Heritability of the Bar-Eye and Ebony-Body Mutations in *Drosophila melanogaster*

FIGURE 1: An upshot of a *D. melanogaster* female (wild-type)

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■ Abstract

The fruit fly, *Drosophila melanogaster*, has been classified among the top model organisms for over a century because of a number of favorable features that allow for ease in experimental design and offer a range of applications to many different areas of research within genetics. These flies are readily available, inexpensive to maintain in a laboratory setting, small in size, they have short life cycles and reproduce rapidly with an abundance of offspring, they have a relatively small genome with ~50% being homologues to the human genome, and perhaps most importantly, they have a variety of easily distinguishable mutations that have been indispensable in studying inheritance patterns, which can be applied to nearly all eukaryotic organisms. We are examining the inheritance patterns of two of these mutations in this study – the ebony-body and the bar-eye mutations. We set up two monohybrid crosses between each of the mutant strains and wild-type flies and also made a dihybrid cross between the mutant strains. F_1 generations were scored and then then self-crossed to produce an F_2 generation, whose phenotypic distribution was similarly recorded. We performed a "goodness of fit" test using the standard Chi-Squared statistical analysis and our results supported the hypothesis that there was no significant difference between our expectations for the phenotypic frequencies in each generation and our collected data. This analysis supported that the bar-mutation exhibited an X-linked dominant pattern of inheritance, while the ebony mutation was transmitted in a pattern typical for autosomal recessive mutations, just as we had anticipated.

■ INTRODUCTION

○ *Background*

The fruit fly, *Drosophila melanogaster*, was one of the earliest model organisms to be used in the field of genetics, making its first experimental appearances in the early 20th century. While it was initially chosen primarily for its wide availability, as it is attracted to ripe fruit and can be caught easily, further study of this common insect indicated that it was surprisingly rare in terms of its favorable qualities for use as a model organism. Furthermore, its striking similarities to other, more complex eukaryotes displayed its potential for illuminating the unresolved questions of inheritance mechanisms as well as many other aspects of transmission genetics. Although *D. melanogaster* experienced a decline in popularity and usage in the mid-century with the introduction of other, more specialized model organisms and microorganisms, which facilitated a range of studies on the molecular basis of genetics, the fruit fly reclaimed its elevated status in recent decades when it received renewed interest for its utility in the study of genetics within developmental biology. Other important roles of *Drosophila* in genetics research include its applications to the fields of cytogenetics, population genetics, and evolutionary biology (Griffiths 2008).

○ *Characteristics*

In addition to its extensive availability *D. melanogaster* shares several other valuable features with other model organisms. Its small size of about 3mm in length allows for the simple storage of even large populations in a laboratory setting. Likewise, they have minimal nutritional needs, requiring only a small amount of potato-based medium as sustenance, which can be provided at negligible cost. Sex determination is fairly easy, as males contain additional anatomical features not present in females, which are easily detected under the microscope (this will be discussed further in the "Methods") Additionally, there in an abundance of interesting, phenotypically distinct, single-gene mutations in this species, of different patterns of inheritance, which can be easily bred and traced through generations in transmission genetics studies.

Next, *D. melanogaster* has a short diploid life cycle that is well suited for genetic analysis. A newly hatched egg will developed through three laval stages, which require about one day to transition to each successive stage. After about three days in the third instar larval stage (see *Fig. 2*), the young fly enters the pupal stage, and just four days later it will reach maturity as a fully functional adult (Griffiths 2008).

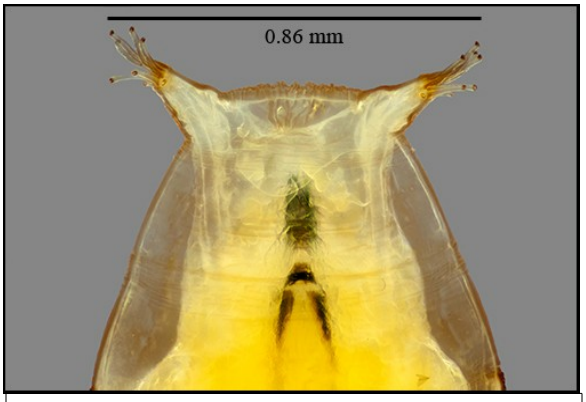

FIGURE 2: a developing fly in the third instar larval stage; notice the anterior spiracles are open to the outside in this stage.

