

# A three point test cross in drosophila: recombination and linkage



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## Abstract

A three-point testcross was performed with *Drosophila* to distinguish the location and relationship of three genes (*y cv f*) on the X chromosome. The parental is a virgin female triply mutant and a wild-type male. This testcross includes mating a trihybrid wild-type F1 female to a mutant F1 male. One hundred F2 offspring were then scored and 8 different phenotypes were observed and assigned reciprocal classes. The order of the three genes was then determined to be (*y cv f*). Recombination frequencies for crossovers in both region 1 and region 2 were calculated for three data sets. A Chi Square examination utilizing the real data (published recombination frequencies) was then directed to decide the exactness of the test recombination frequencies for crossovers in region 1, 2, and the reciprocal crosses for all the data set collections. A linkage map was drawn from the recombination frequencies for region 1 and 2. A linkage map recombination rates to delineate the physical distance between genes on a chromosome. The linkage map related reasonably with the recombination frequencies, anyway there were inconsistencies with the frequency of single crossovers.

## Introduction

Gene mapping is very significant in genetics because of several reasons. Location of the gene provides information about its function, structure, and location. Gene mapping can determine the gene is transmitted from maternal or paternal side. It can also determine how many genes caused an illness transmitted from a parent to kid by using recombination percentage.

Gene mapping in particular gives the location of the gene and it is important to make complex DNA sequences and genomes.

During the Prophase 1 the crossing over takes place between the tetrads. The probability of crossing over increases in the genes that are farther apart. Linkage happens when at least two or more genes are found close-by one another on a same chromosome. In this situation, the genes don't assort independently and they inherited together, as a result, the ratio is different than Mendelian Ratios. Recombination frequency (Rf) is the rate for the number of recombinants out of the total number of progeny in one region. A gene map evaluates the physical distance between two gene loci utilizing information from the measure of recombination. Recombination Frequency can't larger than 50 % and linked genes are less than 50%.

It is beneficial to map three genes at once rather than mapping each gene separately. Mapping three genes at once, gives information about the order and the distance between the genes. This enables understudies to distinguish genes on a gene map despite the fact that it isn't sure whether the sequence is perused from appropriate to left or the other way around. Mapping each pair of gene doesn't give accurate information about the distance and the order of the genes.

A standout amongst the most imperative life forms that have been utilized to ponder hereditary qualities for quite a few years is *Drosophila melanogaster*, additionally called the " Fruit Fly," known to immediately emerge within the sight of matured organic product. They are exceptionally helpful for hereditary investigation since they breed effectively, they have short life

expectancies and generation periods, and they're anything but difficult to keep up and control with respect to sustenance and temperature. Likewise, natural product flies are little enough to gather extensive populaces yet sufficiently expansive to recognize wild compose attributes from mutant characteristics.

The Hypothesis for this experiment is RF measured in lab will be similar to the expected RF based on known map distances. Reciprocal classes will occur and survive in equal numbers. Interference will be a positive value.

#### Methods:

The Experiment started with the cross of two fly: wild type male (+++) and completely mutant virgin females (wfm). The pale coloring, the folded wings, the enlarged abdomens can use to distinguish virgin females, and they have a meconium. Using virgin females in all crosses is very important. Female drosophila can just mate with one male, and they at that point store that male's sperm for the rest of their adult life. In this manner, with a specific end goal to control mating, females must be separated not long after they eclose from their pupa casing, while despite everything they show the highlights recorded above, and have an ensured virgin status. The parental flies were removed and the F1 generation is ready to be crossed. The second cross involves mating 4 heterozygous wild type females to 6 F 1 mutant males. After one week students will score the F2 generation for each of the three traits. This cross is performed to decide if the genes assort independently. If the genes assort independently the frequency for all the phenotypes would be 12.5% in the F generation. If the parental phenotypes

are shown most of the time, or far more prominent than the normal 12.5% (i. e. no requirement for chi squared examination), at that point it can be assumed that genes are linked. Since linked genes are on a same chromosome, it shows that the genes are inherited together most of the time, and it clarifies the absence of independent assortment. Also, the other phenotypes are the result of crossing overs during meiosis. The following phenotypes and Genotypes were observed for F2 progeny: Grey color, cross vein, straight bristles (+++), yellow color, crossveinless, forked bristles (ycvf), Grey, crossveinless, forked bristles (+cvf), yellow, crossvein, straight bristles (y++), Grey, crossvein, and forked bristles(++f), yellow color, crossveinless, straight bristles (ycv+), Grey color, crossveinless, straight bristles(+cv+), and yellow color, crossvein, forked bristles.(y+f). The parental phenotypes which are the non-recombinants are the most numerous in a three-point test cross. Double crossover classes in F2 are usually the least frequent classes of progeny. To conclude which gene is the middle of the three genes, a comparison between non-recombinants and double crossover classes is made. Non-recombinants have parental genotypes +++, and ycvf and double cross over classes has the following genotypes +cv+, y+f. By comparing these two classes you can distinguish the order of gene in middle because in double cross overs the middle gene gets inverted from its initial position in the parental chromosome. Single crossover classes in region 1 had recombinant genotypes differing at the first gene : +cvf, y++. Single cross over classes in region two had recombinant genotypes differing at the third gene : ++f, ycv+.

