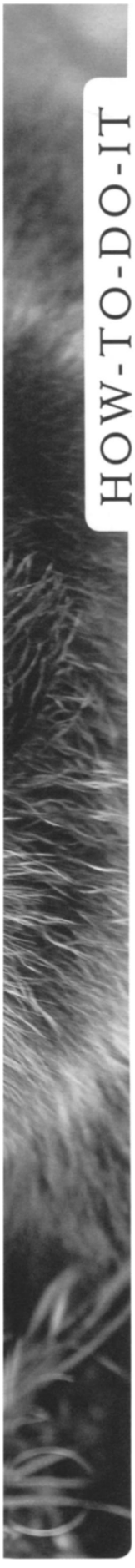

HOW-TO-DO-IT

Mapping Linked Genes in *Drosophila melanogaster* Using Data from the F2 Generation of a Dihybrid Cross

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Drosophila melanogaster is a commonly utilized organism for testing hypotheses about inheritance of traits. There is a wide variety of mutants of *Drosophila* that demonstrate effectively both dominant and recessive traits, as well as autosomal and X-linked inheritance (Flagg, 2005; Winchester & Wejksnora, 1996). Students in both high school and university labs study the genetics of inheritance by analyzing offspring of appropriate *Drosophila* crosses to determine inheritance patterns, including gene linkage. However, most genetics investigations with *Drosophila* analyze offspring patterns of the F2 generation of dihybrid crosses to determine that genes are linked but do not calculate the map units between the linked genes (College Board, 2001; Mertens & Hammersmith, 2007; Scott, 2001).

Calculating map units between linked genes is most straightforward when testcross data is used (Brooker, 2005; Russell, 2006). However, setting up a testcross is not trivial. Constructing a testcross in *Drosophila* requires obtaining an F1 virgin female fly to mate with a homozygous recessive male fly in order to produce the subsequent generation for analysis of traits. In a teaching lab setting in which there are severe constraints on lab time, students have great difficulty in obtaining virgin F1 females to set up the testcross to generate data for mapping. This article describes how to use F2 data generated from an F1 sibmate cross to determine map distances in linked genes.

Lab Method & Data Analysis

Drosophila crosses with virgin parentals are either set up by the instructor (Flagg, 2005) or are ordered from a commercial supplier (Carolina Biological Supply, Burlington, NC or Ward's Natural Science, Rochester, NY). These crosses can be set up either as a coupling cross or as a repulsion cross. The coupling cross is performed by crossing a wild-type fly to one that has both mutant phenotypes in the homozygous form. An example would be crossing a wild type fly to one with sepia eyes and ebony body. The repulsion cross is performed by crossing one homozygous mutant with another. An example of this would be crossing a fly with sepia eyes to one with an ebony body. After one week, the parentals are removed and the larvae are allowed to hatch into the F1 generation.

Students analyze the F1 generation of the appropriate *Drosophila* cross to determine which traits are dominant and which are recessive. The F1 generation of this *Drosophila* cross is then allowed to sibmate in a new vial of *Drosophila* media.

After one week, the F1 generation is removed from the vial and the larvae are allowed to remain. After an additional week, the F2 generation is removed, anesthetized or immobilized, and sorted according to sex and phenotype (College Board, 2001; Flagg, 2005; Mertens & Hammersmith, 2007; Scott, 2001; Winchester & Wejksnora, 1996). Once data are obtained, students use chi-square analysis (College Board, 2001; Brooker, 2005; Russell, 2006) to determine if the genes are linked or independently assorting. They hypothesize that the F2 generation should produce a 9:3:3:1 ratio and use chi-square analysis to determine if the offspring fit the hypothesized ratio (College Board, 2001; Brooker, 2005; Russell, 2006).

To make this exercise more investigative, students can develop hypotheses based upon their knowledge about the chromosomal theory of inheritance, gene linkage, and crossing over (Brooker, 2005) before they count the F2 generation. Once students understand coupling and repulsion crosses, they can predict the outcomes of the F2 generations in the coupling and repulsion crosses for linked genes, if no crossing over has occurred. Students should be able to determine that in a coupling cross, the F2 should produce no offspring demonstrating a single recessive trait unless crossing over occurred. Further investigation should allow the students to hypothesize that in the repulsion cross, the double recessive offspring should not be seen in the F2 unless recombination has occurred.