The entire developmental cycle takes about twelve days from the egg to the adult. Sexual maturity is realized shortly after this, usually within eight hours after its emergence from the pupal stage. To ensure controlled breedings, adult flies are generally separated from the opposite sex within this time frame. After successful mating, females should lay eggs within two days. Adult flies will live for a few more weeks after reaching maturity, and in this time several more generations will emerge .

The rapid life cycle is another one of the advantages of this model organism. Also, there is high fecundity in this organism, so that breeding even just a few adult flies can produce hundreds of eggs, resulting in a sizable F_1 generation. Researchers are thus able to breed many generations within a few weeks, and produce considerably large sample sizes, resulting in a large body of data, desirable for any study.

Furthermore, in the past couple decades, geneticists have begun to appreciate *D. melanogaster* for another reason – its genome. Its genome is relatively small, consisting of approximately 180 Mb, found across a mere four chromosomes (2n = 8) – three pairs of autosomes and an X/Y sex-chromosome pair – which comprise a total of only 13,000 genes. This small genome is more manageable in a way, and its simplicity makes it easier to identify gene interaction. Also, its size indicates that there is less redundancy and non-coding regions, which further simplifies the genetic analysis of this species. Since the D. melanogaster genome was sequenced in 2000, its importance as a model for human genetics is greater than ever. It is now known that ~50% of its genome has human homologs. Additionally, its importance was heightened upon discovering that approximately 60% of disease-causing genes and 70% of oncogenes found in humans have *D. melanogaster* counterparts (Griffiths 2008). Even with all that has been learned about this species over the past century, there is continually excitement about these flies as model organisms and there remains a huge potential for these flies in the future of genetics.

- *Traits of Interest*

Throughout this study, we intend to observe the heritability of two mutations, the ebony-body and the bar-eye, through a series of monohybrid and dihybrid crosses with true-breeding, wild-type populations. After two generations have been bred, cultivated, and scored, we will evaluate our data in terms of our expected phenotypic frequencies based on past literature, using a standard Chi-Squared statistical analysis.

The ebony mutation's phenotypic effects can best be described as an overall darker fly, through increased pigmentation of the body (see *Fig. 3-6)*. Also, affected flies generally have more hair on their body, which is thicker and darker than their wild-type counterparts.

FIGURE 3 (above): A young wild-type male
FIGURE 4 (below): An ebony, female mutant atop a melon

One study suggests that there is significant variation in the intensity of thoracic pigmentation within natural lines of these mutants and also notes that an ebony fly reared in cooler temperatures (below 25° C) results in the darkening of these pigments (Takahashi *et al.* 2007). Similarly we saw some variation among our flies to the extent

that lighter ebony flies were sometimes difficult to distinguish from darker wild-type individuals. Often, we would use their underbellies or wings, which are consistently darker than the wild-type, as indicators of their phenotype during the scoring process.

This mutation is known to be autosomal recessive. One characteristic of autosomal recessive mutations is that the phenotypes are often seen in every other generation. Essentially its effects skip generations in which it is transmitted to the next through heterozygous individuals who are carriers for the trait. Accordingly, in both our monohybrid and dihybrid crosses of ebony mutants with wild-type and bar mutants, respectively, we expect to observe only wild-type body color in the F_1 generation, as all offspring will have heterozygous genotypes. In the second cross, we expect to see F_2 generations with traditional, Mendelian phenotypic ratios (3:1, wild-type: ebony for). These frequencies should be observed for both male and female flies, as is expected for autosomal traits.

The bar mutation has phenotypic effects on the eyes of mutant flies, which reduces their size and changes their shape, giving the mutant eyes the appearance of a vertical bar or slit (see *Fig. 5)*.