Three different traits were recorded for the F2 Drosophila. Y= yellow color body. The wings exhibit a yellow glint in this mutant due to lack of dark melanin pigments. The wild flies are grey color and express melanin pigments.

Cv= crossveinless. The mutant is lacking crossveins in the wing, whereas the wild fly has prominent veins. f= forked bristles. The mutants have shorter bristles with bent segments on the head and thorax regions. Wild flies have longer, straight bristles on their head and thorax regions.

It is unnecessary to record the sex of the F2 since within each of the 8 F2 progeny classes, both genders are phenotypically the same. The F1 males are all mutants because they cannot undergo crossing over because in males the X chromosome can't make a homolog with the Y chromosome. On the other hand, these three genes are located on the X-chromosome not Y so F1 males are hemizygous.

Recombination Frequency is calculated by the division of the sum of all classes of progeny resulting from crossover in one region by the total number of progeny. One percent recombination is the equivalent to 1 map unit. A linkage map uses the recombination frequencies to determine distance between two gene locations. Each crossover has effect on another crossover and they interfere with each other.

C. O. C is calculated by the sum of the observed double cross overs divided by expected number of double crossovers. An interference of value 1 means there wasn't any crossovers and the interference was complete. The value of 0 means the observed number of crossovers is equal to the number of

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expected crossovers. The value of interference between 0 and 1 means some interference happened.

There are 18 different Chi Square calculated for 6 data sets.

1. Chi Square for crossovers in region 1: expected vs observed
2. Chi Square for crossovers in region 2: expected vs observed
3. Chi Square for parental reciprocal cross similarity: expected vs observed
4. Chi Square for single crossovers in region 1 reciprocal cross similarity: expected vs observed
5. Chi Square for single crossovers in region 2 reciprocal cross similarity: expected vs observed
6. Double crossovers reciprocal cross similarity: expected vs observed.

Results:

Actual y \_\_\_\_\_ 13.7 \_\_\_\_\_ cv \_\_\_\_\_ 43 \_\_\_\_\_ f

Small data y \_\_\_\_\_ 16.07 \_\_\_\_\_ cv \_\_\_\_\_ 17.85 \_\_\_\_\_ f

Class data y \_\_\_\_\_ 16.4 \_\_\_\_\_ cv \_\_\_\_\_ 32.83 \_\_\_\_\_ f

Expert data y \_\_\_\_\_ 15.4 \_\_\_\_\_ cv \_\_\_\_\_ 30 \_\_\_\_\_ f

Four linkage maps are shown. The three data sets have different  $R_f$  values between the three genes (y cv f) when compared to published data. The

published RF for distance between y-cv is = 13.7cM. The published Rf for the distance between genes cv-f is = 43cM.

Classes	Genotype	Expert Data	Small data	Class data
Non-recombinant (Parental)	+++	420	35	249
Non-recombinant (Parental)	ycvf	326	4	134
Single cross over in Region 1	y++	70	3	28
Single cross over in Region 1	+cvf	63	4	35
Single cross over in Region 2	ycv+	162	3	83
Single cross over in	++f	161	5	89



Region 2

Double Cross  
Overs      y+f      33      0      23

Double Cross  
Overs      +cv+      29      2      23

total      1264      56      664

Interferen  
ce(l)      0.      0.      0.  
04465      24505      2866  
51      3      9

Calculation:

$$RF = (70+63+33+95)/(1264) = 0.154 * 100 = 15.4\%$$

$$C. O. C = (33+29)/(1264*0.154*0.3045) = 1.0446551$$

$$Interference = 1 - c. o. c = 1 - 1.0446551 = 0.0446551$$

0.      0.      4.  
cross overs in region      294456      26636      14179  
one : expected vs      2      5      3  
observed      P > 0.      P > 0.      P < 0.  
05      05      05

cross overs in region      80.      16.      28.

