Simultaneous lessons in Drosophila genetic mapping, null hypotheses and Chi Squares

Some sources erroneously suggest that Mendel worked with 7 genes located on 7 chromosomes explaining why Mendel never encountered linkage. This suggestion is in fact incorrect!

Check out http://www.nature.com/scitable/content/though-several-of-the-genes-he-studied-18343

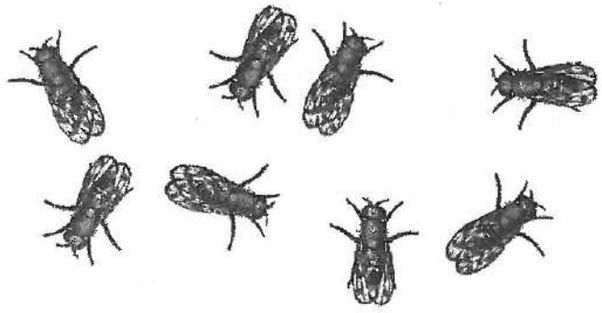
As a matter of fact, 3 of Mendel's traits are all located on chromosome #4, while 2 traits are located on chromosome #1. When genes are far enough apart, they remain unlinked and demonstrate independent assortment even when they are on the same chromosome. Chromosomes can be very long, longer than 100 mu as you will discover, while others can be much shorter.

Enough with Sweet Peas and on to Fruit Flies. Drosophila have 3 autosomal chromosomes and a pair of sex chromosomes. In this activity you will construct a mapping grid just like the ones you used in your last activity to construct linkage maps for 8 Drosophila genes. Before doing this activity, ensure you understand the preceding Chromosome Mapping Practice Activity. Final word: this is a big assignment, you will need to work in groups of 4 and share the work.

If you have access to excel, your teacher can provide a quick program to help calculate Chi Square.

Your teacher hands your group 10 vials of purebred (true breeding Drosophila).
The first 8 vials all have one mutation:

- Vial #1 = flies with crumply wings
- Vial #2 = flies with funky bristles
- Vial #3 = flies with chocolate eyes
- Vial #4 = flies with peg legs
- Vial #5 = flies with plump body
- Vial #6 = flies with warped wings
- Vial #7 = flies with short antennae
- Vial #8 = flies with dark body



As well as the following vials

- Vial #9 = wild type flies; i.e. all normal with no mutations
- Vial #10 = flies with all 8 mutations

Remember that Drosophila only have 4 chromosomes and we have at least 8 mutations, clearly some of these mutations must be located together on the same chromosome.

Your group crossed a one mutant male fly with a female wild type.

P: purebred short antenna ♂ X wild type ♀
F1: all progeny were wild type

What can your group conclude so far? the short antenae mutation is recessive

Your group repeated the above experiment with seven other mutants. Again each and every F1 were wild type. At this point you should be able to assign symbols.

mutation	Symbol for mutation	Symbol for wild type
crumply wings	c	+
funky bristles	f	+
chocolate eyes	e	+
peg legs	l	+
plump body	b	+
warped wings	w	+
short antennae	a	+
dark body	d	+

note to teacher:
It is probably a good idea that the entire class takes a time-out to all agree on identical symbols before proceeding. Any calculated map distances should be rounded to the nearest % or centimorgan.

Your group then took an F1 female from each cross and back-crossed it back to its original purebred parental line. For example,

F1 backcross: Crumply wings, wild-type hybrid F1 Progeny ♀ X purebred wild type male ♂

Redraw the cross above using genetic symbols: $+C$ X $++$

Your results: **All** the F2 progeny are wild type.

Your group determined that the short antenna allele is obviously X-linked / autosomal
Choose one

Draw Punnett Squares to demonstrate your conclusion

X-linked outcomes

	X^+	Y
X^+	X^+X^+	X^+Y
X^a	X^+X^a	X^aY

Autosomal outcomes

♀ \ ♂	+	+
+	++	++
c	+c	+c

Explanation: X-linkage not possible, otherwise F2 ♂ phenotypes would be ½ wild type & ½ mutant

All other F1 Progeny ♀ X purebred wild type male ♂ gave similar results.

