Video Tutorial 9.1: Determining the map distance between genes

Three-factor linkage questions may seem daunting at first, but there is a straight-forward approach to solving these problems. We have described the process in Box 9-6, and in this video we work through a three-factor cross step by step.

First, let's read through the problem, which uses data originally reported by RA Emerson, an American geneticist.

Two different pure-breeding lines of corn were crossed to produce a phenotypically wild-type F_1 that was heterozygous for three alleles: ***br*** (brachytic), ***an*** (anther ear), and ***f*** (fine-striped). We do not know the order of these three genes or the map distances between them. The wild-type F_1 heterozygote was then test-crossed.

The offspring resulting from this test-cross are shown in the table:

Anther ear	355
Brachytic	2
Wild-type	88
Fine-striped	21
Anther ear, brachytic	17
Brachytic, fine-striped	339
Anther ear, brachytic, fine-striped	55
Anther ear, fine-striped	2

Given these data, what is the order of these three genes on the chromosome, what are the map distances between them and what is the interference in this region of the chromosome?

Let's review what the question is telling us.

Three recessive traits are followed in this cross:

Brachytic plants, which have a mutation in the ***br*** gene, have shortened internodes and are dwarfs.

Anther ear plants, the result of a mutation in the ***an*** gene, have the male parts of the flower, the anthers, within the ear of the corn.

Fine-striped corn, with a mutation in the ***f*** gene, has white lines on the leaves.

(Note that if the mutant trait is not mentioned in a phenotypic category, the plant must be wild-type for that trait.)

The general structure of a three-factor cross problem involves mating two homozygous parents to obtain an F_1 triple heterozygote. In our case, the F_1 is heterozygous for all three of the genes – ***an***, ***br***, and ***f***. This wild-type F_1 heterozygote was mated to a plant homozygous for all three mutant genes. The offspring of the cross were counted and grouped by phenotype. Remember that the homozygous recessive parent in the test cross contributes no dominant alleles, so the phenotypes of the offspring represent the gametes produced by the F_1 plant, which may result from zero, one or two cross-overs during meiosis. Analyzing the number of offspring of each phenotype will allow us to determine the order of the genes on the chromosome and the map distances between them.

The first thing to do in solving a three-factor cross is to count the number of phenotypes seen in the offspring. In a test cross of a triple heterozygote, there should be 8 different phenotypes, since the three genes each have two possible alleles – mutant and wild type. If we see fewer than 8 phenotypes, double crossovers could be very rare or some classes of offspring might not survive, so would be absent. We would need to determine which phenotypes are missing before we go further.

In our case, we have 8 phenotypic classes, as expected. Now, we need to list these phenotypes in descending numerical order, as shown here. As we look at the re-organized data, we notice that the phenotypic categories that have a similar number of individuals are related to one another and form a pair. Since these are actual data taken from Emerson's work, the number of offspring in a phenotypic pair may not be exactly the same.

[Anther ear	355
Brachytic, fine-striped	339
Wild-type	88
Anther ear, brachytic, fine-striped	55
Fine-striped	21
Anther ear, brachytic	17
Brachytic	2
Anther ear, fine-striped	2]

For example, there are 355 plants that have the anther ear phenotype but are wild-type for the other traits, while there are 339 plants that are brachytic and fine-striped, but wild-type for anther ear.

We can determine the linkage arrangement in the F_1 heterozygote - and therefore its parents - by finding the most frequent pair of phenotypes. Remember that the parental combinations will always be more common than the recombinants, so the pair that is most abundant has the same phenotypes as the original parents. This tells us which alleles were linked on the same chromosome, but not the actual gene order.

In this case, the most frequent pair consists of plants with anther ear alone and those that are brachytic and fine-striped. We can label these parental phenotypes. One of the original parents had the alleles ***an br+ f+*** while the other parent was ***an+ br f***.

We can now determine which gene is in the middle by finding the least frequent pair of phenotypes, which result from a double crossover during meiosis, making them relatively rare. In our case, the least frequent types are **brachytic** alone and **anther ear and fine-striped** together. We can label these DCO's, for double crossovers.