	73505	56805	0126
two: expected vs			
observed	P < 0. 05	P < 0. 05	P < 0. 05
parental reciprocal		24.	
cross similarity:	11. 84	346	34
expected vs	P < 0. 05	P < 0. 05	P < 0. 05
observed			
single cross over in		0.	
region 1 reciprocal	0. 368	1428	0. 77
cross similarity:	P > 0. 05	P > 0. 05	P > 0. 05
expected vs			
observed			
single cross over in		0.	
region 2 reciprocal	00315	0. 5	0. 209
cross similarity:	P > 0. 05	P > 0. 05	P > 0. 05
expected vs			
observed			
Double cross		2	0
reciprocal cross			
similarity: expected	P > 0. 05	P > 0. 05	P > 0. 05
vs observed			

This table includes the Calculated 18 Chi Square tests for the three-point testcross experiment. Interference and c. o. c are also calculated for each data set.

Discussion:

Usually, traits on non-homologous chromosomes are inherited independently of each

other's locations. However, traits that are linked on homologous chromosomes are often inherited together unless crossing over between the homologous chromosomes occurs. (Klug et. al. 2012) The hypothesis for this experiment was RF measured in lab will be similar to the expected RF based on known map distances. Reciprocal classes will occur and survive in equal numbers. Interference will be a positive value. Based on the results from the experiment the hypothesis is wrong and rejected.

For the parental reciprocal cross there is a similarity in all three data sets and the  $p < 0.05$ . From the table 2, reciprocal classes resulting from single crossovers in region 1 the  $X^2$  is similar and they all have  $p > 0.05$ . For the single crossovers in region 2, the data shows the similar  $X^2$  and the p value is less than 0.05. For the double cross overs the  $X^2$  is different but still they have p value greater than 0.05. For the class data set  $X^2 = 4.14$  but for small data and the expert data the  $X^2$  is similar and the p value is greater than 0.05. For single cross overs in region 2 reciprocal cross similarity the class data set and small data set are similar but the expert data set is different. Reciprocal classes from single cross overs in region 2 and double

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cross overs are selected against and do not survive in equal numbers.

Based on the prediction the reciprocal class non-recombinants had the most frequencies, while the double cross overs occurred the least. Single cross overs in region 1 and region 2 occur in similar numbers for small data and expert data but for class data the frequency is less and it is almost equal to double cross overs.

The data shows the frequency for crossovers in region one is less than region two and it is because the Rf for region one is less than the Rf in region 2. The measurements for Rf for region one is similar in all three data sets but the calculated Rf value in region is vary for all three data. For the expert data set, all but the reciprocal parental cross for reciprocal classes occurred and survived in equal numbers. For the expert data set, interference had a positive value. For the small data set, recombination frequency for crossovers in region 1 measured in lab was similar to the expert and class data but they are different than actual Rf value. For the small data set Interference is a positive value.

For the class data set the Rf measured in lab was not similar to the expected Rf but it is similar to small data and expert data.. This occurrence does not occur by chance, it is likely due to miscoding of the flies. Missing double crossovers that appear to be similar to non-recombinants may have also caused the error.

It is difficult to correctly measure long map distances by recombination frequency because of recombination. If two genes have a small recombination frequency, there will be less chance for recombination to

occur. For more accurate measurements of long map distances two genes must be located farther apart from each other on the same chromosome. When two genes located further away from each other, there is higher chance for recombination to occur.

There are some errors could happened during the experiment. First by anesthetized the flies two times, some of the flies could have died. By putting the flies in the jar, some of them might wedge in the food and died. This mistake reduced the sample size and made it difficult to score phenotypes of some of the flies covered with food. The higher the number of the flies causes the percentage error to decrease. RF measured in lab will be similar to the expected RF based on known

map distances. The Rf value was not similar for all the three data sets. The first part of hypothesis is rejected. Reciprocal classes will occur and survive in equal numbers, is true for all three data sets. The interference is positive for all three data sets.

Conclusion:

The data shows that a three-point testcross can be used to locate the positions of three linked genes on the X chromosome. The Chi square analysis is significant to show the occurrence of recombination in different regions. Experimental data such as recombination frequencies can then be used to map out the physical distance between these genes on the same chromosome. Statistical analysis of Chi-square and map gene can determine the ratio of the progeny, the percent of the crossovers.

Reference:

- Aggarwal, D. D., Rashkovetsky, E., Michalak, P., Cohen, I., Ronin, Y., Zhou, D., ... Korol, A. B. (2015). Experimental evolution of recombination and crossover interference in *Drosophila* caused by directional selection for stress-related traits. *BMC Biology* , 13 , 101. doi: 10. 1186/s12915-015-0206-5