Recombination Rate and Phenotype Ratios Involving Bar-Eyed and Black Body Mutants of *Drosophila melanogaster* in Two Crosses

Alethea Lamb, Yeting Li, & Jonathan Zhu

Abstract

Past research demonstrates a chromosomal linkage between a body color gene and a microsatellite marker in the fruit fly *Drosophila melanogaster*. To confirm this link and calculate the distance between these gene loci, we conducted crosses, isolated DNA, and conducted PCR and gel electrophoresis. Based on the results of this experiment, we show that the distance between the loci for the black body gene and microsatellite molecular marker is 7.9 map units; the sheer amount of data collected provides evidence that the observed genotypes were not due to random chance alone.

Introduction

The fruit fly *Drosophila melanogaster* is a common model organism used in the study of genetics. Its many mutant varieties, short reproductive cycle, and ease of care make it ideal for laboratory usage (Klug et al. 2019; Scott 2008). In this experiment, we studied the *bar* sex-linked trait to make the eyes bar-shaped in addition to the *black* autosomal trait to make the fly's body black. The *black* trait is linked on the same chromosome with a microsatellite marker, so the purpose of this experiment was to illustrate said linkage between the black-body gene and the genetic marker, and to determine the approximate distance between the two loci. The distance between two loci can be determined by looking at the frequency of crossing over events (Klug et al. 2019) as described by:

$$\frac{\#recombinant\ flies}{\#total\ flies} \times 100 = distance\ between\ loci$$

The discovery of this phenomenon is credited to Morgan and Sturtevant in the early 1900s (Klug et al. 2019). Genetic markers can be created in a variety of ways including nucleic acid probes and polymorphisms (Scott 2008). In this experiment we utilized a microsatellite sequence based on polymorphisms present in the DNA sequence that varies in length (Scott 2008). Reciprocal crosses of bar-eyed and black bodied flies were set up amongst the different lab groups which allowed the inheritance pattern of the bar-eyed trait to be determined. It was expected that if the bar-eyed trait was a sex-linked trait with incomplete dominance, our F1 progeny would show all females with kidney bean-shaped eyes and all males with wild-type eyes because bar eyed males were crossed with black bodied females.

The second cross setup involved virgin females from the F1 generation and black bodied males from a maintenance cross. The purpose of this second cross was to determine if the black body trait followed an autosomal recessive inheritance pattern as expected as well as determining if the long and short molecular markers were indeed linked to the wild type and black body phenotypes respectively Our F2 progeny that exhibited the black body trait were then frozen for later PCR testing.

In order to conduct these tests on the F2 progeny, DNA extraction, PCR amplification, and gel electrophoresis were all important steps. PCR in particular is required to amplify the microsatellite markers for observation. The PCR step involved primers created by Michalakis and Venille in their study of glutamine codon tandem repeats (1996). The key aspect of these primers is that they search for length variations in CAG/CAA trinucleotide repeats (Michalakis and Venille 1996). The PCR step was followed by running gel electrophoresis in order to observe

the microsatellite markers. In this experiment, the marker is the Bib gene that is linked to the black body locus (Michalakis and Venille 1996). By observing the gel, the rate of crossing over can be used to determine the approximate distance between the microsatellite marker and the gene controlling body color. The null hypothesis is that there is no linkage between the two genes and any observed difference is due to random chance alone.

Methods

Setting Up Testcross

Two true breeding strains of *Drosophila melanogaster* were sourced from Carolina Biological Supplies for this experiment: black body with wild type eye shape (bb, X^+X^+) and wild type body with the bar-eye shape (++, X^BY). Fly cultures were set up in glass bottles using a mixture of 25 ml of dried fly food and 25ml of water combined with a mold inhibitor. These bottles were capped using a sponge that ensured no flies could escape but allowed for air flow in and out of the bottle. Once flies were placed inside, the cultures were stored at 21ºC.

The P1 cross was set up using virgin females who were homozygous for black body and wild-type eye traits (bb, X^+X^+) and ten males who were homozygous for wild type body color and bar shaped eyes (++, X^BY). An additional maintenance cross was set up using ten black bodied wild-type-eye males (bb, X^+Y) and ten black bodied wild-type-eye females (bb, X^+X^+). To avoid confusion and accidental breeding, the P1 generation was removed before the eclosure of F1 progeny, at the appearance of pupae. From the F1 progeny, twenty females with wild type body color and kidney bean shaped eyes (+b, X^+X^B) were collected and cultured separately. The phenotypes and sex of all the F1 progeny were recorded.

