknown person.

The process of reconstruction proceeds by linking the bloodstain evidence with the other physical evidence and components of the scene. This starts at the scene and continues

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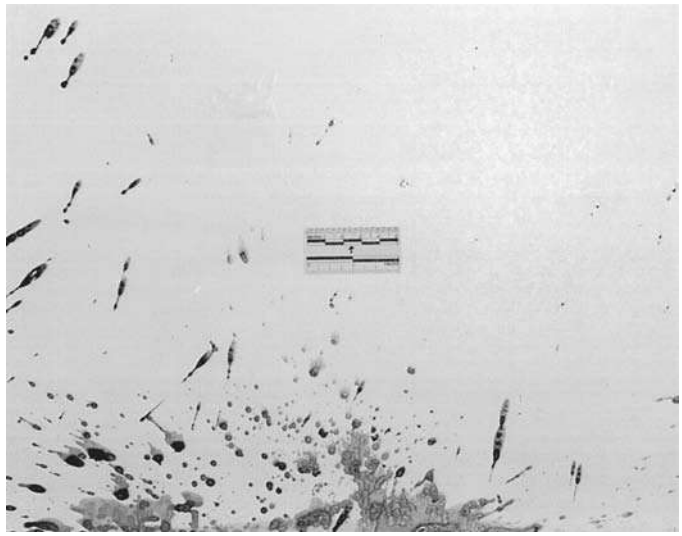


Figure 13.26 Cast-off and impact patterns created by hammer blows. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

at the lab. Items such as furniture may be transported for further analysis in better lighting conditions. Clothing may provide explanations or be an extension of patterns at the scene. Documentation of this evidence is best done in the controlled environment of the forensic laboratory. It is sometimes possible and usually desirable to transport enough of the items related to the evidence to physically reconstruct the scene at the laboratory.

The ultimate goal of reconstruction is to answer the questions of who, what, when, where, and how. Bloodstain evidence analysis plays an important role in those answers. In reference to a specific case, an example of this process would be the analysis of a bloodstain pattern from a reported accidental injury by gunshot to the head. In this case, it was

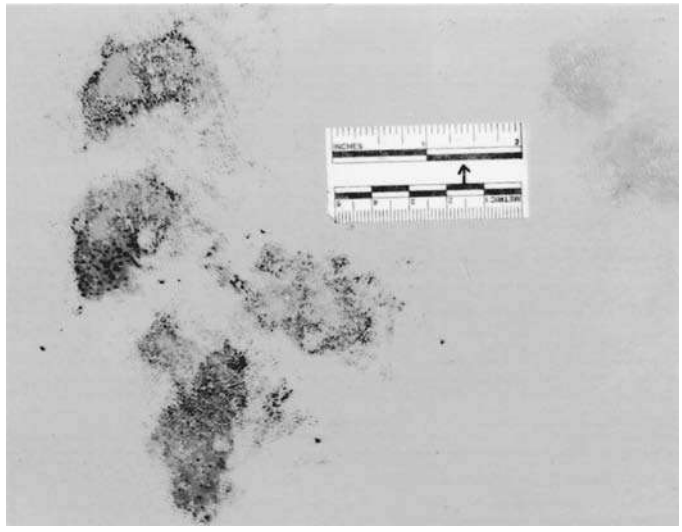


Figure 13.27 Fabric transfer patterns created by a bloody gloved hand. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

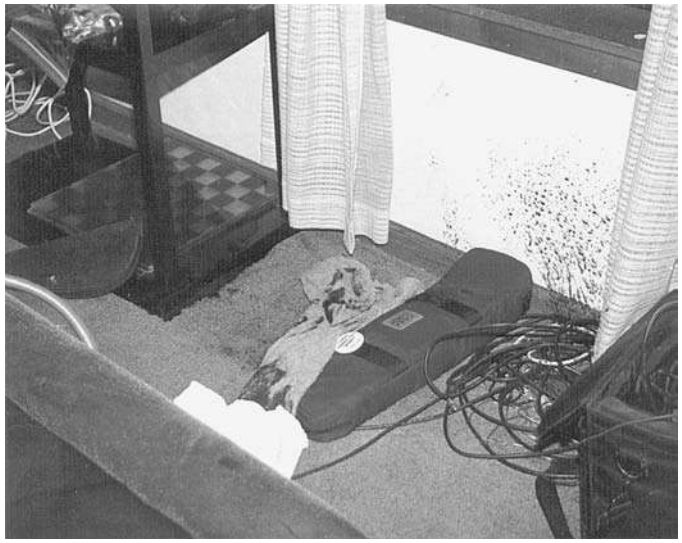


Figure 13.29 The height range from the floor was 12 to 15 in. and the distance range from the wall was 4 to 6 in. The foot of the bed is seen in the lower left corner of the photograph. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

reported that the victim, while leaning over his bed, was shot in the temple area of his head with a 9 mm handgun. An examination of the bloodstain pattern revealed that at the time of the shooting, not only was he not leaning over the bed, but that the fatal shot was fired from the opposite side of the room, which contrasted with the original statements provided to the investigators. Figure 13.29 illustrates that not only is the direction of the bloodstain pattern from left to right to indicate the correct position of the shooter, but from the height of the pattern, the victim had to have been sitting on the floor rather than leaning over the bed.

This case is an example of positive bloodstain evidence that was found not to be consistent with the witness statement. It gave the investigators the ability to eliminate all but a couple of possible scenarios, and greatly assisted in the questioning of the suspect and ultimately determining the truth. In many cases, there are no witnesses and it is the evidence alone that supplies the information of what did or did not take place. However, many times there are other factors that affect the appearance of the bloodstain evidence at a scene, and all possibilities should be considered.

Entomological Artifacts

The following case study will reveal how the physical evidence was consistent throughout the scene except for one area. It is an example of how the unknown, through examination of the evidence and what was known as fact, led to the positive conclusion of the investigation. In January 1998, the Tallahassee Police Department Crime Scene Unit was called to investigate the scene of a death at an apartment complex on the west side of Tallahassee. Neighbors who lived in the adjacent apartment reported the disappearance of a man last seen in mid-December. They had also reported a foul smell that had grown considerably stronger in the last week.

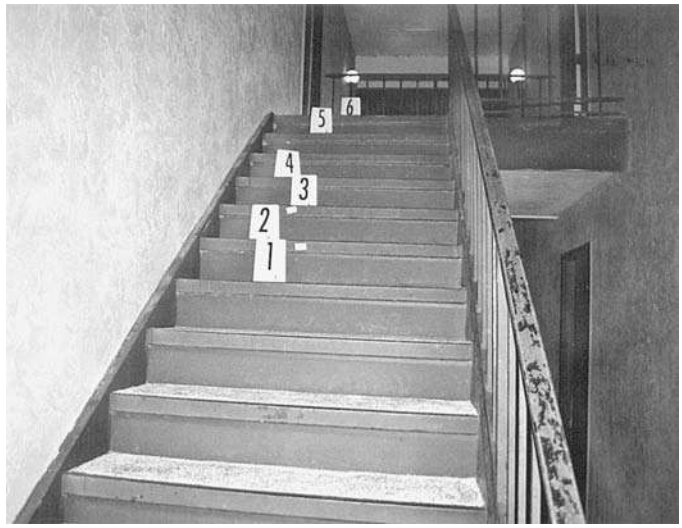


Figure 13.30 Stairway and landing outside the victim's apartment. The numbers mark bloodstain evidence. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

The residence was a second-floor apartment with an entrance foyer, living area, kitchen, hall, and one bedroom with an adjoining bathroom. Upon the first responding officers' arrival, the front door and all of the windows were secured. The officers were unable to generate a response from anyone inside the apartment. Bloodstains were observable on the outside staircase that had 14 steps leading to the landing at the front door (Figure 13.30). The blood was present on several steps and the landing. With all of these circumstances, the officers gained immediate entry into the apartment and found the resident's body.

Upon entering the apartment, Crime Scene Unit personnel observed bloodstain evidence throughout the residence. This included blood drops on the floors, as well as projected and transfer blood on the walls. In the areas of the foyer, living area, kitchen, and hall, the bloodstain patterns were consistent with movement throughout the apartment, but not with any violent actions (Figure 13.31). The reasoning behind this assessment was that these patterns did not show any characteristics of impact force or castoff. The appearance of the bloodstain evidence was also consistent with one bleeding source. The examination of the bedroom and bathroom revealed that the patterns in these areas were consistent with the rest of the apartment (Figure 13.32*). The victim was found in a bathtub lying on his left side. He was positioned with his feet at the drain end of the tub and was in an advanced stage of decomposition (Figure 13.33*). There also were additional patterns such as pooled blood and large areas of transfers. These patterns were produced from the migratory thrid-instar fly larvae exiting the bathtub (Figures 13.34 and 13.35). Items of clothing that contained blood evidence also were present.

There were very large numbers of both living and dead flies throughout the apartment. These flies were mainly concentrated on the windows, windowsills, and on the floors under the windows. Flies and fly pupal cases were present as well in large numbers on the bathroom floor. Pupal cases as well as maggots also were found associated with the body (Figure 13.36).

* See color section insert following page 78.

Figure 13.31 Bedroom floor and bathroom entrance, both displaying bloodstain evidence. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

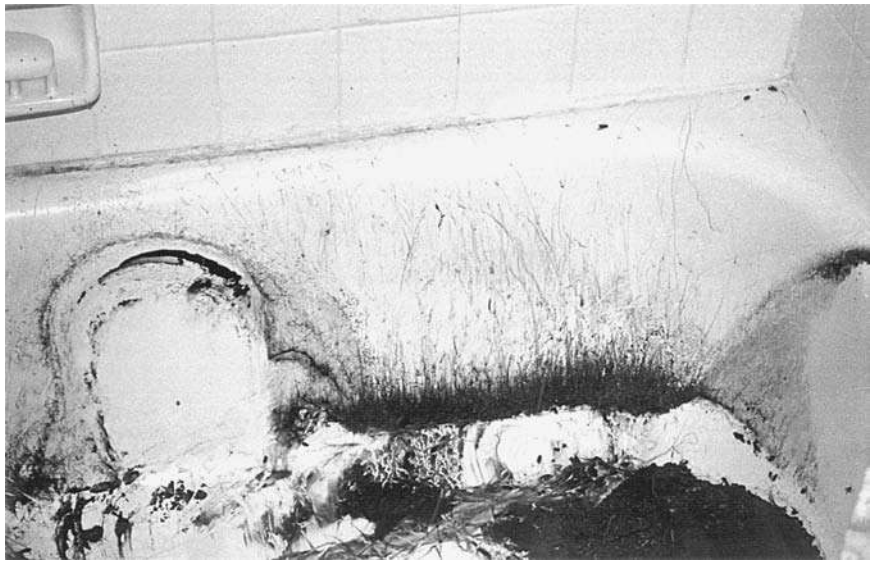


Figure 13.34 View of bathtub after removal of the victim's body. Note trails produced by the fly larvae as they migrated away from the body. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

Figure 13.35 Detail of figure 13.34 showing the segmented pattern in the larval trail produced by the crawling motion of the maggots. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