Compare these phenotypes to those of the most frequent pair. Which trait differs in these two groups?

The gene for this trait must be the one in the middle.

In our problem, we see that **fine-striped** was originally associated with **brachytic** in the parent, but is coupled with **anther ear** in one of double crossover phenotypes. This means that the ***f*** gene must be in the middle.

We can now rewrite the linkage arrangement of the F_1 to reflect this information: ***an f+ br+/ an+ f br.*** It doesn't matter whether the **an** or **br** gene on the right or left; the important thing is that the **f** gene is in the middle.

When we draw in the crossovers on the F_1 genotype, we can see how a double crossover during meiosis gives rise to the least frequent allelic combinations ***an+ f+ br/ an f br+***, which match our DCO's.

We are now ready to determine the map distances between the middle gene and each of the two flanking genes. This step should be familiar from solving problems involving two genes. For each calculation, we will look at two genes at a time, and ignore the third one.

In this case, a crossover between **an** and **f** produces offspring that are ***anther eared and fine-striped*** or are wild type for these two traits. There are 55 plus 2 ***anther eared and fine-striped*** offspring and 88 + 2 that are wild type. We add these together to get the total number of recombinants between ***an*** and ***f:*** 147. There were 879 total offspring, so the recombination frequency between **anther ear** and **fine-striped** is 147/879 which equals 0.167. The distance between **an** and **f** is 16.7 map units.

We can do the same calculation with the other two genes. A crossover between **f** and **br** produces offspring that are only **fine-striped** or only ***brachytic*** but not both. There are 21 plus 2 offspring that are **fine-striped** and 17 plus 2 that are ***brachytic***. We add these together to get a total of 42 recombinants between **f** and **br**. 42 divided by the total number of plants observed, 879, equals 0.048. The distance between **f** and **br** is 4.8 map units.

Notice that the double crossover phenotypes are counted in each of our map distance calculations since they have a crossover in each region of the chromosome.

Let's redraw our map of this region of the chromosome to reflect these distances:

an------**16.7**----------***f***--**4.8**---***br***

an is **16.7** map units from ***f***, which is **4.8** map units from ***br.***

Once we have found these two map distances, we can calculate the amount of interference observed. Remember that interference is a measure of the extent to which one crossover prevents the occurrence of another crossover nearby. We know the probability of a crossover in each region of the chromosome, but what is the likelihood that both would occur at the same time?

We can multiply the probability of a crossover between ***an*** and ***f*** by the probability of a crossover between ***f*** and ***br***, and multiply this by the total number of offspring observed.

This equation is:

0.167 * 0.048 * 879 which equals 7.

This gives us the number of double crossover offspring we would expect to see among 879 offspring, based on our map of the chromosome. How many did we actually find? The observed number of double crossovers, represented by the phenotypes **brachytic** alone and **anther ear and fine-striped**, is 4.

The co-efficient of coincidence (c), is defined as the observed number of double crossovers divided by the expected number. There were four observed double crossovers and we expected to see 7. The co-efficient of coincidence is then 4/7 or 0.57.

Interference is defined as 1 minus the coefficient of coincidence. In our example, 1-0.57 gives us 0.43, or 43%. This means that a crossover in one region of the chromosome reduces the probability of a crossover occurring simultaneously in a nearby region by 43%.

Now we have completed our three-factor cross question. We have determined the linkage arrangement, the order of the genes on the chromosome, and the map distances between the genes. We have also calculated the interference for this region.

Although map distances are generally based on two-factor data, three-factor crosses help us to think about some important concepts, including double crossovers and interference, so it is worth working through problems like this involving three genes on a chromosome.

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brachytic http://www.maizegdb.org/data_center/phenotype?id=64269#variation

anther ear http://www.maizegdb.org/gene_center/gene/an1#!

Fine-stripe http://www.maizegdb.org/data_center/locus/f1

For more information about this database and the genetics of maize, visit http://www.maizegdb.org/