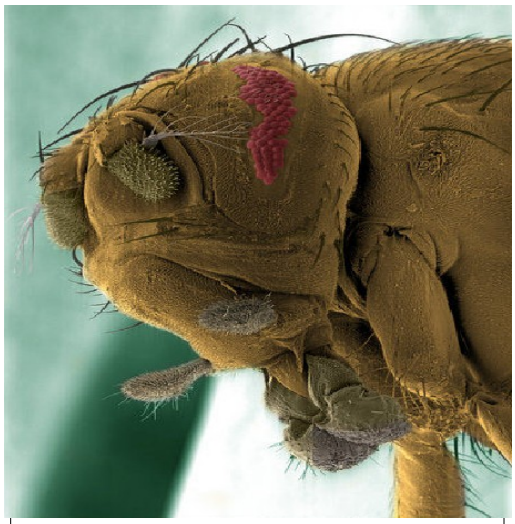

FIGURE 5: A scanning-electron micrograph of a bar mutant *D. melanogaster*

The normal *D. melanogaster* eye, like many insects, is a compound eye, which is made up of hundreds of individual facets or visual-sensory units, called ommatidia, which coordinate their individual signals to function as a single visual apparatus (see *Fig. 6)*. Arthur G. Steinberg identified the mode of development of the mutant bar eye and states that the normal eye contains approximately 770 ommatidia, while a heterozygous individual with the bar allele has a reduction of this number and instead contains about 350 ommatidia (Steinberg 1941). Furthermore, individuals who are homozygous for the bar trait experience a more severe phenotypic effect, and their eyes are further reduced to a mere 75 or so ommatidia (see *Fig. 7*).

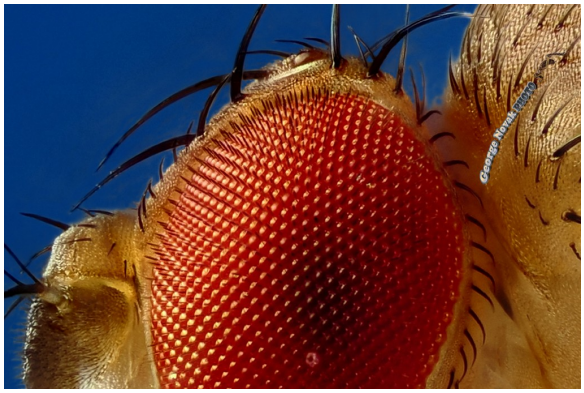

FIGURE 6 (above)*:* The normal *D. melanogaster* eye; notice the individual ommatidia.
FIGURE 7 (below): A comparison of the wild-type, Bar (heterozygote), and "ultra-Bar" (homozygote) eyes

The reason heterozygous individuals with only one bar allele are still affected with the mutation, displaying the bar-eye phenotype, is because the bar mutation is inherited through an X-linked dominant pattern. This

mutation affects a gene on the X chromosome and only one copy of the allele is sufficient for displaying the mutant phenotype. Males are hemizygous for the X chromosome, having only one copy, and thus X-linked disorders generally show higher expressivity among males than females. However, unlike X-linked recessive traits, the dominant traits do not necessarily occur more frequently in males than in females, because females similarly only need one mutant allele to display the trait. The exact pattern of inheritance is instead dependent on which parent the mutation is inherited from. Females that do happen to be homozygous for the bar mutation will likely experience a more severe form of the mutation, as is depicted by the "ultra-bar" eye in *Fig. 7* (which she inherited from an affected father and a mother who is at least a carrier of the defective gene). When the mother is a carrier (heterozygote) of the mutation, she will be affected with the disorder herself and will pass down the disorder to 50% of both her sons and daughters – they will have an equal chance of receiving either of their mother's X chromosomes and thus have an equal chance of inheriting the disorder. When the father is a carrier of the mutation, all of his daughters will receive his single X chromosome and will accordingly be affected by the disorder, while none of his sons will be affected, as they inherit their X chromosome exclusively from their mother. With this in mind, for the first monohybrid cross, between bar-eyed males and wild-type females, we expect all of the daughters to display the bar-eye mutation from the defective allele inherited from their father, but all males should have normal eyes in the F_1 generation. In the F_2 generation, 50% of all offspring from the self-cross of normal males and bar-eyed females will receive a defective X chromosome from their mother and display the trait. In the first dihybrid cross, between bar-eyed females and ebony males, we expect to see 50% of all offspring with the bar mutation. Likewise, in the second cross, inheritance of the bar-eye mutation should be approximately equal between sons and daughters, comprising a 1:1:1:1 ratio.

