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Inheritance of the *ebony* Trait in *Drosophila Melanogaster* through Reciprocal Crosses

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Keyword: Mendel's First Law; Drosophila Melanogaster; Ebony Trait; Autosomal Recessive Inheritance; Reciprocal Cross;

Abstract
Introduction: *Mendel's First Law states that allele pairs segregate during gamete formation, producing predictable inheritance patterns. The ebony body-color mutation in Drosophila melanogaster is a useful model for studying Mendelian inheritance.* ***Objective***: *This study aimed to verify the segregation pattern of the ebony trait and determine whether its inheritance is autosomal or sex-linked.* ***Method***: *A Completely Randomized Design with two reciprocal crossing treatments, female normal × male ebony and female ebony × male normal, was conducted from February to April 2026. Second-generation offspring were classified into normal and ebony phenotypes and analyzed using the Chi-square test.* ***Result and Discussion***: *The female normal × male ebony cross produced 354 normal and 116 ebony individuals, yielding a ratio of 3.05:1. The reciprocal cross produced 578 normal and 195 ebony individuals with a ratio of 2.96:1. All Chi-square values were lower than 3.84, indicating agreement with the expected 3:1 ratio. Similar results from both reciprocal crosses indicate that inheritance of the trait is independent of parental sex.* ***Conclusion***: *The ebony trait in Drosophila melanogaster is inherited as an autosomal recessive allele in accordance with Mendel's First Law of Segregation.*

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Introduction

Genetics is a branch of biology that studies the inheritance of traits from parents to offspring through genetic material. The principles of inheritance constitute a fundamental basis for understanding biological diversity and the transmission of genetic information between generations (Poczai & Santiago-Blay, 2021). One of the most important milestones in genetics was the discovery of Mendel's laws of inheritance by Gregor Mendel through his experiments on pea plants(Cheng, 2022). Mendel's First Law, also known as the Law of Segregation, states that paired alleles separate during gamete formation so that each gamete carries only one allele from a gene pair (Veller, 2022). In monohybrid crosses, this segregation pattern produces a characteristic phenotypic ratio of 3:1 in the F2 generation, which remains a fundamental concept in classical genetics despite the discovery of more complex inheritance mechanisms (Wolf et al., 2022).

Drosophila melanogaster is one of the most widely used model organisms in genetic research (Fischer et al., 2023). Since the pioneering studies of Thomas Hunt Morgan, this species has contributed significantly to the understanding of chromosome theory and Mendelian inheritance (Gayon, 2016). Several biological characteristics make *D. melanogaster* highly suitable for genetic studies, including its short life cycle, high reproductive capacity, simple chromosome structure, and ease of maintenance under laboratory conditions (Ramadhan et al., 2025; Zahrah et al., 2025). In addition, extensive genetic resources and mutant strains have been developed and documented through databases such as *FlyBase,* facilitating experimental investigations of inheritance patterns (Attrill et al., 2016).

Among the available mutant strains, the *ebony* strain is characterized by a dark body color caused by mutations in the *ebony* gene, which is involved in pigmentation pathways (Massey et al., 2021). Individuals carrying the homozygous recessive genotype express the *ebony* phenotype, whereas heterozygous individuals exhibit the wild-type phenotype because of dominant allele expression (Kane et al., 2017; Nikolouli et al., 2025). The clear distinction between *ebony* and wild-type phenotypes makes this trait particularly suitable for studying Mendelian inheritance through monohybrid crosses.

Previous studies have demonstrated that several qualitative traits in *D. melanogaster*, including body color and eye color, generally follow Mendelian segregation patterns (Flatt, 2020). Nevertheless, empirical verification through controlled laboratory crosses remains important for confirming theoretical predictions and providing direct evidence of allele segregation. Reciprocal crossing analysis is also useful for determining whether a trait exhibits autosomal or sex-linked inheritance patterns by comparing the results obtained from reciprocal parental combinations.

Therefore, this study aimed to analyze the inheritance pattern of the *ebony* trait in *D. melanogaster* through reciprocal crosses between *ebony* and wild-type strains. Phenotypic observations of the F_1 and F_2 generations were conducted to evaluate the segregation pattern of the trait, while Chi-square analysis was used to determine the conformity of the observed phenotypic ratios with the expectations of Mendel's First Law of Segregation.

Method

This study was conducted at the Genetics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Indonesia, from February to April 2026. The experiment employed a Completely Randomized Design (CRD). The flies were cultured in glass bottles containing a culture medium consisting of bananas, brown sugar, and fermented cassava in a ratio of 7:2:1. The flies were cultured under controlled environmental conditions, specifically at a temperature of approximately 24°C, a humidity level of 60%, and a 12-hour light-dark cycle.

