Deriving Linkage Distance and Gene Order From Three-Point Crosses

A trihybrid was crossed to a homozygous recessive. The following figure shows the different recombinant products that are possible in the F1 gametes. How many kinds of gametes are possible?



Now if we were to perform a testcross with F_1 , Mendel would expect a 1:1:1:1:1:1:1:1:1:1:1 ratio. Deviation from this expected ratio indicates that linkage is occurring. The best way to become familiar with the analysis of three-point test cross data is to go through an example. We will use the arbitrary example of genes *A*, *B*, and *C*. We first make a cross between individuals that are *AABBCC* and *aabbcc*. Next the F_1 is testcrossed to an individual that is *aabbcc*. We will use the following data to determine the gene order and linkage distances.

This is now a different cross with different gene order.

Genotype	Observed	Type of Gamete
XYZ	390	Parental
хуг	374	Parental
XyZ	27	Single-crossover between genes Yand X
xYz	30	Single-crossover between genes Y and X
XYz	5	Double-crossover
xyZ	8	Double-crossover
Xyz	81	Single-crossover between genes A and C
xYZ	85	Single-crossover between genes A and C
Total	1000	

The best way to solve these problems is to develop a systematic approach. First, determine which of the genotypes are the parental gentoypes. **The genotypes found most frequently are the parental genotypes.** From the table it is clear that the *XYZ* and *xyz* genotypes were the parental genotypes.

Next we need to determine the order of the genes. Once we have determined the parental genotypes, we use that information along with the information obtained from the double-crossover. **The double-crossover gametes are always in the lowest frequency.** From the table the *XYz* and *xyZ* genotypes are in the lowest frequency. **The next important point is that a double-crossover event moves the middle allele from one sister chromatid to the other.** This effectively places the non-parental allele of the middle gene onto a chromosome with the parental alleles of the two flanking genes. In other words, which is the odd allele out – that allele is the middle allele. We can see from the table that the *Z* gene must be in the middle because the recessive *z* allele is now on the same chromosome as the *X* and *Y* alleles, and the dominant *Z* allele is on the same chromosome as the recessive *x* and *y* alleles.

Now that we know the gene order is XZY, we can go about determining the linkage distances between X and Z, and Z and Y. The linkage distance is calculated by dividing the total number of recombinant gametes into the total number of gametes. What is different is that we must now also consider the double-crossover events. For these calculations we include those double-crossovers in the calculations of both interval distances.

So the distance between genes X and Z is 17.9 cM= $[100 \times ((81+85+5+8)/1000)]$, and the distance between Z and Y is 7.0 cM= $[100 \times ((27+30+5+8)/1000)]$.

Sometimes we have a problem when calculating the distance between flanking markers when adding all the recombinant classes: $[100 \times ((81+85+27+30+5+8)/1000)]$ is an underestimate. Remember that we should in fact be counting the total number of cross-overs and that double-cross-overs count twice (double cross-overs = 2 cross-overs)! In other words, the calculated distance for the flanking markers from this data would be:

 $[100 \times ((81+85+5+8+27+30+5+8)/1000)] =$

X-Z Distance Z-Y Distance

Now let's try a problem from *Drosophila*, by applying the principles we used in the above example. The following table gives the results we will analyze.

Genotype	Observed	Type of Gamete
<i>v cv</i> ⁺ <i>ct</i> ⁺	580	Parental
v+ cv ct	592	Parental
v cv ct+	45	Single-crossover between genes and
<i>v</i> ⁺ <i>cv</i> ⁺ <i>ct</i>	40	Single-crossover between genes and
v cv ct	89	Single-crossover between genes and
<i>V</i> ⁺ <i>CV</i> ⁺ <i>Ct</i> ⁺	94	Single-crossover between genes and
v cv+ ct	3	Double-crossover between genes and
<i>V</i> ⁺ <i>CV Ct</i> ⁺	5	Double-crossover between genes and
Total	1448	

Step 1: Determine the parental genotypes.

The most abundant genotypes are the parental types.

These genotypes are ______ and _____. This is different: in this three-point cross dominant alleles and recessive alleles are mixed and matched on different parental chromosomes.

Step 2: Determine the gene order

First draw the Parental chromosome configurations, then figure which allele is the odd one out when compared to parental orientations. That one must be in the middle.

Step 3: Determine the linkage distances.

Step 4. Draw the map.

Remember that in real life – the two distances do not really add up perfectly. For "somewhat" larger distances, undetected double-crossovers are shrinking the <u>apparent</u> distances. For very short distances, undetected interference is also shrinking the <u>apparent</u> distances!

We have also ignored interference. When given two specific recombination rates in two adjacent chromosomal intervals, the rate of double-crossovers in this region should be equal to the product of the single crossovers. That is often not seen. Somehow, cross-overs avoid crowding themselves for some mechanical reasons that are beyond AP Biology when the two genes are very close together. Theoretically, we could measure interference by comparing the expected double cross-overs to the observed double cross-overs.

Unfortunately we do not have enough double-recombinants to test this theory with a \mathcal{X} -squared.