■ Materials and Methods

○ *Preparation*

Vials were prepared for our *D. melanogaster* colonies by combining a small amount of the potato-based "Instant Drosophila Medium" (~1/2" in the bottom of the vial) with equal volumes of cool, tap water. A few grains of yeast were added and netting was placed down into the medium, which will be used by the maturing pupae just prior to becoming adults. We were careful to ensure that the medium was not too wet, which could propose a potential threat of drowning for the larva living in it. It was also confirmed that there was not too much yeast, which could potentially produce enough CO_2 within the vials to effectively sterilize the *Drosophila* or even result in their death. Finally, vials were closed off with a porous foam plug, which contains the flies in the vial but also allows for gas exchange within the culture tube.

Upon receiving the stock flies, All wild-type adults were killed and the eggs were allowed to hatch, at which time flies were promptly separated by sex to ensure female virginity and a clean cross. The same process was used to ensure the bar-females' virginity in the dihybrid cross.

○ *Crosses*

Three crosses were initially set up in the vials – two monohybrid crosses, between ebony males and virgin, wild-type females, and between bar-eyed males and virgin, wild-type females, and one dihybrid cross, between ebony males and virgin, bar-eyed females. Three vials were used in each

cross, which contained four or five flies of each sex per vial. Vials were labeled for the cross type and the date on which it was started. Vials were stored in a climate-controlled refrigerator set at room temperature (~21° C). At this temperature, bacterial growth within the vials should remain minimal, and should not affect the health of the flies. Within a week, females had laid eggs, which began to hatch shortly thereafter. Parental flies were removed from the vials first sign of the emergence of F_1 larva at the bottom of the vials, so that they were not confused with their offspring. After about two more weeks, some F_1 flies had reached adulthood and were scored for sex and phenotype.

◦ *Anesthetizing*

In order to perform microscopic examination of the flies, they were anesthetized using CO_2. CO_2 guns were carefully inserted into vials and a small amount of CO_2 was injected into the vials in order to render the flies unconscious without affecting any permanent damage or death. Once the flies were unconscious (usually after 10 seconds or so), vials were opened and flies were first transferred to a weigh boat where they were separated from any pieces of medium or other debris. The flies were then put on a CO_2 pad, which provides a constant stream of CO_2 to the flies to keep them anesthetized while they are inspected.

◦ *Sexing & Phenotypic Determination*

Paintbrushes were used to delicately manipulate the flies underneath the microscope. The sex of all flies was first determined using a few recognizable anatomical traits present on the males. The genital arch is an elaborate structure on the posterior end of male flies that includes two small protrusions shaped like the letter "Y." This genitalia is usually surrounded by dark bristles and the rear end of males is usually slightly darker than females also. Additionally, male flies feature sex combs on their front legs, which are small black structures that resemble little brushes or combs. Last, male flies are generally somewhat smaller than females.

After the flies were separated by sex, we began scoring the flies for the phenotypic traits of eye-shape and body-color. Data was contained in a lab notebook shared as a group, and shifts were divided equally between group members. While flies were still hatching, adult flies were separated by sex and removed from the vial every eight hours to ensure the new females' virginity, as well as to ensure that the F_1 population did not lay eggs among those that had not yet hatched, which would effectively mix the two generations. Adult flies were either set aside for the second cross in new vials or killed in the "fly morgue." The same process of data collection was used in the second cross as in the first. Once the data was collected, it was characterized by testing for "goodness of fit" using a standard Chi-Squared statistical analysis.