To set up the test cross, the twenty F1 females were divided into two groups of ten and placed in separate cultures. Ten homozygous black bodied wild type eye males (bb, $X^{+}Y$) were then added to each of those cultures from the maintenance cross. In total, this created two replicate cultures. As with the P1 cross, F1 flies were removed once pupae appeared in order to prevent contamination. From the F2 progeny 45 black bodied flies were collected. Their eye shape, sex, body color, and culture vial (A or B) were recorded. To prepare for DNA extraction and analysis, these flies were then frozen in individual tubes and stored.

Phenotyping the Progeny

Two mutant varieties were studied in this experiment: black body color and bar eye shape. The phenotypes were observed by placing the flies under a dissecting microscope. The wild-type body color is brown in the abdomen part, whereas the black body type is dark brown. There are three categories of eye shapes: wild type, kidney shape, and bar shape. The eye shape is determined by the size of the eye. Sex was determined by looking for the presence of the genitile arch (male), vaginal plate (female), sex combs (male), looking at the size and number of striped on the abdomen, and looking to see if the abdomen is rounded (male) or elongated (female) (Scott 2008).

DNA Extraction and Analysis

To extract DNA, the frozen flies were each combined with 50µl of homogenization buffer containing 10 mM Tris-HCl pH 8.2, 1 mM EDTA, 25 mM NaCl, and 200µg/ml Proteinase K (Dr. Rodney Scott, personal communication) and were ground with plastic pestles that were sprayed with 70% ethanol between uses. These samples were then heated to 30ºC for 25 minutes

to activate the Proteinase K and then 95ºC for one minute to deactivate the Proteinase K before being centrifuged for 90 seconds. 2µl of supernatant from each fly sample were placed into individual PCR tubes before freezing in preparation for the next step.

PCR and Gel Electrophoresis

PCRs were carried out in 25µl of a mixture containing 2µl of extracted fly DNA, 10 mM Tris-HCl pH 8.3, 0.75 units of *Taq* polymerase, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix. The samples then underwent PCR as described in Michalakis and Veuville (1996). After denaturation for 5 min at 92ºC, samples were processed through 35 cycles consisting of 1 minute at 92ºC, 1 minute at 60ºC, and 30 seconds at 72ºC. After denaturation, the sample underwent elongation for 3 min at 72ºC. 1.5 µl of each sample was run on a 3% agarose gel consisting of 30ml 0.5X TBE buffer and 1.5µl ethidium bromide, using GeneRuler 50 bp DNA ladder as a molecular size marker. Samples were loaded into the gel alongside a ladder before being run at 130V for 35 minutes. The gels were then viewed under a specialized BIO-RAD viewing machine to identify the banding patterns of each sample.

Genotyping the Progeny

The genotype of each fly was determined by examining the banding pattern on the gel under the BIO-RAD viewing machine. The small microsatellite was seen as a line between the 100 and 150 bp markers on the ladder while the long microsatellite was seen as a line between 150 and 200 bp on the ladder. Heterozygotes exhibited two lines, one at each location. Flies that were black bodied parental types were expected to exhibit a single line between 100 and 150 bp

while black bodied recombinants were expected to exhibit two lines on the gel, one for each marker. The genotypes of the 45 flies were observed and recorded.

Results

Test cross body color phenotypes compared to their marker genotype are shown in Table 1; such results were derived from gels such as the one shown in Figure 1. Our individual gel electrophoresis results showed no crossing over, suggesting the gene loci for body color and the molecular marker are very close together. However, when utilizing the entire lab's dataset, we were able to calculate a more accurate measurement of recombination rate. The genotype of 101 flies was observed and collected based on the results of crosses of three groups within the lab. Based on the gel electrophoresis, there were 89 black body flies with S/S marker, 5 black body flies with L/S marker, 3 wild type flies with S/S marker and 4 wild type flies with L/S marker. Black body with L/S marker and wild type body with S/S marker were the result of crossover, whereas black body with S/S marker and wild type body with L/S marker were parental genotypes. The map units between the locus for the black gene and the locus for the microsatellite molecular marker was 7.9 map units, corresponding to a recombination rate 0.079 (7.9%). The null hypothesis failed to be rejected meaning that the observed distribution is not due to random chance.

