

## Linkage Analysis with three-point crosses

In the homework, you are asked to determine the potential linkage relationships among three recessive, autosomal traits in *Drosophila melanogaster*: javelin bristle (*ju*), striped body (*str*) and pink eye (*pi*). You make a test cross between a female who is heterozygous for all three loci (*ju/+; str/+; pi/+*) and a male who is homozygous recessive for all three traits (*ju/ju; str/str; pi/pi*). When you score the phenotypes of 1000 of their progeny, you get the results shown, you need to use these data to determine if some or all

<u>♂ gametes</u>	<u>♀ gametes</u>	<u>progeny phenotype</u>	<u>#progeny</u>
<b>ju str pi</b>	<b>ju + +</b>	<b>javelin</b>	<b>294</b>
<b>ju str pi</b>	<b>+ str pi</b>	<b>striped, pink</b>	<b>287</b>
ju str pi	ju str pi	javelin, striped, pink	140
ju str pi	+ + +	wild type	139
ju str pi	+ + pi	pink	66
ju str pi	ju str +	javelin, striped	65
ju str pi	ju + pi	javelin, pink	4
ju str pi	+ str +	striped	5

of the genes are linked, and what the genetic distance is between linked genes. Remember that the way to approach a problem like this is to look at the numbers first. What you see is that two kinds of offspring (*ju/ju; str/+; pi/+* and *ju/+; str/str; pi/pi*) are much more common than the others. These are the parentals. Because there are only two common classes, it means that all three genes are linked (close to each other on the same chromosome). They also enable you to determine how the alleles are arranged on the heterozygous females chromosomes: one chromosome is carrying the *ju* allele, but the wild type allele for the other two traits (*ju + +*); and the other chromosome is carrying the wild type allele at the bristle locus, but recessive alleles for the other two traits (*+ str pi*). All of the other types of progeny are recombinant genotypes. The next step is figure out the RF value/genetic distance for each pair of genes. To do this you look at two genes, ignoring the third for each of the three possible pairs (ie, *ju* and *str*, *ju* and *pi*, *str* and *pi*). The parental combination of alleles are the combinations seen in the parental chromosomes (the two common classes); recombinants are different combinations (ie, for *ju* and *str*, **ju +** and **+ str** are parental, **ju str** and **+ +** are recombinant; for *ju* and *pi*, **ju +** and **+ pi** are parental, **ju pi** and **+ +** are recombinant; for *str* and *pi*, **str pi** and **+ +** are parental, **str+** and **+ pi** are recombinant). Calculate the RF value for each pair in turn:

for *ju* and *str* (recombinants in blue):

$\sigma^+$ gametes	$\rho$ gametes	progeny phenotype	#progeny
<i>ju str pi</i>	<i>ju + +</i>	javelin	294
<i>ju str pi</i>	<i>+ str pi</i>	striped, pink	287
<i>ju str pi</i>	<i>ju str pi</i>	javelin, striped, pink	140
<i>ju str pi</i>	<i>+ + +</i>	wild type	139
<i>ju str pi</i>	<i>+ + pi</i>	pink	66
<i>ju str pi</i>	<i>ju str +</i>	javelin, striped	65
<i>ju str pi</i>	<i>ju + pi</i>	javelin, pink	4
<i>ju str pi</i>	<i>+ str +</i>	striped	5

$$RF_{ju, str} = (140 + 139 + 66 + 65)/1000 = 0.41 \quad \text{distance} = RF \times 100 = 41 \text{ cM}$$

for *ju* and *pi* (recombinants in blue):

$\sigma^+$ gametes	$\rho$ gametes	progeny phenotype	#progeny
<i>ju str pi</i>	<i>ju + +</i>	javelin	294
<i>ju str pi</i>	<i>+ str pi</i>	striped, pink	287
<i>ju str pi</i>	<i>ju str pi</i>	javelin, striped, pink	140
<i>ju str pi</i>	<i>+ + +</i>	wild type	139
<i>ju str pi</i>	<i>+ + pi</i>	pink	66
<i>ju str pi</i>	<i>ju str +</i>	javelin, striped	65
<i>ju str pi</i>	<i>ju + pi</i>	javelin, pink	4
<i>ju str pi</i>	<i>+ str +</i>	striped	5

$$RF_{ju, pi} = (140 + 139 + 4 + 5)/1000 = 0.288 \quad \text{distance} = RF \times 100 = 28.8 \text{ cM}$$

for *str* and *pi* (recombinants in blue):

$\sigma^7$ gametes	$\rho$ gametes	progeny phenotype	#progeny
<i>ju str pi</i>	<i>ju + +</i>	javelin	294
<i>ju str pi</i>	<i>+ str pi</i>	striped, pink	287
<i>ju str pi</i>	<i>ju str pi</i>	javelin, striped, pink	140
<i>ju str pi</i>	<i>+ + +</i>	wild type	139
<i>ju str pi</i>	<i>+ + pi</i>	pink	66
<i>ju str pi</i>	<i>ju str +</i>	javelin, striped	65
<i>ju str pi</i>	<i>ju + pi</i>	javelin, pink	4
<i>ju str pi</i>	<i>+ str +</i>	striped	5

$$RF_{str, pi} = (66 + 65 + 4 + 5)/1000 = 0.14 \quad \text{distance} = RF \times 100 = 14 \text{ cM}$$

Assembling the map. On the basis of the distances, *ju* and *str* must be the outer genes, with *pi* in the middle. Because we do not map to a chromosome landmark in *Drosophila*, the order *ju-pi-str* is equivalent to the order *str-pi-ju*. So if we write the genotype of our heterozygous female parent, it is *ju +/+ pi str*. However, when you look at the numbers, there is a problem. Intuitively, you expect that if you add the *ju-pi* distance and the *pi-str* distance you will get the *ju-str* distance. Instead the number is larger—why? The RF value is based on the number of crossover events. When we calculate the distance for *ju-pi* or *pi-str* we count a single crossover with each recombinant offspring. But between *ju* and *str*, we have the possibility of two crossover events. The double crossover class will be rarer than the single crossover class, but we can identify them by looking at the numbers. Of the eight types of testcross offspring, two (*ju pi +/ju pi str* and *+ + str/ju pi str*) are much less common than the others. These are the double crossover events. They appear “parental” for the *ju* and *str* loci (compare to the parental genotypes), but they are also recombinant. When we add twice the number of these classes to the *ju-str* distance, we get 42.8 cM, the sum of the other two distances.

We can also determine the gene order when all three genes are linked by comparing the alleles on the parental and double crossover chromosomes.

Parental	Double crossover
<i>ju + +</i>	<i>ju + pi</i>
<i>+ str pi</i>	<i>+ str +</i>

The locus that changes which allele is present is *pi*, so the order is *ju-pi-str*.