If the data does not fit the 9:3:3:1 ratio, students can analyze their results. Using their predictions outlined above (that in the coupling cross when no crossing over occurs, there should be no F1 offspring demonstrating single recessive phenotypes; and when no recombination occurs in the in the repulsion cross, there should be no offspring displaying both recessive phenotypes), students should be able to determine if the original cross was a coupling or repulsion cross from examining their data.

If the genes are linked, students can also map their data, using the following equations and lookup chart.

A is the number of flies with both dominant phenotypes.

B is the number of flies with one homozygous recessive phenotype.

C is the number of flies with the other homozygous recessive phenotype.

D is the number of flies with both homozygous recessive phenotypes.

For the coupling cross, the equation is: $Z = \frac{B*C}{A*D}$

For the repulsion cross, the equation is: $Z = \frac{A*D}{B*C}$

Using Table 1, students determine the approximate map distance between their two linked genes.

The theory behind this method is that in both the coupling cross and the repulsion cross, certain F2 offspring should not be observed, unless crossing over occurs (Griffiths et al., 2000; Immer, 1930; Macguire, 2005). If no recombination occurs in the coupling cross, no F2 offspring should

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be seen that have a single recessive trait. If no recombination occurs in the repulsion cross, no F2 offspring should be seen that have both recessive phenotypes. However, in linked genes, crossing over does occur. The more crossing over that occurs, the further apart two genes lie from one another (Brooker, 2005). Thus the more F2 offspring in the coupling or repulsion cross that are derived from recombination (in the coupling cross, offspring with a single recessive trait; in the repulsion cross, offspring with both recessive traits), the further apart the linked genes are. The two equations compute a Z value that is a value of the percentage of recombinant offspring. The higher the Z value, the more recombinant offspring, and the further apart the linked genes are. This Z value is used with a lookup table to extrapolate a map distance between two genes (Griffiths et al., 2000; Immer, 1930; Macguire, 2005).

Student Example Using Pooled Class Data

The original cross was: P generation: wild-type X sepia eyes, ebony body.

This is a coupling cross. The F1 generation phenotype was all wild-type. Therefore both wild-type traits (eye color and body color) are dominant.

The F2 generation from the F1 sibmate had four phenotypes:

Wild-type eyes, wild-type body color . . 569

Wild-type eyes, ebony body color 80

Sepia eyes, wild-type body color 63

Sepia eyes, and ebony body color 127

Chi-square analysis indicated that these genes are linked, as the chi-square value for the F2 generation (hypothesized ratio of 9:3:3:1) is greater than 7.815. Thus the hypothesis of independent assortment was rejected (Brooker, 2005).

A is wild-type eyes, wild-type body color.

B is wild-type eyes, ebony body color.

C is sepia eyes, wild-type body color.

D is sepia eyes, ebony body color.

The equation is $Z = \frac{B*C}{A*D} = \frac{80 * 63}{569 * 127} = 0.069745$

Using Table 1, the value is between 19 and 20 map units. In order to determine if this method is appropriate and approximates the results obtained when a testcross is carried out, the class also performed a testcross on the F1 generated from the same starting cross.

The testcross data for this lab was as follows:

Wild-type eyes, wild-type body color 107

Wild-type eyes, ebony body color. 25

Sepia eyes, wild-type body color 35

Sepia eyes, and ebony body color. 100

Map units = $\frac{\text{recombinants}}{\text{Total}} \times 100 = \frac{25 + 35}{267} \times 100 = 22.5$ map units