TABLE 1

The Distribution of F1 Cross Genotype Based On Gel Electrophoresis

	Black Body	Wild Type Body

L/L	0	0
L/S	5	4
S/S	89	3

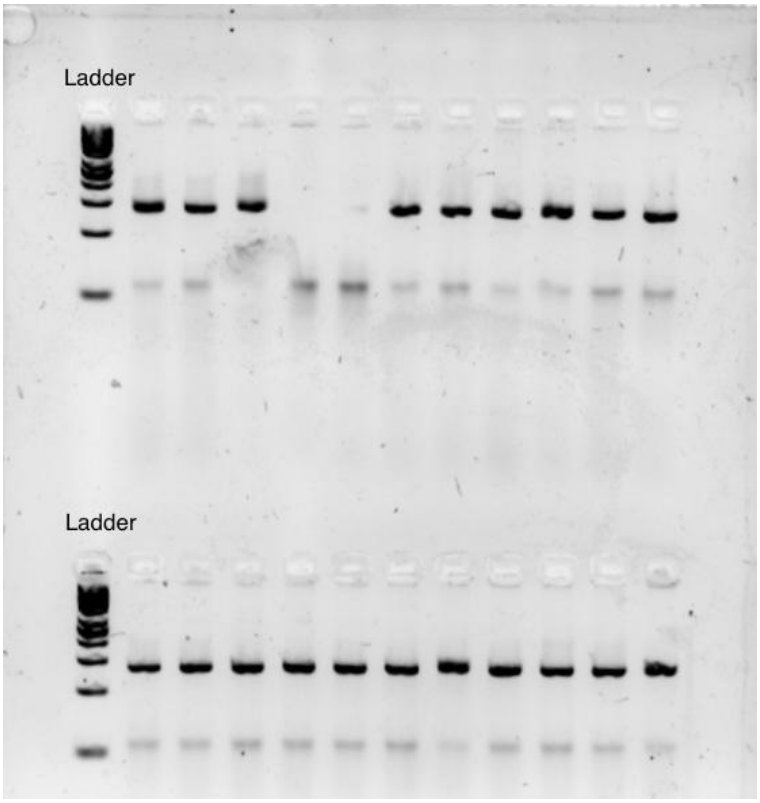

Figure 1. Example of Gel Electrophoresis Results under UV light

Discussion

Based on a low calculated recombination rate, our results indicate a short distance between the loci of the body color gene and the marker. Data pooled from other crosses conducted by other lab sections further supports this conclusion. Despite this relatively strong conclusion based on many data points, we identified one main source of error, that being the

possibility that some fly crosses were conducted incorrectly. While the fly holding vials were inspected almost daily, it was impossible to guarantee the virginity of female flies, which would lead to contamination from unintended flies and a misconducted cross. We attempted to account for this by removing data with unexpected results, but it is possible that other contaminants went unnoticed.

This experiment demonstrates the necessity of a large data set in order to fully examine recombination rates as our individual data set of 45 showed no recombination. Had we used our data alone, we would have calculated a distance of zero map units, which would imply that the loci are the same; past research has shown that this was not the case.

Despite the difficulties presented in conducting this experiment, the procedure is proof of concept that the two-generation crossing, PCR, and gel electrophoresis works to demonstrate the process of confirming gene linkage and calculating map units between gene loci. As such, future experiments could be useful in future studies examining the linkage of other traits in *Drosophila*. After demonstrating the linkage between the black body locus and the microsatellite marker it would be interesting to see if there are any other genes linked to this marker and the respective locus, or if there are genes linked to one but not the other.

References Cited

Klug, W.S., Cummings, M.R., Spencer, C.A., Palladino M.A., & Killian, D.J. (2019). *Concepts of Genetics*. Pearson.

Michalakis, Y. & Veuille M. (1996). Length Variation of CAG/CAA Trinucleotide Repeats in Natural Populations of *Drosophila melanogaster* and Its Relation to the Recombination Rate. *Genetics Society of America*, pages 1713-1725.

Scott, R.J. (2008). *Contemporary Genetics Laboratory Manual*. Bent Tree Press.