The inheritance of the *ebony* trait was investigated through reciprocal crosses between wild-type and *ebony* strains of *D. melanogaster* **(Picture 1)**, consisting of wild-type females >< *ebony* males (♀N ><♂e) and wild-type males >< *ebony* females (♂N >< ♀e). The resulting F_1 progeny were reared to adulthood and subsequently intercrossed within their respective groups to generate the F_2 generation. All F_2 individuals were phenotypically classified as either wild-type or *ebony* based on body coloration, and the observed segregation patterns were analyzed to evaluate their conformity with Mendelian inheritance expectations.

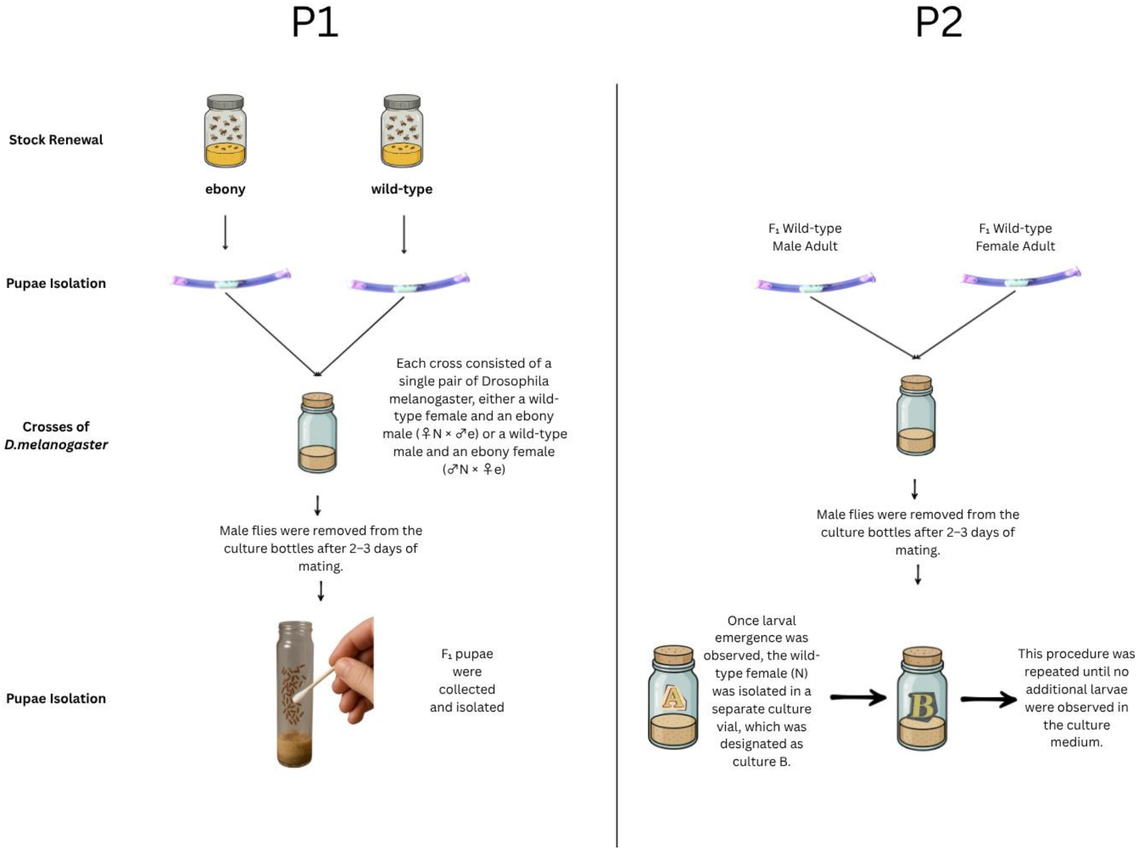


Figure 1. Workflow of a Mendel's First Law Demonstration Project Using *D. melanogaster*