Are any of the mutant genes located on the X chromosome? Explain: all mutations are recessive & autosomal

Drosophila Genetics can be very laborious and time consuming! Your teacher saved you a lot of work by kindly provided your group more Vials including **Vial #11**, identified as dihybrid short antenna, chocolate eyed flies.

Your teacher wants you to determine whether the genes for short antenna and chocolate eyed are on the same chromosome or not. You are told to pick one of the original 10 vials to make the appropriate cross. Which one will your group choose and explain why:

Vial # 10 because mapping requires a test cross i.e. dihybrid crossed to homozygous recessive

Vial #11 Dihybrid crumply wings, chocolate eyed flies. Draw cross using genetic symbols:

+c,+e X cc,ee

Your results:

+,+	+,chocolate	+, short antenna	chocolate, short antenna
213	237	245	225

Are these the results that Mendel would expect? Explain Yes, Mendel expected independent assortment as exhibited here

Checking a variety of sources, your group agrees on the following working hypothesis:

Vial #11 - Working Hypothesis: " The two genes for crumply wings and chocolate eyed are/~~are not~~ on the same chromosome because the two genes exhibit no apparent linkage"
Null Hypothesis: the 4 phenotypes show a 1:1:1:1 ratio
Degrees of Freedom: 3
Chi Square value: 2.56
Your group rejects/fails to reject the Null (level of significance = 0.05)

What can your group conclude so far? We cannot rule out independent assortment
Our working hypothesis may be correct.

Your teacher hands your group four more vials. These are your results after you did the appropriate crosses. Remember, you are attempting to map the genes.

Vial #12= Dihybrid short antennae, chocolate eyed flies. Draw the cross using genetic symbols:

+a, +e X aa, ee

Results:

+,+	+,chocolate	+, crumply wings	chocolate, crumply wings
151	178	159	163

Vial #12 - Working Hypothesis: " The two genes for crumply wings and chocolate eyed are/are not on the same chromosome because ditto "

Null Hypothesis: ditto

Degrees of Freedom: 3

Chi Square value: 2.36

Your group rejects/(fails to reject) the Null (level of significance = 0.05)

What can your group conclude so far? ditto

Vial #13: Dihybrid peg legs, chocolate eyed flies. Draw the cross using genetic symbols:

+L, +e X LL, ee

Results:

+,+	+,chocolate	+, funky bristles	chocolate, funky bristles
271	237	277	251

Vial #13 - Working Hypothesis: " The two genes for crumply wings and chocolate eyed are/are not on the same chromosome because ditto "

Null Hypothesis: ditto

Degrees of Freedom: 3

Chi Square value: 3.92

Your group rejects/(fails to reject the Null) (level of significance = 0.05)

What can your group conclude so far? ditto

Vial #14: Dihybrid funky bristles, chocolate eyed flies. Draw the cross using genetic symbols:

+f, +e X ff, ee

Results:

+,+	+,chocolate	+, peg legs	chocolate, peg legs
366	350	378	348

Vial #14 - Working Hypothesis: " The two genes for crumply wings and chocolate eyed are/are not on the same chromosome because ditto "

Null Hypothesis: ditto

Degrees of Freedom: 3

Chi Square value: 1.67

Your group rejects/(fails to reject) the Null (level of significance = 0.05)

What can your group conclude so far? ditto

Vial #15: Dihybrid dark body, chocolate eyed flies. Draw the cross using genetic symbols:

+d, +e X dd, ee

Results:

+,+	+,chocolate	+, black body	chocolate, black body
267	243	279	241

Vial #15- Working Hypothesis: " The two genes for crumply wings and chocolate eyed are/are not on the same chromosome because ditto "

Null Hypothesis: ditto

Degrees of Freedom: 3

Chi Square value: 4.02

Your group rejects/fails to reject the Null (level of significance = 0.05)