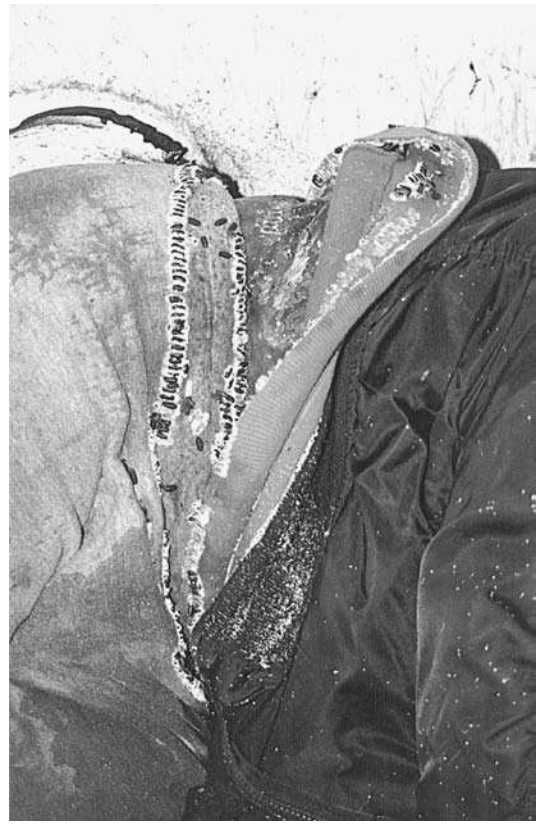


Figure 13.36 Victim's body in bathtub. Pupae of *Synthesiomyia nudiseta* (Van Der Wulp) are in rows where the elastic of the shirt and jacket contacted the pants. Pupae of *Phormia regina* were randomly distributed on the body and at various locations in the room. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

Figures 13.37 Bathroom closet doors where apparent high-impact patterns were observed. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)

Along with the bloodstain evidence, there were several items indicating that this case was a suicide. These items included several empty medical prescription bottles and a few empty razor blade packages next to the bathtub. There was a notepaper tablet containing a suicide note in the bathtub with the victim. The only aspect of the evidence that was not consistent within the scene was one particular bloodstain pattern. This concentrated pattern was on the bathroom closet doors (Figures 13.37 and 13.38), mirrors, and portions

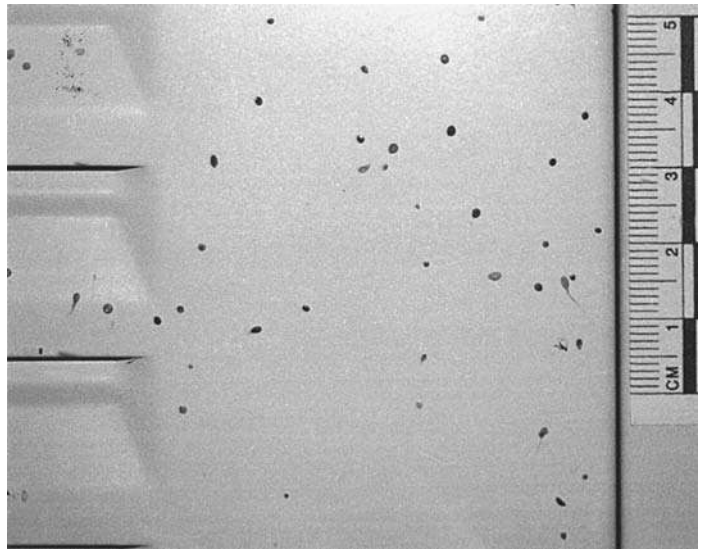
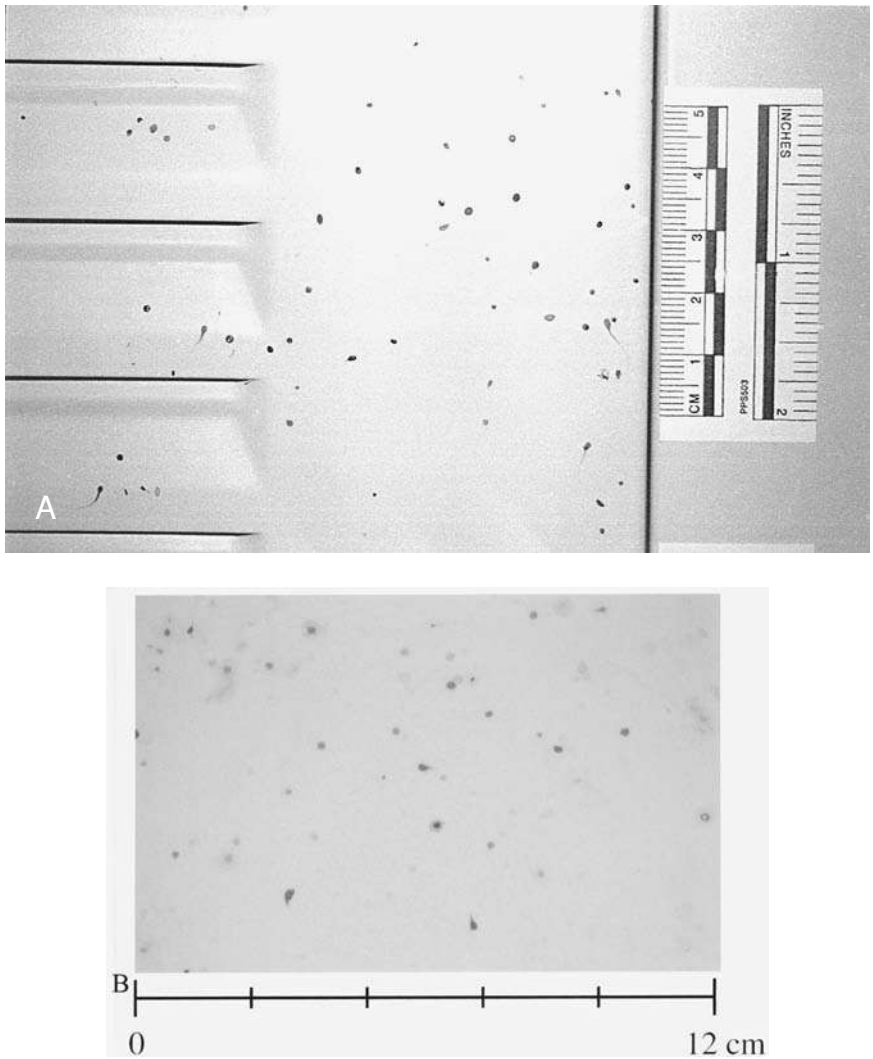


Figure 13.38 Closeup of one area of the doors in Figure 13.37. (Photo courtesy of Tallahassee, FL Police Department Crime Scene Unit.)



Figures 13.39 (**a**) Scene photo of unknown bloodstain on closet door. (**b**) Fecal deposits (fly-specks) and food regurgitate produced under laboratory conditions. ((**a**) Courtesy of Tallahassee, FL Police Department Crime Scene Unit. (**b**) Courtesy of James L. Castner.)

of the walls. The stains appeared to be all at a 90° angle to the surface and had a very small diameter. They were consistent in appearance with the patterns that would be indicative of high-velocity impact trauma to the victim. However, there was no evidence of gunfire and there were no guns found at the scene.

After considerable analysis of these bloodstain patterns, a forensic entomologist was contacted. Through proper documentation, examination, and consultation with the forensic entomologist as part of the forensic science team, it was determined that the patterns originated from the insects present, and that no violent force created these patterns. Proper macrophotography of the blood droplets found at the scene was essential in documenting the patterns for later comparison with known samples produced by entomological activity. The scene photographs of different droplet sizes and flow patterns (Figures 13.39a and 13.40a) were compared with blood droplets produced by the mechanical transmission and

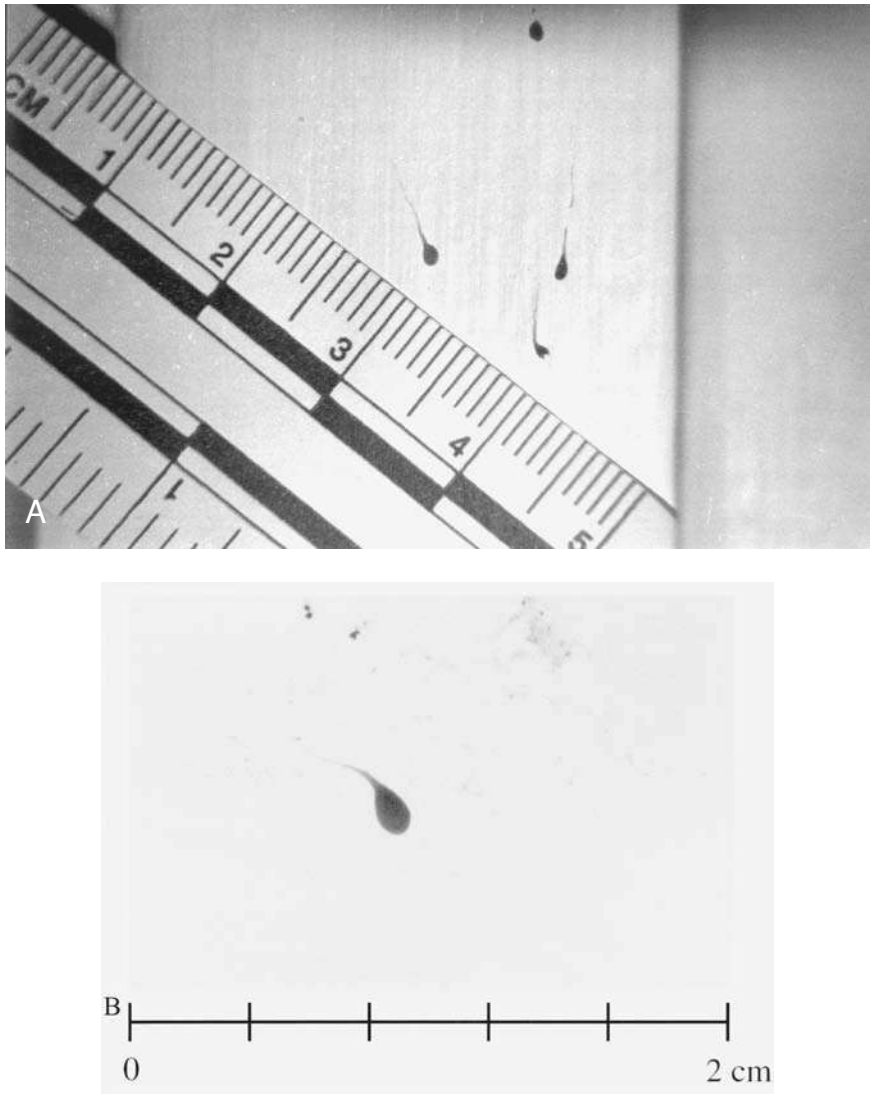


Figure 13.40 (**a**) Detail of bloodstains on closet door. (**b**) Fly fecal deposits (flyspecks) produced under laboratory conditions. ((**a**) Courtesy of Tallahassee, FL Police Department Crime Scene Unit. (**b**) Courtesy of James L. Castner.)

digestion of flies (Figures 13.39b and 13.40b) contained in colonies at a forensic entomology laboratory. These latter samples were found to be consistent with the photographs and observations made at the scene.

Conclusion

Blood has long been used as evidence to substantiate a crime and to link suspects with a particular scene or violent event, and the recent developments of blood recovery techniques has made it almost impossible to erase or hide traces of bloodstain evidence. However, much information can be obtained from blood relating to the occurrence and sequence

of events in a violent crime without chemical analysis, based solely on the expert interpretation of bloodstain patterns. Bloodstain interpretation has developed into a distinct discipline among the forensic sciences, and it emphasizes analysis of the size, shape, and pattern of bloodstains observed at a crime scene. However, insects present at the scene may alter the blood evidence and create artifacts that are easily misinterpreted. Careful examination of the shape and pattern of such blood spots will usually reveal the particular characteristics that indicate their entomological origin. The information in this chapter was presented to help identify common characteristics, and to familiarize the forensic investigator with the basics of bloodstain pattern analysis.

Suggested Readings

Bevel, T. and R. M. Gardner. 1997. *Bloodstain Pattern Analysis*, CRC Press LLC, Boca Raton, FL.