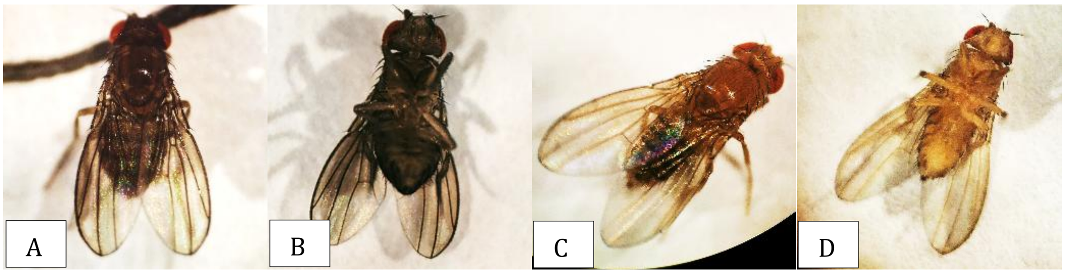
The data collected in this study consisted of the phenotypes of all F_2 offspring that emerged from each reciprocal cross. Each individual was classified as either wild-type or *ebony* based on body coloration. The number of offspring exhibiting each phenotype was recorded for every replicate and used to determine the phenotypic segregation ratio. Data were analyzed descriptively by summarizing the frequency distribution of normal and *ebony* phenotypes. Phenotypic ratios obtained from the F2 generation were compared with the Mendelian expectation of 3:1 (Snustad & Simmons, 2016). The goodness-of-fit between observed and expected frequencies was evaluated using the Chi-square (χ^2) test, calculated as:

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

where *O* represents the observed frequency and *E* represents the expected frequency. The null hypothesis stated that the observed segregation ratio did not differ significantly from the expected Mendelian ratio. The hypothesis was accepted when the calculated χ^2 value was lower than the critical χ^2 value at a significance level of $\alpha = 0.05$ (Brooker, 2022; Griffiths et al., 2020).

Result and Discussion

The parental strains used in this study were the wild-type (normal) and *ebony* strains of *D. melanogaster* **(Figure 2)**. The wild-type strain exhibited the typical phenotype characterized by red eyes, a light brown body color, and wings extending beyond the abdomen (Hotimah et al., 2017). In contrast, the *ebony* strain displayed a dark brown to black body coloration while retaining normal eye morphology and wing structure (Takahashi et al., 2007). The clear difference in body pigmentation between the two strains provided a reliable phenotypic marker for inheritance analysis (Massey & Wittkopp, 2016).



Picture 2. Morphological Characteristics of Parental Strains of *D. melanogaster*. (A) *ebony* (dorsal view), (B) *ebony* (ventral view), (C) normal (dorsal view), (D) normal (ventral view) (Source: Personal Documentation, 2026)

Chi-square (χ^2) analysis was used to compare observed and expected phenotypic frequencies according to Mendelian expectations. A χ^2 value lower than the critical value of 3.84 at $\alpha = 0.05$ and one degree of freedom indicates that the observed deviation is not statistically significant (Snustad & Simmons, 2016). The calculated χ^2 values for all successful replicates and pooled datasets were lower than this critical value (**Table 1**).

Table 1

Chi-square analysis of F_2 segregation

Replicate	♀N × ♂e		♂N × ♀e	
	χ^2 value	**Decision**	**χ^2 value**	**Decision**
A	0.013	Accepted	0.128	Accepted
B	0.149	Accepted	0.113	Accepted
C	0.223	Accepted	-	-
Total	**0.025**	**Accepted**	**0.021**	**Accepted**

The Chi-square (χ^2) analysis showed that all calculated χ^2 values were lower than the critical value of 3.84 at $\alpha = 0.05$, indicating no significant difference between the observed and expected phenotypic frequencies. The pooled χ^2 values for the ♀N × ♂e and ♂N × ♀e crosses were 0.025 and 0.021, respectively, both supporting the acceptance of the Mendelian segregation hypothesis. The complete distribution of the data obtained in this study is presented in **Figure 3**.