Map Units	Coupling Cross Z Value	Repulsion Cross Z Value	Map Units	Coupling Cross Z Value	Repulsion Cross Z Value
1	0.0001356	0.00020005	31	0.2328	0.2465
2	0.0005516	0.0008008	32	0.2538	0.2672
3	0.001262	0.001804	33	0.2763	0.2899
4	0.002283	0.003213	34	0.3002	0.3127
5	0.003629	0.005031	35	0.3259	0.3377
6	0.005318	0.007265	36	0.3532	0.3643
7	0.007366	0.009921	37	0.3823	0.3927
8	0.009793	0.01301	38	0.4135	0.4230
9	0.01262	0.01653	39	0.4467	0.4553
10	0.01586	0.02051	40	0.4821	0.4898
11	0.01954	0.02495	41	0.5199	0.5266
12	0.02375	0.02986	42	0.5603	0.5660
13	0.02832	0.03527	43	0.6034	0.6081
14	0.03347	0.04118	44	0.6494	0.6531
15	0.03915	0.04763	45	0.6985	0.7013
16	0.04540	0.05462	46	0.7510	0.7529
17	0.05240	0.06218	47	0.8071	0.8082
18	0.05972	0.07033	48	0.8671	0.8676
19	0.06787	0.07911	49	0.9313	0.9314
20	0.07670	0.08854	50	1.0000	1.0000
21	0.08628	0.09865	51	1.0736	1.0738
22	0.09663	0.1095	52	1.1526	1.1533
23	0.1078	0.1211	53	1.2373	1.2390
24	0.1198	0.1334	54	1.3282	1.3316
25	0.1328	0.1467	55	1.4260	1.4317
26	0.1467	0.1608	56	1.5312	1.5400
27	0.1616	0.1758	57	1.6446	1.6574
28	0.1777	0.1919	58	1.7668	1.7848
29	0.1948	0.2089	59	1.8989	1.9234
30	0.2132	0.2271	60	2.0417	2.0742

Table 1. Table for determining map distance between two linked genes in a dihybrid cross. Determine whether the cross is a coupling or a repulsion cross and perform the mathematics to calculate the Z value. Use the table to lookup the Z value, and then determine the map units between linked genes. Modified from Table 2, Immer (1930) and McGuire (2005).

Although both of these values are low as compared to the published value of the map units between the two genes (Brooker, 2005; Flagg, 2005), these values are in reasonable agreement. This method of calculating map distance between linked genes using F2 data is an effective method of generating mapping data for students to use in lab.

Discussion & Conclusion

I have used this calculation method successfully in a sophomore-level Fundamentals of Genetics Lab when the students were not able to generate testcross data. The calculations are straight-forward and the lookup table (Table 1) is easy to read. The most difficult element

for the students is to determine whether the cross is a coupling cross or a repulsion cross, so we discuss this as a group in lab before they analyze their F2 data. Additional confusion can occur when the students are trying to determine the variables for the equation (A, B, C, or D). We also discuss this in lab as a group so that the students are clear on which numbers to use in the formula.

This method calculates map distances between two linked genes using data generated from the F2 generation of an F1 sibmate cross. Students routinely generate and analyze for gene linkage the F2 generation of *Drosophila* dihybrid crosses using chi-square analysis but do not map the genes if they are determined to be linked (College Board, 2001; Mertens & Hammersmith, 2007; Scott, 2001). Laboratory exercises in high school, college general biology, and genetics routinely mate *Drosophila* to study the genetics of inheritance. The procedure described in this article will permit students to more comprehensively analyze the F2 data they generate without adding any additional expense and will add only minimal instruction time in lab or class. This method can easily be implemented in any *Drosophila* inheritance lab in which F2 data is generated from crosses performed on linked genes. Although I describe our specific class example in which we use this method with the F2 data from a dihybrid *Drosophila* cross, this method could easily be adjusted for analysis of a *Drosophila* trihybrid cross as well, mapping each pair of genes individually (Brooker, 2005).

As indicated with our data, map units generated in the teaching lab are often lower than those on the published maps. As an extension of this exercise, students could speculate as to why the map distance calculated in lab did not agree with the published map distance. Topics they might consider include: experimenter error (for example, the ebony body takes time to develop and newly-emerged flies with ebony bodies can be mistaken for wild-type body color; red-green color blind students cannot differentiate wild-type from sepia eyes), small sample size, double and triple crossovers, and mutant strains that are less robust than wild-type (in our hands, vestigial flies are less robust than wild-type).

Utilizing the procedure outlined in this article should increase students' interest in their *Drosophila* inheritance lab. Students will be able not only to determine that two genes are linked to each other using F2 data and chi-square analysis, but also to calculate the map units between linked genes using their F2 data. Inclusion of this procedure in a *Drosophila* lab will provide additional opportunities for students to utilize mathematics to analyze their data and to formulate additional conclusions from their crosses about the distance between linked genes.

Acknowledgments

I thank the reviewers for their helpful comments that strengthened this manuscript.

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