What can your group conclude so far? ditto

Genetics Time Out! How many autosomes does Drosophila have again? 3

Can short antenna, crumply wings, funky bristles, peg legs and black body all be on a different chromosome than chocolate eyes? Explain
These 5 genes are located on a maximum of 3 chromosomes. Some genes are on same chromosome

Statistics Time Out!
There are a number of reasons why statisticians insist on using the expression "fail to reject the null" instead of "accept the null". Basically statistics can often lead us all astray by "confirming the right answer for the all the wrong reasons" or "confirming the wrong answer for all the right reasons". That said, we need to revisit what we wrote above. That's OK, science works like this all the time, as new data requires scientists to revisit hypotheses.

Rewrite the Working Hypotheses:
Working Hypothesis: " The two genes for short antenna and chocolate eyed are unlinked and maybe on same chromosome (>50 m.u.) or on different chromosomes

Working Hypothesis: " The two genes for crumply wings and chocolate eyed eyed ditto

Working Hypothesis: " The two genes for funky bristles and chocolate eyed eyed ditto

Working Hypothesis: " The two genes for peg legs and chocolate eyed eyed ditto

Working Hypothesis: " The two genes for dark body and chocolate eyed eyed ditto

Can any of the results above be used for mapping? Yes/(No) Explain: Even if we could determine which genes are on same chromosome, this data only proves no linkage & provides no mapping information.

Again, your kind and patient teacher hands your group some vials with various dihybrids. There is a lot of work to do here, so remember to delegate and share the workload. At his point, you want to do Chi Square on a scientific calculator or an excel spreadsheet (a very valuable skill indeed).

Vial #16 dihybrid = short antenna, crumply wings.
Draw the cross using genetic symbols: $\frac{aw}{++} \times \frac{aw}{aw}$ (coupling)

+,+	+, short antenna	+, crumply wings	short antenna, crumply wings
222	37	28	212

Vial #16 - Working Hypothesis: We are observing linkage of 2 genes on the same chromosome
Null Hypothesis: We are observing a 1:1:1:1 phenotype ratio expected of no linkage

Degrees of Freedom: 3
Chi Square value: 274
Your group (rejects)/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? the data is not consistent with independent assortment
Parental classes are ++ & aw Recombinant classes are +a & +w
Your results above provide a map distance of 13 between a & w

Statistics Time Out!
When genes appeared to assort independently, our working hypothesis implied a ratio of 1:1:1:1 and our null agreed with our working hypothesis. Our null accordingly assumed a 1:1:1:1 ratio. However, when genes appeared to NOT assort independently, our null AGAIN assumed a 1:1:1:1 ratio; even when this time, our null appeared to contradict our working hypothesis! That means, the statistical null hypothesis and the scientific hypothesis may or may not be "in agreement."

That means, sometimes "Our theory leads us to expect some particular proportions" and sometimes "Our theory leads us to contradict some particular proportions." We can't measure how different or similar our proportions are without fixing these proportions in advance.

Quick take-home message: "Statistical Null Hypotheses" are always written as equalities.

Vial #17 dihybrid = warped wings, plump body.
Draw the cross using genetic symbols: $\frac{w\ +}{+\ b} \times \frac{w\ b}{w\ b}$ (repulsion)

+,+	+, warped wings	+, plump body	warped wings, plump body
21	318	330	28

Vial #17 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 515
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are +w & +b Recombinant classes are ++ & wb
Your results above provide a map distance of 7 mu between w & b

---

Vial #18 dihybrid = plump body, chocolate eyes.
Draw the cross using genetic symbols: $\frac{b\ e}{+\ +} \times \frac{b\ e}{b\ e}$ (coupling)

+,+	+,chocolate	+, plump body	chocolate, plump body
282	71	83	265

Vial #18 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 222
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are be & ++ Recombinant classes are +b & +e
Your results above provide a map distance of 22 mu between b & e

