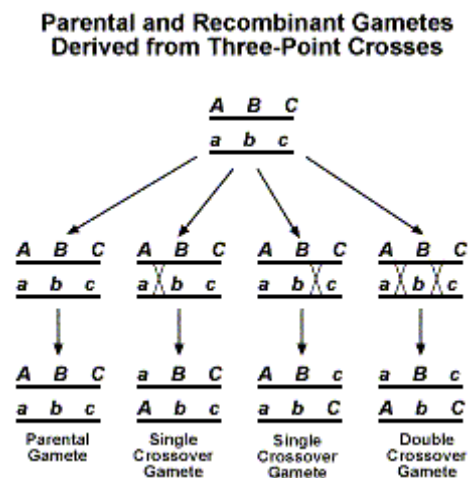


## Deriving Linkage Distance and Gene Order From Three-Point Crosses

This activity is modified with some changes and additions from Phillip McClean's excellent introduction found on ([link](#))

A trihybrid was crossed to a homozygous recessive. The following figure shows the different recombinant products that are possible in the F<sub>1</sub> gametes. How many kinds of gametes are possible?



Now if we were to perform a testcross with F<sub>1</sub>, Mendel would expect a 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<sub>1</sub> 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
<i>ABC</i>	390	Parental
<i>abc</i>	374	Parental
<i>AbC</i>	27	Single-crossover between genes <i>C</i> and <i>B</i>
<i>aBc</i>	30	Single-crossover between genes <i>C</i> and <i>B</i>
<i>ABc</i>	5	Double-crossover
<i>abC</i>	8	Double-crossover
<i>Abc</i>	81	Single-crossover between genes <i>A</i> and <i>C</i>
<i>aBC</i>	85	Single-crossover between genes <i>A</i> and <i>C</i>
<b>Total</b>	<b>1000</b>	

The best way to solve these problems is to develop a systematic approach. First, determine which of the the genotypes are the parental genotypes. **The genotypes found most frequently are the parental genotypes.** From the table it is clear that the *ABC* and *abc* 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 *ABc* and *abC* 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 *C* gene must be in the middle because the recessive *c* allele is now on the same chromosome as the *A* and *B* alleles, and the dominant *C* allele is on the same chromosome as the recessive *a* and *b* alleles.

Now that we know the gene order is *ACB*, we can go about determining the linkage distances between *A* and *C*, and *C* and *B*. 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 *A* and *C* is 17.9 cM [ $100 \cdot ((81+85+5+8)/1000)$ ], and the distance between *C* and *B* is 7.0 cM [ $100 \cdot ((27+30+5+8)/1000)$ ].

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<sup>+</sup> ct<sup>+</sup></i>	580	Parental
<i>v<sup>+</sup> cv ct</i>	592	Parental
<i>v cv ct<sup>+</sup></i>	45	Single-crossover between genes ____ and ____
<i>v<sup>+</sup> cv<sup>+</sup> ct</i>	40	Single-crossover between genes ____ and ____
<i>v cv ct</i>	89	Single-crossover between genes ____ and ____
<i>v<sup>+</sup> cv<sup>+</sup> ct<sup>+</sup></i>	94	Single-crossover between genes ____ and ____
<i>v cv<sup>+</sup> ct</i>	3	Double-crossover between genes ____ and ____
<i>v<sup>+</sup> cv ct<sup>+</sup></i>	5	Double-crossover between genes ____ and ____
<b>Total</b>	<b>1448</b>	

### Step 1: Determine the parental genotypes.

The most abundant genotypes are the parental types. These genotypes are *v cv<sup>+</sup> ct<sup>+</sup>* and *v<sup>+</sup> cv ct*. What is different from our first three-point cross is that one parent did not contain all of the dominant alleles and the other all of the recessive alleles.

### Step 2: Determine the gene order

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

### Step 3: Determine the linkage distances.

### Step 4. Draw the map.

Notice that in real life – the two distances do not really add up perfectly. One reason is that double cross-overs cancel each other out and underestimate the larger flanking distances. However, Three-point crosses also allows one to measure **interference (I)** among crossover events within a given region of a chromosome. Specifically, the amount of double crossover gives an indication if interference occurs. The concept is that given 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 not what we saw.

Interference must have occurred. Biologically, cross-overs somehow avoid crowding themselves for some mechanical reasons that are beyond AP Biology.

Let's just leave it at that.