Eckert, W. G. and S. H. James. 1989. *Interpretation of Bloodstain Evidence at Crime Scenes*, Elsevier Science Publishing, New York.

Laber, T. L. and B. Epstein.1994. *Experiments and Protocol Exercises in Bloodstain Pattern Analysis*, Midwestern Association of Forensic Scientists. St. Paul, MN.

MacDonell, H. L. 1993. *Bloodstain Patterns*, Laboratory of Forensic Science, Corning, NY.

Wolson, T. L. and J. R. Johnson. 1994. *Bloodstain Pattern Analysis Workshop Manual*, Miami, FL.

The Forensic Entomologist as Expert Witness

14

ROBERT D. HALL

Contents

Introduction

Evidence from medicocriminal entomology can affect investigative or legal proceedings in various ways. Oral and written anecdotes pertaining to insects may be useful as investigators piece together a present or retrospective look at pertinent circumstances. Occasionally, insect evidence may lead to other lines of investigation, which in turn may reveal the truth. Insect-derived data may simply corroborate other real or testimonial evidence. In fact, litigation seldom turns solely on insect evidence. Sometimes, however, it does.

As the medicocriminal entomologist becomes increasingly involved with the judicial system, the procedures governing such involvement become progressively more stringent. For example, the aforementioned anecdotes, verbal comments or suggestions, during the processing of a crime scene may represent informal involvement. However, entomological

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opinions by written report (usually discoverable by the opposition), affidavit, deposition, or in-court testimony represent formal procedures ultimately characterized by testimony under oath, where prescribed penalties for perjury attach.

If entomological evidence is to have any impact on the outcome of litigation, it somehow must find its way into the proceedings. In virtually all instances, insect evidence is evaluated by state or federal courts under the general rubric of scientific "expert testimony." In brief, this means that the entomological data must be analyzed by someone qualified to render an opinion regarding how such evidence fits the facts of the case at hand. Such "expert testimony" is governed by several Federal Rules of Evidence, or the codification thereof that exist in state jurisdictions (Giannelli and Imwinkelried, 1993).

Theories of Admissibility

The guidelines governing whether or not evidence will be admitted, that is, evaluated by the trier of fact (a jury in criminal proceedings), depend upon several fundamental tests which appear deceptively simple (Imwinkelried, 1992). First, the proffered evidence must be *relevant* — both logically and legally. Irrelevant evidence is not admissible because it would not serve to make the trier of fact believe that any certain set of circumstances was more or less likely. Therefore, evidence about the presence of a certain species of butterfly at a crime scene would be considered logically irrelevant if such information had no scientific or other bearing on the analysis of the case. The issue of legal relevance is treated under Federal Rule of Evidence 403, where evidence may be excluded by the trial judge if "its probative value is substantially outweighed by the danger of unfair prejudice, confusion of the issues, or misleading the jury, or by considerations of undue delay, waste of time, or needless presentation of cumulative evidence." This powerful rule obviously gives the judge wide latitude.

In addition to being relevant, proffered evidence and witnesses must also be *competent*. Witnesses may be declared incompetent for violation of various procedural rules. As an example, if the court has adopted an "exclusionary rule," witnesses must remain outside the courtroom until called. If an expert violates this procedure, he or she may be barred from testifying in that proceeding.

To qualify as competent, witnesses and evidence must not be protected by any of the various common law, statutory or constitutional *privileges* (such as spousal privilege, attorney-client privilege, privilege against self-incrimination, and so forth), and evidence must be *trustworthy*. It is argument over trustworthiness that absorbs the most time when admissibility, or weight of insect evidence, is contested. If it can be shown that evidence is untrustworthy, it follows as a matter of law that it is incompetent, and if incompetent it should not factor into the trier of fact's decision-making process. Such a straightforward analysis, however, can lead to emotionally charged argument because the legal terms of art employed can be interpreted as hurtful when perceived as an *ad hominem* (i.e., against a person rather than against the evidence) attack. In fact, most affidavits, pretrial motions to exclude, and oral argument directed toward exclusion of evidence affirmatively use such terminology as the legally proven way to achieve the most direct defense against entomological and, indeed all, expert testimony — by simply keeping it out of the courtroom altogether.

Federal Rule of Evidence 702, followed in federal courts and virtually all states, is entitled "Testimony by Experts." It is remarkably broad, stating that "(i)f scientific,

technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise." The question, then, becomes, "who is an expert?" Whereas educational credentials may not be important in qualifying a witness whose "expertise" was gained through experience, such as carpentry or driving a truck, they are critical in the qualifications of an expert in science. An expert lacking such credentials may be barred from testifying.

Legal Tests of Admissibility

Whether the analysis is under a federal *Daubert*[⋆] standard or any of the various state standards typified by *Frye,*[⋆⋆] the same four broad elements seem to shape decisions regarding whether or not scientific, including entomological, evidence will be admitted (Cantor, 1994). First, is the "expert" qualified? Second, is the opinion supported by scientific principles? Third, is the opinion based on reliable data? Fourth, is the opinion so confusing or prejudicial that it should be excluded? The first three questions may be restated in another way, which can be regarded as a "three-pronged" test for admissibility. Is it (first) good science, incorporating (second) reliable data, as applied to the facts of the case at hand, by (third) a qualified expert? If the answer to any of these is no, a good legal argument can be made to keep the jury from hearing the evidence.

Good Science

Although the federal and state standards are at variance with each other on minor points, the issue of whether a field, technique, or procedure constitutes "good science" is fairly straightforward. Under the *Frye* "general acceptance" principle, in order to qualify as good science, the relevant scientific community must have generally accepted a theory. Publication, peer review, and reliance all are elements to be examined and weighed when making a decision about whether something constitutes such "good science." Additional elements under *Daubert* include evaluation of known error rates, and any standards or indices of reliability. The latter decision makes the trial judge the "gatekeeper" deciding what testimony enters the courtroom. While a judge has arguably more discretion under the latter standard — and many have used it since *Daubert* was announced — most scientists, attorneys, and the judiciary seem to understand obvious differences between documented and accepted science, scientific uncertainty, and pure speculation.

From a practical standpoint, medicocriminal entomology has enjoyed more than a century of judicial acceptance in regard to its fundamental scientific principles, including temperature-dependent insect development, necrophilous ecological behavior patterns, and the generally predictable succession of decomposer fauna. These also have been well documented in the scientific literature. It is, therefore, highly unlikely an assertion would prevail in any U.S. jurisdiction today that medicocriminal entomology does not constitute "good science" per se and, thus, should be excluded. When entomological evidence is important to a case then, the first prong of the three-part admissibility test is essentially unassailable. The same cannot be said for the remaining two.

⋆ *Daubert v. Merrell Dow Pharmaceuticals,* 509 U.S. 579 (1993).
⋆⋆ *Frye v. United States,* 293 F. 1013, D.C. Cir. (1923).

Qualifications of the Expert

Whether or not an individual is qualified to render an opinion in a case involving medicocriminal entomology depends principally on their educational and experiential background. As described in the Introduction of this book, the modern trend in U.S. courts is to view postgraduate education in entomology, such as the Master of Science (M.S.) or Master of Arts (M.A.), or doctoral degrees such as the Doctor of Philosophy (Ph.D.) or Doctor of Science (Sc.D.) as the educational minima to qualify an expert. Organizations such as the American Board of Forensic Entomology require that applicants possess an earned (in contrast to honorary) doctoral degree with a major in medical entomology, and this seems to be recognized as reasonable by the judiciary. However, testimony by masters-prepared "experts" is still frequently admitted in many state courts. The progression, however, is inexorable: the expectation that the best-credentialed expert will be best received by the jury militates toward the highest academic degrees. There is, of course, ample precedent for this pattern in expert medical testimony.

In addition to demonstrating educational qualifications, the putative expert, to be considered fully qualified, also must show consistent involvement with research and/or teaching of forensic entomology. This can be done by exhibiting publication lists of the expert's scientific papers germane to the subject, and documenting involvement with classroom teaching or workshops. The latter sort of "hands on" association is important to show that the expert is involved day-to-day with the so-called "wet work" in a manner analogous to medical experts in surgery and related clinical areas. The goal here is to identify and eliminate so-called experts who are "book smart" but without actual experience.

Another important aspect of expert qualification is absence of genuine or perceived bias. That is, the jury may perceive, rightly or wrongly, an expert whose record shows testimony only for the defense in criminal cases as biased toward the defense in all cases. Such a pattern is certain to be brought up during cross-examination of qualifications. On the other hand, a record of cases where the testimonial history shows involvement with either prosecution or defense without regard for the type of entomological evidence tends to demonstrate a lack of bias.

In practicality today, unless an individual offered as an expert witness in medicocriminal entomology can be shown to be patently unqualified on educational grounds (such as no college degree altogether) or to be unconscionably biased, it is likely they will be allowed to testify. This is only one reason why expert testimony on one side must usually be countered by expert testimony on the other, resulting in a predictable "battle of the experts."

Application of Reliable Data to the Facts

It is, therefore, the third and last prong of the three-part test; that is, the application of entomological science to the facts of the case, where most arguable points arise and most controversy centers. In addition to presenting as tight and scientifically valid an entomological analysis as possible, another function of the expert witness is to identify weak points in the opponent's analysis and educate counsel so that they may be thoroughly attacked. As will be explained later, this involvement does not constitute advocacy. Although the science of medicocriminal entomology per se will be considered "good science" by virtually all courts, application of scientific principles to individual cases can readily be seen as

fraught with opportunity for error. Absent measurements or evidence of repeatable and documented phenomena, the expert witness quickly moves from science to guesswork. While a prerogative of the expert is to render an opinion, such an opinion must be supported by good science, and guesswork is speculation, not science. One of the tasks of our adversarial legal system is to identify and attack such speculation, thus exposing shortcomings and showing the trier of fact why it is unreliable and incompetent. Commonly argued weak points in the application of reliable entomological data to the facts of a case include, but are by no means limited to, the following.

Were the insects or other arthropods identified correctly? Accurate taxonomic identification is the foundation upon which the remainder of the entomological analysis rests, yet it is seldom challenged. It is obvious that errors here can have a fundamental impact on the validity of any estimates made because different species of flies, for example, may have different developmental times and different proclivities. If a postmortem interval is calculated on the mistaken assumption that it was species X developing, but it can be shown that it was, in fact, species Y, it is easy for the jury to conclude that the entomological analysis is flawed. Further, demonstration of such misidentification can be damaging to the credibility of the expert who made the mistake. "Why, you couldn't identify species Y correctly when it was right in front of you, could you, Doctor?" can be fatal to an entomological analysis on re-cross examination, in addition to being personally humiliating. To reduce the chances of this sort of attack being successful, prudent medicocriminal entomologists "backstop" identifications by getting second opinions from other specialists before completing analysis of the case. In the future, it is likely that molecular techniques such as polymerase chain reaction or DNA sequencing may be useful as taxonomic "litmus tests" in species identification of forensically important insects, especially hard to identify eggs and larvae. At present, attempts to rear a subsample of specimens to the adult stage, which is most reliably identified, represent a prudent course of action.

Temperatures from remote sites don't accurately reflect temperatures at the scene. When temperature-dependent insect development is used to gauge postmortem interval, so-called "retrospective" weather records usually have to be employed. What this means is that the entomologist or investigator will get weather records from the nearest, or several nearest, weather recording stations for the time frame in question. Weather conditions are recorded routinely at many sites, but most often at airports, agricultural experiment stations, water treatment facilities, and other municipal facilities. Such data may range from complete hourly temperatures, precipitation, relative humidity, wind speed, and cloud cover to the starker record of daily maximum and minimum temperatures. The entomologist uses these temperatures, known to have prevailed during the time when the case at hand was transpiring, to estimate the temperatures under which the insect evidence developed. It is immediately apparent to any lay observer that the basic assumption — that the remote, retrospective temperatures in fact represent what actually prevailed at the crime scene — is tenuous at best.