---

Vial #19 dihybrid = funky bristles, short antenna.
Draw the cross using genetic symbols: $\frac{f\ +}{+\ a} \times \frac{f\ a}{f\ a}$ (repulsion)

+,+	+, funky bristles	+, short antenna	funky bristles, short antenna
71	279	265	83

Vial #19 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 219
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are +f & +a Recombinant classes are fa & ++
Your results above provide a map distance of 22 mu between f & a

---

Vial #20 dihybrid = crumply wings, peg legs.
Draw the cross using genetic symbols: $\frac{c\ l}{+\ +}$ X $\frac{c\ l}{c\ l}$ (coupling)

+,+	+, crumply wings	+, peg legs	crumply wings, peg legs
294	59	67	281

Vial #20 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 288
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are c l & ++ Recombinant classes are +c & +l
Your results above provide a map distance of 18 mu between c & l

---

Vial #21 dihybrid = crumply wings, funky bristles.
Draw the cross using genetic symbols: $\frac{c\ +}{+\ f}$ X $\frac{c\ f}{c\ f}$ (repulsion)

+,+	+, crumply wings	+, funky bristles	crumply wings, funky bristles
15	177	188	21

Vial #21 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 271
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are +c & +f Recombinant classes are ++ & cf
Your results above provide a map distance of 9 between c & f

Vial #22 dihybrid = funky bristles, peg legs.
Draw the cross using genetic symbols: $\frac{f\ l}{+\ +}$ X $\frac{f\ l}{f\ l}$ (coupling)

+,+	+, funky bristles	+, peg legs	funky bristles, peg legs
219	25	20	235

Vial #22 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 336
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are fl & ++ Recombinant classes are +f & +l
Your results above provide a map distance of 9 mu between f & l

---

Vial #23 dihybrid = dark body, peg legs.
Draw the cross using genetic symbols: $\frac{d\ +}{+\ l} \times \frac{d\ l}{d\ l}$ (repulsion)

+,+	+, dark body	+, peg legs	dark body, peg legs
50	241	249	58

Vial #23 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 244
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are +d & +l Recombinant classes are ++ & dl
Your results above provide a map distance of 18 mu between d & l

---

Vial #24 dihybrid = warped wings, dark body.
Draw the cross using genetic symbols: $\frac{w\ d}{+\ +} \times \frac{w\ d}{w\ d}$ (coupling)

+,+	+, warped wings	+, dark body	warped wings, dark body
104	44	37	116

Vial #24 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 65.5
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are wd & ++ Recombinant classes are +w & +d
Your results above provide a map distance of 27 mu between w & d

---

Vial #25 dihybrid = dark body, plump body
Draw the cross using genetic symbols: $\frac{d\ +}{+\ b} \times \frac{d\ b}{d\ b}$ (repulsion)

+,+	+, dark body	+, plump body	dark body, plump body
127	237	225	112

Vial #25 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 72.0
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are +d & +b Recombinant classes are ++ & db
Your results above provide a map distance of 34 mu between d & b

---

Vial #26 dihybrid = chocolate eyes, warped wings.
Draw the cross using genetic symbols: $\frac{w\ e}{+\ +}$ X $\frac{w\ e}{w\ e}$ (coupling)

+,+	+,chocolate	+, warped wings	chocolate, warped wings
170	76	69	184

Vial #26 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 89
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are ++ & we Recombinant classes are e+ & w+
Your results above provide a map distance of 29 between e & w

---

Vial #27 dihybrid = funky bristles, dark body.
Draw the cross using genetic symbols: $\frac{f\ +}{+\ d}$ X $\frac{f\ d}{f\ d}$ (repulsion)

+,+	+, dark body	+, funky bristles	dark body, funky bristles
101	264	248	88

Vial #27 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 150
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are +f & +d Recombinant classes are ++ & fd
Your results above provide a map distance of 27 between f & d

---

Vial #28 dihybrid = peg legs, warped wings.
Draw the cross using genetic symbols: $\frac{l\ w}{+\ +}$ X $\frac{l\ w}{l\ w}$ (coupling)

+,+	+,chocolate	+, peg legs	chocolate, peg legs
185	163	152	201

Vial #28 - Working Hypothesis: ditto

Null Hypothesis: ditto

Degrees of Freedom: 3
Chi Square value: 8.2
Your group rejects/fails to reject the Null (level of significance = 0.05)
What can your group conclude so far? ditto

Parental classes are ++ & lw Recombinant classes are +l & +w
Your results above provide a map distance of 45 between l & w

---

Everyone of these genes is less than 50 map units from at least one other gene in the list! That can only mean one thing!