■ RESULTS

F_1 Generation:

Monohybrid Cross	Male	Female
Bar Eye/Normal Body	8	175
Normal Eye/Normal Body	181	6

TABLE 1: Monohybrid crosses – bar-eye males x wild type females; ebony males x wild-type females

Dihybrid Cross	Male	Female
Bar Eye/Normal Body	6	197
Bar Eye/Ebony Body	0	6
Normal Eye/Ebony Body	3	2
Normal Eye/Normal Body	203	2

TABLE 2: Dihybrid cross – bar-eye males x ebony females

Monohybrid Cross	Observed	Expected
Male Bar Eye/Normal Body	8	0
Male Normal Eye/Normal Body	181	185
Female Bar Eye/Normal Body	175	185
Female Normal Eye/Normal Body	6	0
TOTALS	370	370

TABLE 3: Chi-Squared analysis for monohybrid crosses of F_1 generation; p-value = 0.6775

Dihybrid Cross	Observed	Expected
Male Bar Eye/Normal Body	6	0
Male Bar Eye/Ebony Body	0	0
Male Normal Eye/Ebony Body	3	0
Male Normal Eye/Normal Body	203	209.5
Female Bar Eye/Normal Body	197	209.5
Female Bar Eye/Ebony Body	6	0
Female Normal Eye/Ebony Body	2	0
Female Normal Eye/Normal Body	2	0
TOTALS	419	419

TABLE 4: Chi-Squared analysis for dihybrid crosses of F_1 generation; p-value = 0.80702

F_2 Generation:

Monohybrid Cross	Observed	Expected
Male Bar Eye/Normal Body	68	79
Male Normal Eye/Normal Body	83	79
Female Bar Eye/Normal Body	81	79
Female Normal Eye/Normal Body	84	79
TOTALS	316	316

TABLE 5: Chi-Squared analysis for monohybrid crosses of F_2 generation; p-value = 0.5517

Dihybrid Cross	Observed	Expected
Male Bar Eye/Normal Body	87	89.875
Male Bar Eye/Ebony Body	25	29.875
Male Normal Eye/Normal Body	83	89.875
Male Normal Eye/Ebony Body	32	29.875
Female Bar Eye/Normal Body	97	89.875
Female Bar Eye/Ebony Body	32	29.875
Female Normal Eye/Normal Body	85	89.875
Female Normal Eye/Ebony Body	37	29.875
TOTALS	478	478

TABLE 6: Chi-Squared analysis for dihybrid cross of F_2 generation; p-value = 0.75128

Throughout the eight weeks in which we were running our crosses and collecting data, we were able to accumulate fairly large sample sizes. We bred, sexed, and scored a total of 589 flies in the F_1 generation, and 794 flies in the F_2 generation. As seen in *Tables 1-6*, we analyzed our three crosses in each generation with a Chi-squared statistical analysis. Analyses of the F_1 generation yielded p-values of 0.6775 and 0.80702 for the monohybrid and dihybrid crosses, respectively. This supports our hypothesis, that inheritance would follow Mendelian rationos for the ebony trait, and that the bar-trait showed vertical transmission of the mutation from one generation to the next, as one would expect for an X-linked dominant trait. The results also reinforce the idea that there are no significant differences between our observed phenotypic frequencies and our expected distribution of traits. This also supports the notion that we were successful in all of the technical aspects involved in running the crosses and breeding the flies, since everything within the populations functioned exactly how it was expected to. As for the F_2 generation, we obtained p-values of 0.5517 and 0.75128 for the monohybrid and dihybrid crosses, respectively. These data support our hypothesis that the bar mutation is X-linked recessive and that ebony is autosomal recessive, as the results of the self-crossed F_1 populations match our expectations closely. These results also confirm that there are no significant differences between the two categories of data (expected and observed).