If the weather recording station was generally proximate (say, within 20 miles or so) to the crime scene, it is reasonable to assume that prevailing conditions were at least similar at the two locations. As one judge remarked, "When it's hot in Oklahoma City, it's hot in Stroud (a small town about 60 miles away), and when it's cold in Oklahoma City, it's cold in Stroud." This proposition is generally true when two locations are fairly close together, as long as there is no major difference in elevation, no big geographical feature, such as a mountain range or body of water, separating the two, and the situation under evaluation

is out-of-doors. Trivially, everyone has witnessed rain on one side of a street while the other side was sunny, or rapid changes in temperature as a cold front moved through.

The weakness in using remote temperature data is when microclimatic factors introduce unknown but consistent variables. Weather stations are usually designed to measure air temperatures in unshaded sites with the thermal equipment protected from direct solar radiation. Suppose a body was found hidden in a deep, wooded ravine where midsummer overstory created intense shade? The combination of shade with cool air currents, especially in the evening, might produce a microclimate measurably cooler than the temperatures measured at an airport several miles away. On the other hand, a body found inside a closed car trunk, when the car was parked in direct summer sunlight, might have been exposed to temperatures much higher than those recorded at a remote weather station.

Because temperatures are so important to many entomological analyses and because in most cases there is no way to escape using retrospective, remote temperature data, it is important to measure concordance, or lack thereof, between the remote site and the crime scene. This can simply involve taking temperatures, hourly or daily maximum–minimums, for about a week at the crime scene, at the same time of year and under conditions identical to those prevailing when the crime occurred, and subsequently colocating the thermometer with the site producing the retrospective weather data. This technique will determine if the two instruments measure identically, and allow correction if not. Furthermore, it will allow correction of the appropriate thermal units for any consistent microclimatic differences noted between the crime scene and the remote temperature site. Absent such measurements, questions on cross-examination can draw attention to flaws.

Q. *Doctor, you used the daily maximum and minimum temperatures from City Airport when you calculated the fly development in this case, is that right?*
A. *Yes.*
Q. *How far is City Airport from where the body was found?*
A. *20 miles, more or less.*
Q. *Well, Doctor, we've all experienced situations where the weather was different over a 20-mile distance, right? How do you know they were the same?*
A. *You're right about the weather changes, but in this case I'm quite sure that the temperatures were similar.*
Q. *Did you make any measurements?*
A. *No.*
Q. *Did you have any temperature data available to you other than those derived from City Airport, 20 miles from the crime scene?*
A. *No.*
Q. *Then you cannot say for sure that the temperatures were identical, can you?*
A. *No, but …*
Q. *In fact, you had to guess they were the same, isn't that correct?*
A. *Yes.*

Maggot Mass May Have Affected the Temperature

A fascinating aspect of studying the thermal development of maggots is that under certain circumstances they can generate heat. Although insects are cold-blooded and generally

must develop as a function of ambient temperature, the teeming, writhing mass of maggots that sometimes occurs during decomposition may produce significantly elevated temperatures (Greenberg, 1991). These higher temperatures have the effect of shortening the developmental time of the insects themselves in relation to what analysis of ambient temperatures would suggest. Obviously, the occurrence of any maggot mass is important and should be taken into account. Problems arise, however, when there is no evidence that a maggot mass occurred. Absent eye-witness testimony, the existence of fly pupal cases in large numbers (resulting from the maggots in the mass), or in some cases the disarticulation of skeletal remains in the absence of scavenging animals, conjecture about the effect of maggot mass temperatures may represent speculation. Conversely, failure to account for maggot mass temperatures when one obviously occurred (documented in scene photographs, for instance) also represents error. When actual measurements of maggot mass temperature are absent, such "refinement" of ambient thermal data generally involves some amount of guesswork. The proper way to reflect this uncertainty is to present conclusions describing a range, rather than a discrete number.

Accumulated Degree-Hours May Give the Impression of Precision Without Substance

The thermal units necessary for well-known necrophilous flies to progress through their various life stages have been recorded in various data sets. The theory of application is simple: if one knows how many so-called "thermal units" were available at a crime scene, and also knows the thermal units necessary for a given species of fly to develop from the stage deposited by the female (generally egg or first instar larva) to the stage collected, by putting the two together, the inference can be made that the decedent must have been dead *for at least that period of time*. This interval is termed the "minimum postmortem interval" or "minimum PMI."

The climatological theory of accumulating "degree hours" or "degree days" is widely accepted in both agricultural science as well as in heating and cooling engineering, and is readily applied to insect development. In brief, it involves cumulative additions of temperatures (as either degrees Celsius or Fahrenheit) on an hourly or daily basis (see Chapter 9). The attractiveness of such an approach is that it can be quite precise if applied correctly. From the standpoint of litigation, it typically *appears* to be quite precise when presented to a jury. Some entomologists count only those thermal units over a "base" of, for example, 6 or 10 degrees Celsius, depending on the fly species involved. Accumulated degree hours, or ADH, are calculated appropriately only from hourly temperature data. Daily maximum and minimum temperatures can be used to calculate a daily average and accumulated degree-days (ADD), but no more. Some analysts have attempted to calculate ADH by multiplying the daily average (calculated from a maximum and minimum temperature) by 24. This calculation is valid only if the daily temperature change from warm in the afternoon to cool in the early morning fluctuates as a sine curve about that average. While this presumption may have some validity when considered over a lengthy time span, it is obviously problematic over shorter times such as the few days generally considered in most crime analyses. It is, of course, possible for temperature to "dwell" more on the hot or cool side of a daily average and this cannot be calculated when only maximum and minimum temperatures are analyzed. Misuse of the ADH developmental models thus puts the entomologist at risk of attack.

Did Other Temperatures Affect Insect Development?

Bodies exposed outdoors soon become an insect habitat thermally affected by ambient temperature and perhaps by maggot mass. However, complications can arise when bodies, along with the maggots on them, are refrigerated. As the temperature falls, maggot development slows. When the temperature is low enough, for example, under 6 to 10°C for many common blow flies, development, for all practical purposes, ceases. Therefore, prolonged periods in air-conditioned ambulances, morgues, and so forth can have major effects on thermal calculations. Whereas body temperature drops in fairly predictable fashion shortly following death when actual temperature measurements (which constitute recommended procedure) are absent, there is no way to know for sure how rapidly a corpse's temperature dropped. In some cases, engineers have calculated cooling curves applied to this question on an *a posteriori* basis, but they remain vulnerable to criticism for possible inaccuracy.

When Did the Insects Arrive at the Decedent? When Did They Lay Their Eggs or Larvae?

The most-often asked question in relation to medicocriminal entomology is: "how long does it take for flies to arrive at a corpse?" The typical answer is: "within minutes." This seemingly incredible ability of necrophilous flies has been documented repeatedly over many years and constitutes a repeatable phenomenon widely accepted by forensic entomologists.

The problem is that when this observation is applied to the facts of a particular case, the assumptions necessary may not be met. Three assumptions are required to support the assertion that flies will arrive "within minutes." First, it must be the season of the year when flies are active. To assume that flies will arrive within minutes at a decedent during wintertime depends upon the climatic conditions prevailing at the moment. Cold weather hinders and finally stops fly activity. Second, it must be during daylight hours. Necrophilous flies are generally inactive nocturnally and so the arrival "within minutes" must usually occur during daylight. Third, the flies must have ready access to the corpse.

The repeated observations regarding prompt arrival of necrophilous flies have inevitably been made when the corpse or surrogate was exposed in open air to fly activity. Thus, a decedent lying on the ground surface, such as on the side of a rural road or in a city park, would meet this criterion. The basic assumption regarding the out-of-doors decedent is that there are "no barriers" to fly access and "no barriers" to dispersal of the decomposition odors recognized as an attractant by the flies. At the other extreme, it is easy to conceive of a decedent, otherwise highly attractive to necrophilous flies, which would never exhibit any fly activity at all. A body sealed within a closed casket, zipped inside a tight body bag, or stuffed inside a tightly sealed automobile trunk would qualify. This is one of the reasons why dismembered bodies wrapped in plastic garbage bags often prove refractory to successful entomological analysis. Frozen bodies constitute a similar problem.

Between the two extremes, one of complete exposure and the other of complete protection, lies an infinite number of gradations in accessibility of flies to corpses. What about corpses inside houses with doors and windows closed? With a window open? What about corpses wrapped in various numbers of blankets? Waffle-weave blankets vs. thick wool blankets? It will quickly be seen that evidence of any major "barrier" to fly access is liable to introduce insurmountable uncertainty to estimates made therefrom. In many such

cases, estimates about when flies actually accessed decedents represent nothing more than guesswork, although some experiments have been conducted (Goff, 1992).

Further, access to corpses by flies does not necessarily mean that oviposition or larviposition occurred at that time. Given the major assumptions enumerated earlier (season, time of day, and access), it is generally accepted that necrophilous flies make access to and utilization of the corpse for their offspring reasonably contemporaneous. This has not been demonstrated equally convincingly when major barriers come into play. Thus, estimates derived under these circumstances often may be challenged as speculation, as in the following illustration of cross-examination.

Q. *Now, the decedents in this case were found inside a closed building, correct?*
A. *Yes.*
Q. *The screens were shut, the blinds were down, and the doors were all shut and locked, is that right?*
A. *Yes.*
Q. *And your assumption, if I understood you correctly, is that the flies — the ones that laid the eggs producing the maggots you used to estimate the PMI in this case — arrived at the decedents within 1 hour after the decedents had died, correct?*
A. *Yes, that was my conclusion.*
Q. *Doctor, do you think the decedents in this case had any maggots on them before they were dead?*
A. *No, that's very unlikely.*
Q. *And the crime scene report reflects that the decedents were covered with maggots and flies when they were found, do you remember that?*
A. *Yes.*
Q. *So what we know for sure is that the decedents didn't have any maggots when they died, and they had plenty of maggots when they were found, right?*
A. *Yes.*
Q. *Now, Doctor, were you in the house when the flies arrived on the decedents?*
A. *Of course not.*
Q. *So you don't know exactly when the flies actually got there?*
A. *No.*
Q. *So your statement that the flies arrived within an hour is really a guess, then, isn't it?*
A. *Yes.*

When Were the Insect Specimens Actually Preserved?

The theory behind using temperature-dependent insect developmental times to calculate a PMI is to "work back" from a known point in the insect's life history. That is, if the insects collected were mid-stage third instars, as evidenced by gross length, length-to-crop ratio, or other factors, it is possible by knowing prevailing temperatures to estimate how long it would have taken the species in question to grow from the stage (egg or larva) deposited to the stage collected. This "minimum PMI," as mentioned previously, is the length of time that the decedent in question *had to be dead*. Infestation of living humans with necrophilous fly maggots (myiasis) is comparatively rare today. Such PMI estimates are routinely analyzed by the entomologist in accordance with time of day because nocturnal oviposition by necrophilous flies seldom occurs.

The validity of these "had to be dead at least so long" estimates depends upon knowing, as medicocriminal entomologists are fond of saying, when the "clock was stopped" on the insects. That is, when were they actually killed and preserved so that their development terminated? Written records by crime scene investigators or medical examiners to the effect that "maggot samples obtained and preserved in 80% alcohol at 0900 hours this date" leave little room for argument. Fluids as exotic as special entomological fixatives or as simple as embalming preservative have similar effect. Perhaps the best maggot specimens are obtained by dropping them alive into boiling water, which causes them to extend full length and kills them, after which they are moved to preservative. Freezing the samples, although not recommended, also will serve to kill them quickly and preserve them. The common denominator documenting all of these techniques is the written record.