Explain these genes are linked one to another on one chromosome

Now comes the tricky part – transfer all this on to a grid and create a genetic map

A reduced and simplified grid:

funky bristles f	9 x21						
chocolate eyes e							
peg legs l	18 x20	9 x22					
plump body b			22 x19				
warped wings w			29 x26	45 x28	7 x17		
short antennae a	13 x16	22 x19					
dark body d		27 x27		18 x23	34 x25		27 x24
	crumply wings c	funky bristles f	chocolate eyes e	peg legs l	plump body b	short antennae a	warped wings w

Don't panic. The first time is always a little tricky, but thereafter genetic mapping is easy.

Follow this logic. Find any landmark, let's say you have a land mark called "A". Now find two distances from A to two other land marks. Let's say

- A to B is 9 map units
- A to C is 18 map units.

To complete the map you need to search the grid and find the distance from B to C from the grid. Say

- B to C is also 9 map units,

...then you have a map with 3 landmarks:

```
    9       9
A---------B---------C
          18
```

Now check if <u>any</u> two of these genes is connected to other so-called landmarks.

Say the grid tells you that

- A to D is 13 map units
- B to D is 22 map units

- A to B is 9 map units (You know that already, that is why you strategically chose this pair to start with.)

You can now construct a map with C & D as flanking markers and A in the middle. Draw the map below:

```
      13        9
D-------------A---------B
a        22c      f
```

← same values as data table but, different letters

You now have two maps that overlap.

```
    9       9                      9        13
A---------B---------C      &   B---------A-------------D
c         f         l            f        c             a
          18                     22
```

Superimpose the two maps (flip the second map before superimposing)

```
      13        9        9
D-------------A---------B---------C
a          c31      f        l
```

You could now draw the new total 31 in the appropriate spot in the grid if indeed that spot is empty. Fill in your data first, then fill your calculated distances. Now follow this procedure with your data. At this rate, you will soon have a map that can exceed 50 or even 100 map units!

Draw a map to scale:

Congratulations on completing your first genetic map.

In your own words explain how it is possible that three of the independently assorting chromosomes that Mendel studied were all on the same chromosome:

Once genes are 50+ m.u. apart, they demonstrate independent assortment i.e. 50:50 probability of assortment looks like 50 m.u.

According to your completed grid and genetic map, how far apart are

Chocolate Eyes and Short Antennae 105 mu & Chocolate Eyes and Dark Body 56 m.u.

Now look at your results from vials #12 & #15. Explain the discrepancy:

Vial #12 = 50 m.u.?! ≠ Vial #15 = 50 m.u.?! See answer immediately above. Vials 12 & 15 provide no mapping information due to independent assortment

One final word on Statistics. (Groan – last one, I promise)

We already know how peg legs and warped wings map on the chromosome. Now check out these results:

Vial #29 dihybrid = peg legs, warped wings.

Draw the cross using genetic symbols: **X**

+,+	+,chocolate	+, peg legs	chocolate, peg legs
142	107	118	133

Vial #29 - **Working Hypothesis:** We are observing linkage of two genes on same chromosome.

Null Hypothesis: we are observing 1:1:1:1 ratio expected of no linkage

Degrees of Freedom: 3

Chi Square value: 5.70

Your group rejects/fails to reject the Null (level of significance = 0.05)

What can your group conclude so far? data is statistically consistent with independent assortment

Parental classes are ? & ? Recombinant classes are ? & ?