■ Discussion

The results confirmed that the bar-eye mutation was transmitted through an X-linked dominant inheritance pattern. The large p-value represents that there is very close correspondence between our observed values and our expectations based on previous studies on this mutation as well as on our understanding of the distribution patterns of these sort of traits from one generation to the next. The results of the monohybrid cross between the bar-eyed males and the wild type females was precisely how we envisioned it. Nearly all of the F_1 females displayed the bar trait with very few exceptions. There were only 6 outliers, females displaying the normal eye trait, out of a total female population of 181. These are very favorable results because, in theory, all daughters of an affected male should inherit his defective X chromosome and also display the mutant X-linked dominant trait. Likewise, none of the males should have displayed the bar-eye trait in this generation, as they only receive their single X chromosome from their mother, and all of the parental female flies in this cross were true-breeding, virgin, wild-type females. Only 8 males out of a total of 189 displayed the bar-eye mutation, which again is very good data. The few exceptions we had could have been due to a number of factors. The most likely explanation is that at some point, we waited too long to remove the adult flies of the F_1 generation from the vials, and one of the mutant females bread with one of the wild-type males, producing a few bar-eyed males and wild-type females, both of which conflict with our expectations as well as with the X-linked dominant pattern of inheritance for our crosses. These flies would essentially be part of the F_2 generation. Never-the-less, our hypothesis was supported, and aside from these minor exceptions and irregularities, we had really good data. Within the F_2 population, our results showed a 1:1:1:1 phenotypic ratio, which makes sense for a self-cross involving wild-type males and bar-eyed females. Every offspring would have an equal chance of inheriting either one of its mother's X chromosomes and thus approximately equal numbers of mutant and wild-type individuals should be seen in both the males and females of the F_2 generation. This is exactly what is displayed in our results.

Additionally, the results confirmed our hypothesis that the transmission of the ebony mutation from one generation to the next would take the form of an autosomal recessive pattern of inheritance. In our F1 generation, all offspring were phenotypically wild-type, and as would be verified by our F2 population, we believed the offspring of the first cross should all be genotypically heterozygous. We couldn't have asked for a better result, and although we all knew this would occur in theory, it is still amazing to see how two individuals with such strikingly different physical characteristics can produce offspring that look exclusively like one of them. This is especially fascinating to see on such a large scale, being displayed by hundreds of offspring. The F_2 population resulted from self-crossing the heterozygous flies from F_1, and the results of the second cross supported a 3:1 Mendelian ratio, just like we expected. This is reinforced with the large Chi-Squared p-value, giving us a high level of confidence for our statistical support. Within the F_2 population, the phenotypic ratio was expressed equally in both males and females, further indicating the presence of a Mendelian autosomal recessive inheritance pattern.

Aside from the possible experimental error previously discussed, other sources of error that may have contributed to the deviations within our results might include the use of non-virgin females in crosses. Even though

we did well to check these flies every eight hours, it is possible that a small number of female flies develop to sexual maturity faster than what we accounted for. Other systematic error may have occurred in the data collection or in the separation of flies by sex. Especially early in the semester, we had not yet mastered sex determination and it is feasible that a somewhat ambiguous fly was mis-sexed and either scored inaccurately, skewing our data, or added to the wrong vial and perhaps mating with some otherwise virgin females. Other sources of error might lie in the misidentification of the mutant phenotypes. It was mentioned previously that the lighter variants of the ebony mutation sometimes appear to simply be a darker colored wild-type. In the same way, while most of the bar-eyed flies are easily detected, others have eyes that are only partially reduced, and without careful examination these flies could easily pass for a wild-type. Overall, I was extremely pleased with our results and by the end of the project I felt extremely competent in running this sort of experiment effectively and producing reliable data.

■ Contribution

My contribution to the study included the preparation of some of the crosses, regular visits to the lab about once a day while eggs were still hatching, and about once or twice a week on the off-weeks. Because I live off-campus and about 30 miles from school, it was not feasible for me to come into the lab on weekends. Since my fellow group members all either live on campus or extremely close to it, they divided the weekends between themselves and I took on extra shifts throughout the week to help balance the responsibilities for each person. Perhaps my biggest contribution was continuing data collection over spring break, while my group members were all out of town. I drove to Pepperdine a total of four times throughout that week and probably spent 4-5 hours scoring flies overall, as there were several hundreds of flies reaching maturity around that time. As a result of this contribution, we were able to finish collecting data completely by the Tuesday after spring break, because we had gotten so far ahead over spring break.

■ References

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