Absent specimens killed and preserved at known times, the resulting entomological analysis can be fatally confused. In some cases, investigators retain insect evidence with no attempt to kill or preserve it. As an example, maggots collected into empty plastic 35 mm film containers can continue to develop for variable times afterward, even molting to the next stage. With this sort of evidence, the entomologist can only guess at the time that the insects developed to the stage identified, and this sort of speculation is vulnerable to attack. Similarly, insect evidence retained in paper bags with no preservative may be examined years later. Attempts at speculation that specimens were "crushed" and thus "preserved" at known times, such as when a decedent was moved from the crime scene to autopsy, represent no verifiable phenomena and are rightly attacked in court.

In addition, mistakes are frequently made by misinterpreting the time when the specimens were actually preserved. Bodies may be found but not autopsied until a day or so later. It is an obvious error to calculate a PMI by using an insect specimen collected and preserved at autopsy, but employing the date the body was found as the starting point for analysis.

How Were the Collections Made? Were These the Oldest Specimens?

From the foregoing, it should be clear that reliable estimation of the minimum PMI from insect evidence is contingent upon several assumptions. First, it must be known when the specimens were preserved, so that one knows when to start to "work backwards" in time. Second, there must be concordance between the temperatures used and those prevailing at the scene. Finally, the "minimum" PMI represents the time it would have taken the *oldest specimens on the body* to get to the stage identified. Of course, if a body has 4-day-old insects on it, it is possible for that same body to have younger, say 3-day-old, insects on it as well. Because necrophilous flies ovi- or larviposit over a period of time, an accurate estimation of minimum PMI rests upon analysis of the oldest insects associated with the corpse. All else being equal, "oldest" in the context of necrophilous flies generally means "largest." Thus, the procedure at crime scenes is to collect the largest specimens available. Because this typically is done by personnel other than the medicocriminal entomologist, the latter must usually depend on the skill of the collector as one fundamental assumption in the analysis. Shortcomings may come out during cross-examination.

Q. *So, Doctor, you identified the insects in this case as third instar* Phormia regina, *is that correct?*

A. *Yes.*

Q. *And you used the temperatures available to you to calculate that these maggots were 4 days old, is that right?*
A. *Yes, 4 days.*
Q. *And did you base your estimate of how long the decedent had been dead on this 4-day interval?*
A. *Yes.*
Q. *Now, Doctor, if the maggots you examined were not the oldest available on the body, then your estimate would be incorrect, would it not?*
A. *Yes, it would tend to underestimate the PMI.*
Q. *And you don't know for sure that the specimens you examined were in fact the oldest available.*
A. *They were the oldest of the ones I looked at.*
Q. *My point is, there might have been older ones on the body that were not available to you, isn't that possible?*
A. *Well, I'm sure that the investigator who collected them followed proper procedure.*
Q. *But you didn't collect the specimens yourself did you, Doctor?*
A. *No.*
Q. *In fact, you never actually examined the decedent in this case at all, did you?*
A. *No.*

Another shortcoming in basing analyses on evidence collected by someone else is the appearance of unfamiliarity with the crime scene in question. If possible, it is always best to visit the scene during the acquisition of evidence and, if this is impossible, to view the scene personally before rendering a final opinion. As a last resort, photographs of the scene may be examined and this has become more convenient with the advent of CD-ROM computer disks containing many image files. A forced admission of having not visited the crime scene can be especially damaging if the opposing expert has done so.

Q. *Now, Doctor, have you had a chance to examine the basement where the decedent was found and where Dr. X made the extensive insect collections?*
A. *I've seen some photographs.*
Q. *Have you been to the house where all this took place?*
A. *Not personally, no.*
Q. *So you haven't seen with your own eyes any of the things we're talking about here today, have you?*
A. *No.*

Serving as Expert Witness

The medicocriminal entomologist must be prepared to function in the adversarial legal system if his or her analyses are to affect the outcome of litigation. The initial contact may stem from being part of a crime scene investigation team, where the entomologist physically collects insect evidence, documents and analyzes it, or delivers it to someone else for analysis. In other cases, the entomologist may be called to the necropsy and be involved with collection and preservation of specimens. Most frequently, however, the entomologist is contacted at some point during the investigation when it has become apparent that

insect evidence is important. In times past, and fortunately becoming less frequent today, the entomologist might not be contacted until insect evidence surfaced during a new trial, often years later.

The rubric of "expert witness" attaches when the attorneys handling either side of a case make an oral or written formal agreement to retain an individual in that capacity. A lawyer would say that the legal theory here "sounds in contract law" because of the actual or implied contractual relationship. This may be either as a "testifying" or "nontestifying" expert, the distinction being whether the identity of the expert need be disclosed to the opposing side. Whereas experts anticipated to testify in court must be disclosed, occasionally an expert will be retained in a nontestifying status as a second-opinion backup or to deny his or her services to the opposing side. In any event, the attorneys involved should make the status clear. During initial dialog, the actual work to be performed should be discussed and the rate of compensation, payment schedules, and billing particulars set (Cantor, 1997). Expert witnesses can expect reasonable compensation for their services, but exorbitant fees affect credibility. The amount of fees, expenses, and other compensation is fair game during cross-examination, and it is important for the expert to keep in mind that such fees represent compensation for his or her expertise and expenses, not for the testimony itself. It is good practice for the expert to keep stringent records of time spent on a case, usually to a fraction of an hour. "Book" billings should always be avoided, such as: "I always charge $1000 for an opinion in a murder case" or "I always bill for 3 hours for an insect identification, no matter how long it takes me." Unless the retaining side represents a governmental or other entity likely good for any amount due, it is prudent for an expert to ask for advance payment sufficient to cover initial expenses. Further, if travel is anticipated, agreement about reimbursable expenses should be reached early in the relationship. As trial nears, the time-value of money often makes it expedient to request, for example, that airline tickets be forwarded directly to the expert or that hotel bookings be billed directly to the retaining party, rather than to depend on reimbursement at some time in the future.

It is unethical for expert witnesses to enter into fee agreements contingent upon the outcome of the case or for a percentage of any settlement. Therefore, an arrangement where, "we'll pay your fee, but only if we win," is forbidden as unconscionably biased, as is an arrangement where the retaining side says, "we'll pay you 20% of whatever damages we recover." Such arrangements put lawyers in jeopardy of disciplinary action. No ethical attorney will suggest such a fee arrangement and no expert witness should accept one. From a purely practical standpoint, such agreements are considered void as against public policy; thus, they are unenforceable and an aggrieved expert will have no legal remedy.

Experts are not Advocates

Perhaps no other line becomes blurred so easily as that between detached, scientific expert and biased advocate for one side or the other. The function of the expert is to assist the trier of fact in understanding the circumstances of the case, and this cannot be done except in a neutral and detached capacity. This is one rationale for the court-appointed expert, but in U.S. courts litigants have the right to retain experts of their own choosing. The public perception of "hired gun" expert witnesses who will say anything if the price is right has some basis in reality, although by far the majority of scientific experts do their best to present unbiased reports and testimony.

When contacted initially by law enforcement authorities, the prosecuting attorney, or by defense counsel, the responsibility of the medicocriminal entomology expert is to gather available information, perform an entomological analysis, and apply the analysis to the facts of the case as they are known — "take it or leave it." The same can be said for civil matters. Whether or not the expert's analysis supports the plaintiff's or defense's position should be immaterial in the analysis itself. Whether it is scientifically valid is everything. Most cases are settled before trial and the support of experts, or the lack of such support, is often critical in decisions to settle. When performing this initial analysis, it is probably best for the expert to know only the minimum necessary about unrelated facts of the case, so that potential sources of bias can be avoided. At this point, if the entomological analysis runs counter to the "case" being made by counsel, the expert can be compensated for time spent with no further involvement anticipated. Alternatively, the expert may be put "on hold" as a nontestifying witness. In either event, the information generated by the expert to that point might be protected under various confidentiality doctrines. Therefore, the expert should not discuss the case with outside parties until it has been resolved. In particular, experts should not discuss a case in the presence of an opposing expert except during formal proceedings with counsel present.

The danger lurking in the background here is twofold: one is that the expert witness will tend to identify excessively with the particulars of the case as they favor his side, and the other is that prospective remuneration will be enough to sway objectivity. Probably analogous to the psychological identification of prisoners with their captors, it is not unusual to see scientific experts adopt biases as they increasingly identify with "their side" in a case. Highly insidious, such bias is unethical and prejudicial to fair resolution of controversies. As an example, an entomologist routinely contacted by law enforcement agencies may begin to adopt the "us or them" attitude frequently a product of street survival. "Help us put this dirtbag behind bars, where we know he belongs," may be enough to induce an impressionable expert to stretch his analysis to fit a theory which will do just that. Similarly, it is a rare defense team unable to make the compassionate statement, "There's just no way that this guy could have done that crime. We're sure of that; all we have to do is prove it." Under this type of pressure, skewed analyses may result and, thus, negate the theory behind admissibility of entomological, and indeed all expert, testimony: that it will lead to discovery of the truth.

Under the ethical codes of virtually every scientific society, including the American Academy of Forensic Sciences and the Board Certified Entomologist category of the Entomological Society of America, slanting or skewing analyses to favor one side or the other, without a valid scientific reason, is actionable. On the other hand, the expert will be expected to educate those responsible for retaining them in regard to weak points in the opposition's case. As will be amplified later, this requires walking a fine line between advocacy and education. If the expert remembers that his or her job is to perform an unbiased analysis and apply it to the facts of the case, and that it is the job of counsel to present that application in the light most favorable to one side or the other, things generally will go smoothly.

Occasionally, an expert witness may be retained in the capacity of *consultant*. In this instance, there should be no expectation of actual testimony. The job of the consultant is to assist counsel in putting together the best possible case. An expert functioning as a consultant properly may be regarded as an advocate, and bias in such cases in not problematical because the expert will not testify or give sworn statements in the proceedings.

The Entomology Expert and Formal Legal Proceedings

As the entomologist deploys his scientific expertise toward the resolution of a litigated controversy, there are three principal ways in which such expert opinion can be documented. These include the filing of an *affidavit*, the giving of a *deposition*, and *courtroom testimony*. The common denominator is that all constitute sworn statements. In addition to the expectation of intellectual honesty (which of course pervades day-to-day activities in science and scholarship), penalties for perjury attach.

Affidavit

An affidavit is a written, voluntary declaration of fact or opinion made before one authorized to administer an oath. Most entomology experts become associated with affidavits as they are used in support of pretrial motions. Typically, discussion between the expert and attorneys results in some consensus regarding the "fit" between the expert's opinion and the fact pattern of the case. When the expert's opinion can be used to support any of the many pretrial motions possible in legal proceedings, she may be asked to sign a notarized affidavit, which then accompanies the motion. In essence, the expert's opinion is used as testimony in support of the motion. Perhaps the example most common in medicocriminal entomology is the pretrial motion to exclude expert testimony. The sequence of events usually takes the following pattern. If one side or the other proposes to use entomology evidence in support of their case, that intent plus evidence accumulated and analyzed to that point must be disclosed. This disclosure may involve the name of the entomologist, any reports she has filed, and an accounting of the entomological evidence such as specimens and weather data.

Upon such disclosure, the opposing side has the opportunity to respond to such evidence, and often does so by retaining a separate expert to review it. If the latter expert's analysis points out flaws or deficiencies in the entomological analysis, then a motion to exclude can be made under the legal standard appropriate in that jurisdiction. The attorneys making the motion to exclude will draft it. What often comes as a surprise to the entomologist is that they also will draft an affidavit (a voluntary, written statement of facts made under oath) for his or her signature. This procedure is efficient in several ways, because the affidavit will reflect the entomological results and conclusions as they have been discussed between the expert and the attorneys and will be in the proper format. Most entomologists have no background in preparation of affidavits.