Your results above provide a map distance of ? between l & w

These results contradict Vial #28. Which results are more believable? Explain:

Vial #28 counted 201 more flies! Bigger data set is more believable

What exactly is the "rule of 10" for Chi-Square? :

No category should be smaller than 10 when using Chi-Square. (Vial #29 indicates that more than 10 is still no guarantee of a successful test)

In your own words explain why statisticians do **NOT SAY** they 'accept' the null hypothesis, but **SAY INSTEAD** there is insufficient evidence to reject the null hypothesis.

Chi-Square can raise a "red flag" or fail to raise a "red flag"

Statistical "testing" is always indirect and cannot really confirm or contradict cause-effect relations.

One final word on statistics. Our latest series of Chi square tests indeed ruled out independent segregation but in fact did not test whether our hypotheses of those calculated map distances were really statistically defensible. In real life, further statistical testing would be required. For now this is good enough and let us leave it at that. We should leave something for university or higher level stats courses.

Last word on Genetics: The exercise was greatly simplified for beginners. In theory this exercise could have been accomplished with just six vials! In real life, even half that!

Check out:

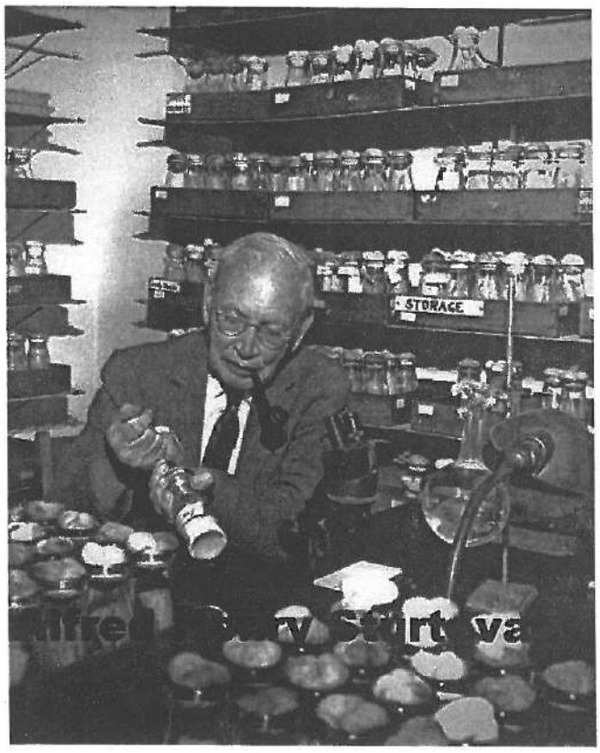
http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/T/ThreePointCross.html

and

http://www.ndsu.edu/pubweb/~mcclean/plsc431/linkage/linkage3.htm

Another simplification was jigging the data to make map distances perfectly additive. This does not happen in real life as explained in the two links above.

Who was it who first figured how to construct the first genetic map even though map distances did not add up perfectly?



Alfred Henry Sturtevant was a poor farm-boy teenager whose hobby was amateur genetics. Sturtevant sent Morgan his notes on horse genetics based on his observations of horse breeding on the family farm. Morgan immediately offered the young Sturtevant a coveted undergrad spot in his lab.

http://www.dnaftb.org/11/bio.html

They did not have Ritalin back then and Sturtevant was easily bored and distractible. One night, Sturtevant punted his homework assignment to examine six genes on the X chromosome. In his own words:

"In the latter part of 1911, in conversation with Morgan, I suddenly realized that the variations in strength of linkage, already attributed by Morgan to differences in the spatial separation of genes, offered the possibility of determining sequences in the linear dimension of a chromosome. I went home and spent most of the night (to the neglect of my undergraduate homework) in producing the first chromosome map."

Here is a copy of Sturtevant's thesis, that was a result of his missed homework assignment.

http://www.nature.com/scitable/content/The-linear-arrangement-of-six-sex-linked-16655