However, the entomologist should be keenly aware that the affidavit they are asked to sign becomes their own statement. It will be drafted by the attorneys to give the best support to the motion, and will typically contain legal terms of art. As mentioned previously, such terms are included solely to achieve a desired legal result. The entomologist should read the affidavit carefully before signing it, to ensure that it presents a scientific analysis she can support in good conscience. If there are misstatements or other problems, the affidavit should be edited and revised so that the entomologist (the one "making" the statement) is completely in agreement with it. Once notarized and filed, affidavits become part of the official record and may be used to impeach the affiant in the present or future proceedings.

> **Q.** *Doctor, you have testified just now on direct examination that in your opinion it is impossible to perform an entomological analysis solely from photographic evidence, is that correct?*

A. *Yes.*
Q. *And that is your expert opinion in this case?*
A. *Yes, it is.*
Q. *I have here your affidavit filed in the case of State v. Smith. Do you remember that case, Doctor?*
A. *Well, I think that's been several years ago.*
Q. *Paragraph four of this affidavit, which you signed under oath, Doctor, reads as follows, "My conclusion from examination of the crime scene photographs, which were all the entomology evidence available, is that the insects present were probably migrating third instar black blow flies." Do you remember that assertion?*
A. *Yes.*
Q. *So your conclusion in State v. Smith was, in fact, based solely on photographic evidence, wasn't it?*
A. *Yes.*
Q. *And that's directly counter to what you've just said in this courtroom, isn't it?*
A. *Yes.*

Depositions

There is considerable misunderstanding surrounding depositions and the entire associated process by those outside the legal system. Current federal rules actually limit the number of depositions in civil cases. A deposition is testimony — out of court, to be sure, but under oath — in response to questions posed, most often, by attorneys for the opposing side. It is part of the carefully regulated exchange of information called "discovery." A deposition may be taken in the attorney's office, but more often is conducted in the expert's office or conference room. An authorized court reporter will administer the oath and will record the deposition word-for-word for transcription. The expert, as deponent, has the right to read and correct the transcription before affixing his notarized signature. Occasionally, a deposition is so straightforward that review is waived. Videotaping of depositions is becoming increasingly popular and places an additional burden on the deponent: that of a visual, in addition to a written, record.

A deposition by a medicocriminal entomologist typically focuses on the entomology report and conclusions. At the beginning, prefatory remarks usually include a statement and spelling of the expert's name, introduction of the attorney who will be asking the questions, and a reminder that clarification of questions may be sought and that responses must be verbal (court reporters cannot record grunts, nods, or shakes of the head).

Because the questioning attorney will generally have studied the entomology report and the expert's curriculum vitae, the trend of medicocriminal entomology depositions is fairly predictable. They usually begin with background questions in regard to the expert's academic qualifications and performance history. This may be glossed over or may become the subject of in-depth questioning. It some cases, questions may be asked about the particulars of every scientific article the expert has published. Thus, it is important to be well prepared.

Sooner or later, though, questioning will turn to the entomology report and the application of entomological science to the facts of the case. Because responses on deposition can be used to impeach subsequent in-court testimony, the deponent must take care to provide accurate and supportable responses to questions asked. Although the expert often will be allowed to provide a discourse ("narrative") on a particular topic, it is good

advice to limit one's responses to the best and most straightforward answer to the question asked. The more one rambles on, the greater chance that such information will be inconsistent with later statements. Although minor inconsistencies and "fine points" may be clearly understood as such by fellow entomologists, they may be perceived by the jury as undermining the expert's credibility.

Most depositions are straightforward, although they may be lengthy. If a break is needed, simply make a request. One major difference between depositions and courtroom testimony is that no judge is present. Therefore, objections cannot be ruled upon at the moment. Still, if the questioning attorney asks an objectionable question, the attorney representing the side retaining the expert (a critical point is that she does not represent *the expert*) may register an objection, saying "subject to that, you can answer." The effect of this give-and-take is that much information usually comes out during a deposition, and each bit can be fairly brought up during future testimony, where objections may have to be argued or where it may be inconsistent with responses offered at that time.

Depositions may or may not be done under *subpoena*. A subpoena, especially a *subpoena duces tecum*, commands a party to appear in court or for deposition at a certain date and time, and to bring relevant documents. This court order must be obeyed, or contempt sanctions may result. Most expert witnesses are willing to have depositions scheduled without compulsion and the subpoena is often dispensed with. If this is the case, it is wise to ensure that payment for time and expenses will be forthcoming despite the lack of a subpoena. In some cases, an expert may be compelled to testify under subpoena with only the statutory compensation for travel expenses and appearance in court. This situation is far from ideal and is comparatively rare, but it underscores the wisdom of arriving at written expert fee and compensation agreements early in the relationship. The party requesting the deposition, either the prosecution or defense in criminal matters, is responsible for paying for the deponent's time and travel expenses, if any. Needless to say, they also are responsible for the transcription fee, which is often greater than the expert's charges.

In addition, the attorney with whom the expert is associated should examine all documents the expert expects to bring to the deposition. Often, this is not done and can constitute a major mistake. Such documents are discoverable and stray notes, memoranda, and correspondence may contain statements or information adverse to one's party. Inadvertently bringing harmful documents may constitute negligence. The best procedure is to avoid creating these in the first place; thus, the expert should give much thought before making any notes, memoranda, letters, reports, or other written materials.

Courtroom Testimony

When entomological evidence is going to be argued in court, whether at trial or in relation to pretrial motions, the expert witness will generally be needed on the stand. As with affidavits and depositions, courtroom testimony is under oath. Also similar to other statements under oath, trial transcripts are historical documents available to future litigants. What this means in practice is that testimony from one trial can be used to impeach a witness in a second proceeding. Therefore, the expert should be aware of and avoid inconsistent statements, and if these become necessary, for instance, because of new scientific knowledge, he or she should be prepared to explain inconsistencies.

Good preparation is fundamental to success in litigation and expert testimony is no exception. Prior to trial, the expert should review all reports, notes, and associated documents relevant to analysis of the case. Further, there should be a pretestimony meeting

between the expert and the attorney who will be representing the side for which the expert is testifying. A trial lawyer's maxim is "never ask a question when you don't know what the answer will be." This applies to expert testimony and represents good preparation and rehearsal. It does not constitute advocacy for the expert to review the salient scientific aspects of his or her analysis and alert the attorney as to which points need to be made, and to go through a question-and-answer session to ensure that both are in accord. Similar to depositions, all documents that the expert expects to bring into court should be screened beforehand. Like the situation with depositions, a subpoena may or may not be issued by the court; if one is issued, absent an agreement to the contrary with appropriate counsel, the expert is entitled only to the statutory compensation for his or her appearance.

On the day of courtroom testimony, the expert should arrive with sufficient lead-time for a final session with counsel, if required. Sometimes, an expert can request to be put "on call" so that it is not necessary to wait at the courthouse. If an exclusionary rule is in effect, it will be necessary to wait outside the courtroom (in a hallway or in a witness room) until called. This time can be used for final review of relevant documents. If the exclusionary rule has been waived, the expert may and should listen to the testimony of opposing experts. If in doubt, consult with the appropriate attorney.

While an expert witness need not adopt the formal attire of trial lawyers, she should present a professional appearance. This means, at a minimum, coat-and-tie for men and its equivalent for women. Remember that expert testimony is valueless unless it affects the decision-making process of the trier of fact. The goal is for the jury to believe the scientific opinion of the expert. Therefore, inappropriate attire or mannerisms can have an adverse effect by causing the jury to reject the expert and his or her theories. In extreme circumstances, attorneys may request a recess and have a clerk or paralegal purchase a change of clothing for an expert, to be paid for from the fee owed.

While on the witness stand, the expert should strive to maintain composure even under pressure. Anger, argumentative responses, and annoying mannerisms are usually counterproductive. The best results are obtained by assuming a relaxed but alert attitude and making eye contact with the questioning attorney, judge, or jury as appropriate. It is especially important that the expert not "look to" the attorney with whom he has been working for support when difficult questions are asked on cross-examination.

The initial questioning of the expert in court is called *direct examination* and is done by the attorney representing the party calling the expert. This critical period is relatively friendly, because it consists of the attorney and expert who recently rehearsed precisely for this occasion. The initial portion of the question-and-answer period will be devoted to the expert's qualifications, in order to convince the trial judge to admit the testimony under the appropriate rules of evidence pertaining to such expert witnesses. In the case of medicocriminal entomologists, the focus will be on academic preparation and degrees, academic appointments and other professional positions, and research contributions including papers published, students advised, and grants awarded. It is important to document qualifications in the field of forensic entomology — the fact that one is an expert in another entomological field, control of insect pests on corn, for example, is irrelevant when seeking qualification as a medicocriminal entomologist. A medicocriminal entomologist with experience testifying as an expert witness can effectively point out their academic and professional background in narrative form. If one is not so experienced, it is best to allow the attorney to take the lead by asking pertinent questions. In either event, it is important to ensure that the judge and jury understand the full impact of the expert's

credentials. Whereas misrepresentation of one's credentials, by affirmative misstatement or by omission, is an ethical violation and not tolerated, this is no time to be modest about one's honestly earned background. The issue here is believability, and the expert the jury considers best qualified is often the one believed. Thus, one's professional title should be stated and all academic degrees along with the institution where each was earned. After that, a coherent presentation covering professional stature, number and type of publications, major grants or endowments, membership in professional and scientific societies, and significant honors should be provided by narrative or questioning. In particular, the manner in which the expert's background makes him or her uniquely qualified to enlighten the jury should be emphasized. Frequently, opposing counsel will attempt the old ploy of stipulating to the expert's credentials. The purpose of this tactic is to cut short the litany so the jury will not hear it. Inevitably, the attorney seeking to qualify the expert will request permission to proceed (to preserve the matter on the record) and this is typically granted.

After the expert has been qualified by the court, the next function of the direct examination is to present the expert's theory of the case to the jury. This will usually start with the expert's written report, which has earlier been disclosed. As in the qualification phase, the expert may proceed by testifying in narrative form, or in direct response to questions from counsel. Typically, direct examination consists of responses elicited by nonleading direct questioning and, therefore, in some instances opposing counsel may object to narrative testimony. If this objection is sustained, it constitutes a major reason for adequate preparation between the expert and associated counsel. The goal is for the expert to be able to teach the jury and instruct them why his theory of the case is correct and should be believed. Two points become important here. One is that the expert may refresh his or her memory by referring to a wide range of materials. Because accuracy is critical to expert testimony, it is not a sign of weakness to ask permission to refer to notes, documents, or other written sources to ensure that testimony is factually correct. The second point is that responses during direct examination impact the scope of cross-examination, in that the latter phase is a derivative of those questions answered during direct examination.

As the expert testifies in narrative form or in response to questions, it is important to include the jury in the discussion by eye contact. It is not necessary to look at the jury to the exclusion of everyone else in the courtroom (indeed, this would appear awkward) but a relaxed demeanor in which the expert looks at counsel when questions are asked and at the jury as they are answered is often effective. Within the strictures of good science and ethical limits, the expert should appear positive and forthright, and able to explain the biological variability limiting the precision of his or her answer.

Another often-fatal error of scientific experts is to infuse their responses with excessive technical jargon. It is a mistake to assume that reliance on mystical sounding terminology will be taken by the jury to represent education or wisdom. In fact, often the opposite occurs. If an expert confuses responses with arcane jargon, thinking that it sounds "technical" and that the jury will believe that someone who knows so many technical terms must also know the correct analysis of the case, a fundamental mistake has been committed. While the use of some jargon or technical language may be unavoidable, it is best to couch answers in terminology that anyone can understand. Remember that the job of the expert is to educate the trier of fact, and it is impossible to provide doctoral-level education in an hour or even many hours on the stand. Usually, exactly the opposite results: the jury becomes bored with the testimony and simply ignores it. Therefore, the key is to use clear

language while avoiding "talking down" to the jury. This sort of presentation involves craftsmanship and can be learned with practice.

Q. *Now, Doctor, can you tell us what your entomological analysis of this case was?*
A. *Yes, the climatological data were applied retrospectively to the thermal developmental profile for putatively late but premigratory third instar* Phormia regina *collected in this instance by the medical examiner at necropsy. At least 3472 accumulated degree hours are required for this species to enter the prepupa; thus, I calculated that …*

Whereas this response would be intelligible to another medicocriminal entomologist, it is one only an entomology graduate student could love. Unless much time is taken to define each term and make it understandable, the jury will fail to learn much, if anything, from it. Without "talking down" to the jury, a better response might be as follows.

A. *The insects tell us a good bit about how long Mr. Smith had been dead. I identified the flies found on his body and their stage of development. I also checked the temperatures for that time from Central City airport and performed a short test that showed it was valid to use them. Knowing that flies grow up at different rates depending on how warm it is, the insect evidence here tells me that Mr. Smith had to have been dead for at least 4 days when he was found.*

If use of scientific terminology is unavoidable, such as when discussing the various species of flies, it may be useful to prepare a list of arcane terms to hand to the court reporter before testifying. This will at least ensure that the terms are spelled correctly in the trial transcript. Although the fine points of the analysis, such as latinized names of species, use of thermal data like ADH, and so forth will surely be argued on cross-examination, the effect of simple initial responses is for the entomologist to transmit their result to the jury in a fashion they will understand and remember.

It has been well documented that retention and learning improve with visual, in addition to auditory, input. Therefore, many experts enjoy success with well-designed courtroom presentations, which fall under the category of *demonstrative*, rather than testimonial, evidence. These may be as simple as chalkboards or flip charts, as straightforward as slide presentations from a projector or television, or as sophisticated as preserved or living exhibits, computer imagery, or videotapes. Although possibly smacking a bit of "theater," there is no question that thoughtful visual aids are very effective in getting the expert's point across. Often, they can be left in place after the expert testifies and, thus, serve to remind and reeducate the jury as the proceedings continue. Because these sorts of aids or exhibits must be disclosed before they will be permitted in the court, be sure to advise counsel of what you intend to present. Do not wait until trial or opposing counsel will likely object to the surprise and will probably be successful in keeping such demonstrative evidence out of court. This can be especially damaging if the expert is building a critical presentation around the visuals.

At the conclusion of the direct examination, opposing counsel is given the opportunity to *cross-examine* the expert witness. A principal difference between the form of questions on direct vs. cross-examination is that the latter may be *leading*; that is, the question itself may suggest the answer. The expert must be especially alert during cross-examination because an experienced opposing attorney will have identified all possible inconsistencies

arising as a result of the direct examination, affidavits, depositions, or former testimony. These inconsistencies may then be pointed out to the jury and serve to impeach (to make less credible) the expert's opinion. Experts should pay particular attention to the following points because cross-examination is such a critical phase of testimony.

Listen carefully to the question asked. If the question is not clear, ask for clarification. It is good practice to develop a habit of pausing deliberately before responding to any question on cross-examination, in order to give counsel on your side time to recognize and register an objection. Generally, if the question is unclear, poorly phrased, argumentative, or exhibits similar defects, counsel will register a timely objection. If the question is not legally objectionable but is unclear scientifically, it is appropriate to request restatement or clarification.

Q. *So, as I understand it, it is your opinion that the insect larvae in this case support a PMI estimate of 4 days, is that right?*
A. *I'm sorry, there were three insect species involved: two species on one decedent and one species on the other. I am not sure which species you mean.*
Q. *I'm talking about the species that was found on Mr. Jones. I think that decedent had only one kind of fly maggot on him.*

On cross-examination, an experienced expert will respond as truthfully and briefly as possible to the questions asked. While it may be tempting to offer additional explanation, it is best to refrain from doing so. The opportunity may seem particularly tempting when a well-phrased question has apparently exposed some weakness in the expert's response. The best way to handle this is to permit the attorney representing the side retaining the expert to *rehabilitate* the expert on *redirect examination*. Of course, a redirect may be followed by *recross examination*, but the number of iterations of these decreases rapidly as the scope of possible questions narrows.

It is imperative that the expert retains his or her composure during cross-examination. This may be difficult to do when the questions posed constitute a direct attack on the expert's credentials, scope of practice, and professional competence. Answers given in anger are often regretted. Resist the temptation to "match wits" with the attorney asking the questions and remember that the courtroom is their professional habitat. Making a witness angry is only one of many strategies employed by trial lawyers. An experienced attorney will never try to match an expert one-on-one when arguing the fine points of entomological science. Similarly, an expert who tries to "outsmart" the attorney in the courtroom is generally doomed to failure.

Q. *Doctor, the data you used to analyze this case were generated at the "Body Farm" in Tennessee, weren't they?*
A. *I see what you're getting at. There's no proof that data from the Body Farm reflect faunal enrichment or are otherwise unreliable.*
Q. *Doctor, did I ask you about "faunal enrichment"?*
A. *No.*
Q. *Did I ask whether or not such data were unreliable?*
A. *No.*
Q. *Well, would you please simply respond to the questions that I ask?*

In this manner, the attorney has made it clear to the jury who is in charge during the cross-examination. The expert in this example has not educated the jury; worse, their esteem has been lessened because the jury will perceive that he "lost" this confrontation.

Another way in which expert witnesses get into difficulty is by venturing outside their discipline. What this means is that the expert must be highly cognizant of the boundaries demarcating their scientific specialty. The medicocriminal entomologist is an arthropod expert qualified to render an opinion about the identity of insects and related species, their biology including reproductive behavior, successional occurrence in relation to geography, season and time of day, rate of development, and so forth. The entomologist may be extremely familiar with closely allied fields, such as forensic pathology, but must be sensitive to questions that call for an opinion outside the area of qualification.

Q. *Doctor, may I refer you to the photograph marked "State's Exhibit 43," which you have previously testified depicts third instar larvae of the black blow fly?*
A. *Yes, I have that photograph.*
Q. *If you will examine the decedent's forehead in the photo? Can you see the forehead, Doctor?*
A. *Yes, I can see it.*
Q. *What appear to me to be fly maggots are depicted crawling around a hole in the forehead, are they not?*
A. *I see them around a hole, yes.*
Q. *Doctor, does that hole appear to have been made by a 9 mm bullet?*
A. *I don't know. I'm not an expert in regard to bullet holes.*

Venturing outside one's area of expertise can be tempting, especially during heady moments on the witness stand. Be assured that experienced trial attorneys will know this and may lead into it simply to "dull" the expert's luster and erode their credibility with the jury.

An important role of the expert witness, as emphasized previously, is to educate the attorneys involved so that they can elicit the truth during the direct and cross-examinations for which they are responsible. This role can become especially interesting if the exclusionary rule is waived. Then, the opposing experts are present in the courtroom during testimony and are expected to provide expert insight into the responses provided. In some cases, the expert will sit at the appropriate counsel's table and take notes as his or her counter-expert undergoes direct or cross-examination. While this appears close to advocacy (it certainly has all the visual trappings of it), it is important to remember that the expert's role continues to be one of education. That is, the expert is used to alert the appropriate counsel to inaccurate statements of fact, misrepresentations, subtly artful responses, and so forth. This allows clarification on redirect or recross examinations. As might be expected, the plainly adversarial nature of such participation can elicit hard feelings and misunderstandings between experts. If this happens, it is a good idea to deal with it immediately so that interpersonal bad feelings do not become a major issue. In fields such as medicocriminal entomology where there are relatively few experts, long-term associations with colleagues work best on a positive, rather than negative, note.

Malpractice

An area seldom considered by expert witnesses is malpractice liability. Whereas the attorneys involved in a case are invariably well insured, most experts are not. While it is true

that certain immunities attach to testimony under oath, such as immunity from slander and similar charges, many vulnerable areas remain. The theory most commonly applied to malpractice of expert witnesses is common law *negligence*, which is a tort. In order to establish a negligence cause of action, the plaintiff must prove the existence of a duty, breach of such duty, cause-in-fact and proximate cause, and damages. Often, these elements are not difficult to establish in malpractice cases.

The agreement in contract between the expert and the side employing him establishes duties owed and the expert's appearance in court is evidence of awareness. The issue of causation is also straightforward: whether or not the expert's actions caused the loss of a criminal case or civil lawsuit, for instance, and whether or not it was reasonably foreseeable that such a result would occur. The issue of damages is often easily determined, in that criminal penalties or civil monetary awards are clear. Most argument centers on whether or not the expert, in fact, breached his duty to the side retaining him.

Common mistakes by expert witnesses that can be considered breach of duty and, thus, incur malpractice liability include factual misstatements or errors, breaches of confidentiality, and inadvertent disclosure of documents. The expert should take care to ensure that all testimony is factually accurate. The truth is a powerful defense against malpractice. Further, the expert should not discuss an ongoing case outside the courtroom with anyone other than counsel for the side retaining him. Narrow exceptions exist for personnel coming under the umbrella of the confidentiality doctrine, such as technicians and other employees of the expert. Expectations of confidentiality extend to these personnel and any breaches may be actionable against them or against the expert himself under the theory of *respondeat superior*. As stated earlier, negligent disclosure of damaging documents may be considered malpractice. In addition to following good practice, the prudent medicocriminal entomologist will carry adequate malpractice insurance and may seek legal advice regarding methods to limit potential exposure by skillful use of certain business organizations.

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Glossary

a posteriori "After the fact." A method of reasoning from observed facts.

ADD Abbreviation for accumulated degree-days.

ADH Abbreviation for accumulated degree-hours.

ad hominem Appealing to personal considerations rather than to logic or reason.

Aedeagus The reproductive organ of a male insect.

Affiant An individual who makes the statements contained in an affidavit.

Affidavit A written declaration made under oath before a notary public or other authorized officer.

Alleles One member of a pair or series of genes that occupy a specific position on a specific chromosome.

Ametabolous An insect life cycle lacking distinct life stages. Without metamorphosis, or "development without change." The immature forms are similar to the adults, differing only in that they are smaller and not yet sexually mature.

Antemortem Before death.

Antenna (*pl.* antennae) One of a pair of segmented sensory appendages located on the head of an insect.

Anterior Front; in front of.

Anthropologist An individual who studies the origin, behavior, and the physical, social, and cultural development of humans.

Anthropophagi An organism that consumes human flesh.

Apterous Without wings.

Archaeologist An individual that engages in the systematic recovery and study of material evidence of past human life and cultures, such as tools, buildings, pottery, and graves.

Artiodactyl Any of the various hoofed mammals of the order Artiodactyla. This includes cattle, deer, sheep, goats, etc.

Arista A bristle-like appendage or "hair" located near the tip of the antenna of certain flies.

Aristate Bristle-like, with an arista.

Arthropod Any of the invertebrate animals in the phylum Arthropoda, which includes the insects, crustaceans, and arachnids. These animals are characterized by a chitinous exoskeleton and a segmented body to which jointed appendages are articulated in pairs.

Bioaccumulate The accumulation of a substance, such as a toxic chemical, in the various tissues of a living organism.

Blow fly (or bottle flies) A fly in the family Calliphoridae.

Botanist An individual that studies plants.

Bucca A sclerite on the head below the compound eye and just above the mouthparts. The region on the head of a fly, analogous to the "cheeks" of the face.

Capitate With an apical knob-like enlargement.

Cercus (*pl.* cerci) One of a pair of terminal sensory appendages found at the tip of the abdomen in certain insects.

Chitin A nitrogenous polysaccharide formed primarily of units of N-acetyl glucosamine, occurring in the cuticle of arthropods.

Claspers Appendages of certain male insects used to hold the female during copulation.

Clingers Aquatic insect representatives that have behavioral and morphological adaptations for attachment to surfaces in stream riffles and wave-swept rocky zones of lakes (e.g., Ephemeroptera, Heptageniidae; Trichoptera, Hydropsychidae).

Coarctate An insect pupa totally enclosed in a hard outer shell formed by the last larval skin.

Compound eye A type of eye found in insects and some crustaceans that is composed of numerous light-sensitive individual elements, each having its own refractive system and each forming a portion of an image.

Consonant Values being in agreement or accord.

Conspecifics Members of the same species.

Coxa The basal segment of the leg of an arthropod.

Cursorial Adapted or specialized for running.

Cuticle The outer covering of insects and arthropods that is made of chitin.

Degree-day A measurement of heat. One degree (any scale) in average daily temperature above an appropriate developmental minimum, cf. degree-hour. Data in degree-days cannot be converted to degree-hours.

Degree-hour A measurement of heat. One degree (any scale) in average hourly temperature above an appropriate developmental minimum, cf. degree-day. Data in degree-hours may be consolidated and expressed as degree-days.

Deposition Oral or written testimony under oath but outside the courtroom. A written transcript is made of any such oral testimony. Depositions are an important aspect of the discovery process in legal proceedings.

Desiccate To dry out thoroughly; to remove all moisture.

Diapause A period during which growth and development is suspended. Both physical and physiological activity is either ceased or greatly diminished. In some insects, this is a response to adverse environmental conditions.

Disarticulation To separate at the joints.

Discovery The process in which data or documents that a party to a legal action are compelled to disclose to another party either before or during a legal proceeding.

Diurnal To be active during the daytime rather than at night.

Ecdysis Molting. The process of an insect shedding its exoskeleton.

Elytra The leathery, chitinous forewings of a beetle that serve to protect the thin membranous hind wings used for flight.

Entomotoxicology The use of arthropods, which consume and bioaccumulate drugs and toxins within their bodies, for forensic toxicological analysis in place of human tissues. This method is frequently used when sufficient amounts of human tissue are not available due to decay and skeletonization.

ETOH An abbreviation for ethyl alcohol.

Eutrophic The state of nutrient enrichment as a result of the natural or artificial addition of nutrients to bodies of water, especially lakes, often resulting in high productivity and low transparency.

Exarate An insect pupa in which the appendages of the body are free and not contained within the main body case.

Exoskeleton A skeleton on the outside of the body whose inner walls serve as a point for the attachment of muscles. All arthropods possess chitinous exoskeletons.

Exuvium The cast larval or nymphal skin of an arthropod.

Facultative myiasis Myiasis (q.v.) caused by a fly species not biologically requiring a living host. Most necrophilous flies are capable of causing facultative myiasis. Also referred to as secondary (2^0) myiasis.

Flesh fly A fly in the family Sarcophagidae.

Fossorial Adapted or specialized for burrowing or digging.

Frass The excrement of some insect species.

Haltere A small knob-like structure found on each side of the metathorax of flies. It is formed from the modified hind wings.

Hemimetabolus Incomplete metamorphosis. The developmental life cycle of an insect that lacks the pupal stage. The nymphs are usually aquatic.

Hindgut The posterior portion of the insect alimentary tract, between the midgut and the anus.

Holarctic The zoogeographic region that includes the northern areas of the Earth. It is divided into the Nearctic and the Palearctic regions.

Holometabolous Complete metamorphosis. This is the most complex type of metamorphosis. The developmental life cycle of an insect that goes through four distinct stages of growth the egg, larva, pupa, and adult.

Instar An insect or arthropod between successive molts (i.e., the first instar occurs between hatching and the first molt).

Intertidal The region of a large body of water between the high tide mark and the low tide mark.

Invertebrate Any organism lacking a spinal column.

Lamellate Having plate-like structures or segments.

Larva (*pl.* larvae) The immature stage of an insect (having complete metamorphosis) which occurs between the egg stage and the pupal stage.

Larviposition The act of laying or "depositing" living first instar larvae instead of eggs.

Lentic Standing water habitats, such as ponds, lakes, and marshes.

Locus (*pl.* loci) The position that a gene occupies on a chromosome.

Lotic Running water habitats, such as rivers and streams.

Maggot A legless larva without a well-developed head capsule.

Maggot mass An aggregation of maggots wherein the number of fly larvae developing becomes sufficiently large so that the friction caused by movement and the metabolic heat of digestion generates heat greater than that afforded by environmental conditions.

Malpractice Improper or unethical conduct by the holder of a professional or official position.

Mandible A mouth organ of invertebrates (especially in the arthropods and insects) used for seizing, biting, and manipulating food. With vertebrate organisms, it is recognized as the lower jaw.

Meconium The substance excreted from certain insects after emergence from the pupa.

Mesothorax The middle of the three divisions of an insect thorax, which bears the second pair of legs and the first pair of wings.

Metamorphosis A change in form during development.

Metathorax The hind most of the three divisions of an insect thorax, which bears the third pair of legs and the second pair of wings.

Methylated compound An organic compound in which the hydrogen of the hydroxyl group of the methyl alcohol is replaced by a metal.

Microclimate The long-term weather pattern in a small and localized area.

Morpho-behavioral adaptations Insect morphological structures (e.g., hairs, setae, mouth brushes, fans, silk secretions) and behavioral mechanisms (e.g., shredding, filtering, gathering, scraping) used in feeding, as seen in aquatic insects.

Mouth hooks The paired maxillary oral structures of a maggot used for shredding tissue so that it may be digested more effectively.

Multiplexer A hardware circuit for selecting output from multiple inputs.

Myiasis A disease condition caused by the infestation of a living human or other animal with fly larvae. The description may be further refined to indicate location affected (e.g., nasal myiasis, rectal myiasis) or the predisposing cause (e.g., traumatic myaisis in a suppurating wound). Myiasis may be classified as either primary or secondary (facultative), q.v.

Natatorial Adapted or specialized for swimming.

Nearctic The biogeographic region that includes the arctic and the temperate areas of North America and Greenland.

Necrophilous Feeds on dead bodies or decaying tissue.

Neotropical The biogeographic region from the south of the Tropic of Cancer, including southern Mexico, Central and South America, and the West Indies.

Nocturnal Active at night.

Ocellus (*pl.* ocelli) The simple eye of an adult insect or arthropod.

Odontologist An individual who studies the structure development and abnormalities of teeth.

Old World A term used to refer principally to the Palearctic, Ethiopian, and Oriental Regions, as opposed to the "New World" Nearctic and Neotropical Regions.

Oviposit To lay or deposit eggs.

Oviposition The act of depositing eggs, typically through the use of an ovipositor.

Ovipositor The egg-laying apparatus of a female insect.

Palearctic The biogeographic region of the Earth that includes Europe, the northwest coast of Africa, and Asia (North of the Himalayas).

Peritrophic membrane A membrane in insects secreted by the cells lining the midgut. The membrane is secreted when food is present and forms an envelope around the food. In some cases it pulls loose from the gut wall and is passed out with the feces.

Phenology The study of periodic biological phenomena (i.e., flowering, breeding, and migration) in relation to climatic conditions.

Photoperiod The duration of an organisms daily exposure to light, especially when considered with regards to the effect of exposure on growth and development.

Plumb bob A conical metal weight suspended from the end of a string. This device is used to generate a perfectly vertical line.

PMI (Postmortem Interval) The time period between death and the discovery of the body.

PMSI (Postmortem Submersion Interval) The time period the body remained under the water surface before surfacing due to natural means.

Poikilothermic (Cold-blooded) An animal whose body temperature fluctuates with environmental temperature.

Polymorphism The occurrence of different forms, stages, or types in individual organisms, or in organisms of the same species, independent of sexual variation.

Posterior Hind or rear.

Predacious Feeding as a predator.

Primary myiasis Myiasis (q.v.) by a fly species biologically requiring a living host. Known also as obligatory (1^0) myiasis, species exhibiting this developmental pattern are seldom associated with decaying materials.

Pronotum The dorsal sclerite of the thorax.

Prothorax The anterior division of an insect thorax, which bears the first pair of legs.

Punctate Pitted or beset with punctures or small depressions.

Pupa (*pl.* pupae) The stage between the larva and the adult in insects having complete metamorphosis. This is an inactive and nonfeeding stage.

Pupariation The act of forming the puparium.

Puparium (*pl.* puparia) A case formed by the hardening of the last larval skin of a fly in which the pupa forms.

Pupate To transform into a pupa.

Pupation To change form to the pupal stage from the larval stage.

Raptorial Fitted or adapted for grasping prey.

respondeat superior The legal doctrine wherein a master (e.g., employer) or principal can be held liable under certain circumstances for the wrongful acts of a servant (e.g., employee) or agent.

Riparian zone Streamside or relating to the bank of a stream.

Saltatorial Adapted or specialized for leaping.

Saprophagous Feeding on dead or decaying plant or animal material, such as carrion, corpses, dung, or rotting wood.

Sarcosaprophagous Feeding on or consuming dead flesh.

Sclerite A chitinous plate of an invertebrate, many of which form part of the exoskeleton.

Sclerotin A protein that hardens and darkens the insect exoskeleton by the process of cross-linking the chitin protein molecules.

Sclerotized The completed process of hardening an insect's cuticle by the formation of sclerotin.

Shagreen Having a rough, granular surface.

Species A group of individuals that are similar in structure and physiology, and are capable of interbreeding and producing fertile offsping.

Spiracle A respiratory aperture, or tracheal opening, in the exoskeleton of an insect. A breathing pore.

Spiracular plate A plate-like sclerite next to or surrounding the spiracle.

Sprawlers Aquatic insect representatives that inhabit the surface of floating leaves of emergent plants or fine sediments, usually with modifications for staying on top of the substrate and maintaining the respiratory surfaces free of silt (e.g., Ephemeroptera, Caenidae; Odonata, Libellidae).

Stratification The actual observable sequential layering of soil, rocks, or surface debris.

Stratigraphy The sum total of the processes where the layers of deposits (or strata) of soil or rock have accumulated.

Stria (*pl.* striae) A groove or depressed line.

Subpoena A writ requiring appearance in court to give testimony.

Substrate The surface on which an organism is attached, or the surface or substance on which an organism can grow or feed.

Synanthropic Used to describe organisms (particularly insects) that have an affinity, or preference, for human association.

Tarsal claw A claw at the apex of the tarsus.

Tarsus (*pl.* tarsi) The terminal segment of an insect leg immediately beyond the tibia.

Taxon (*pl.* taxa) A taxonomic group, such as a phylum, order, family, genus, or species.

Tergite A dorsal sclerite. In particular, a dorsal sclerite on the abdominal segment.

Tergum (*pl.* terga) The dorsal surface of any body segment of an insect.

Thorax The middle region of an arthropod between the head and the abdomen. In insects, this body region bears the true legs and the wings.

Tibia (*pl.* tibiae) The fourth segment of an insect leg between the femur and the tarsus.

Trachea (*pl.* tracheae) A tube of the respiratory system that ends at the spiracle externally, and terminates at the tracheoles internally.

Trochanter The second segment of an insect leg between the coxa and the femur.

Truncate Cut off square at the end.

Tubercle A small knob-like or rounded protuberance.

Ubiquitous Widespread, seeming to be (or occurring) everywhere at the same time.

Vermiculite A micaceous hydrated silicate mineral used as a planting medium and as insulation.

Vestigial Small remnants or rudimentary structures of a previously functional body part.

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