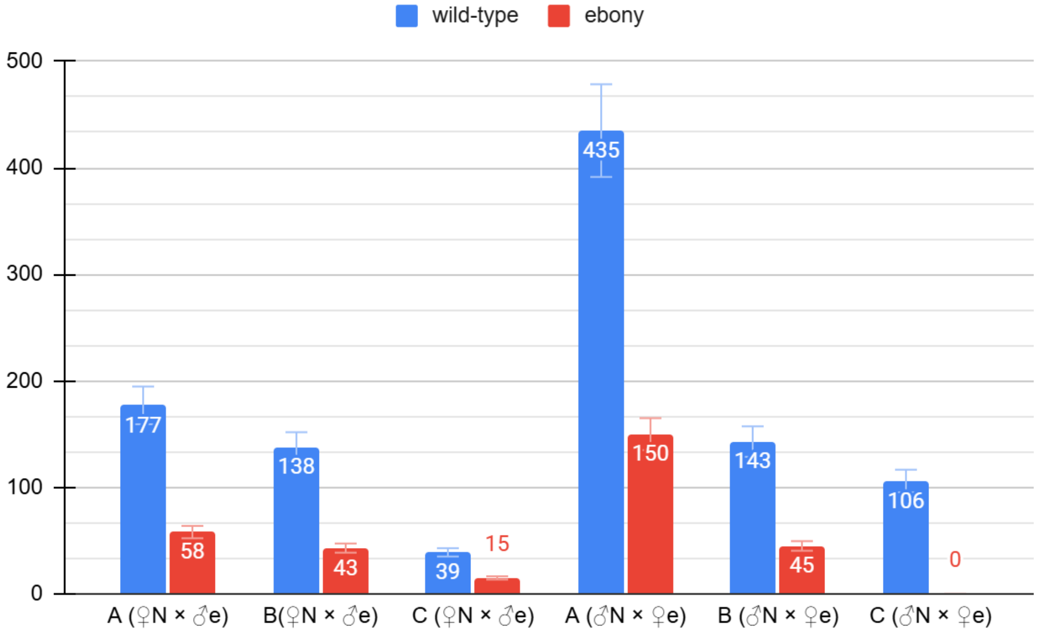


Figure 3. Illustrates the distribution of data across different crosses and experimental replicates.

The pooled F_2 data from the ♀N × ♂e cross (354 wild-type and 116 ebony individuals; 3.05:1) and the reciprocal ♂N × ♀e cross (578 wild-type and 195 ebony individuals; 2.96:1) closely matched the expected Mendelian ratio of 3:1. The very low pooled χ^2 values (0.025 and 0.021, respectively) indicate that the small deviations between observed and expected frequencies are attributable to random sampling rather than non-Mendelian inheritance. These findings demonstrate that the ebony allele behaves as a recessive allele and that segregation occurred according to Mendel's First Law. Furthermore, the nearly identical segregation ratios obtained from both reciprocal crosses indicate that the direction of the cross did not influence offspring phenotype, providing experimental evidence that the ebony locus is autosomal rather than sex-linked. The observed 3:1 phenotypic ratio indicates that the wild-type body-color allele is dominant over the ebony allele. Under this inheritance pattern, crosses between pure-breeding wild-type and ebony strains produce heterozygous F_1 offspring expressing the wild-type phenotype, while segregation of alleles during meiosis and random fertilization generate the expected F_2 genotypic ratio of 1:2:1 and phenotypic ratio of 3:1(Strome et al., 2024). These results are therefore consistent with Mendel's Law of Segregation, which states that allele pairs separate during gamete formation and recombine randomly during fertilization (Najafabadi et al., 2023).

The observed segregation pattern is supported by the cytological mechanism of meiosis. Before homologous chromosomes segregate, DNA replication during the S phase produces sister chromatids that remain attached at their centromeres (Bergero et al., 2021). During meiosis I, homologous chromosomes undergo synapsis and align at the metaphase plate before segregating to opposite poles, ensuring that each gamete receives only one allele of each gene (Ito & Shinohara, 2023; Láscarez-Lagunas et al., 2022). This pairing ensures that homologous chromosomes are correctly aligned for subsequent separation. This reductional division provides the cellular basis for Mendel's Law of Segregation and explains the 3:1 phenotypic ratio observed in the present study (Bergero et al., 2021; Zhang et al., 2026).

The reciprocal cross analysis further demonstrated that the direction of crossing did not affect the inheritance pattern of the *ebony* trait. The phenotypic ratios obtained from the ♀N × ♂e and ♂N × ♀e crosses were similar and closely approximated the expected Mendelian ratio of 3:1. This observation suggests that the *ebony* gene is not sex-linked but is located on an autosome (Signor et al., 2016). If the gene were linked to a sex chromosome, differences in phenotypic segregation would be expected between the two reciprocal crosses due to the distinct inheritance patterns of sex chromosomes in male and female offspring (Johnson & Lachance, 2012). Therefore, the comparable results obtained from both crossing directions provide strong evidence that the *ebony* trait follows an autosomal pattern of inheritance (Paulo et al., 2025).

The *ebony* gene plays an important role in the metabolic pathway responsible for cuticular pigmentation in *Drosophila melanogaster*. The *ebony* phenotype results from mutations in the *ebony* gene located on chromosome 3 at locus 70.7 (Chyb & Gompel, 2013). Mutations in the *ebony* gene lead to the accumulation of dark pigments, causing affected flies to exhibit a darker body coloration than the wild-type phenotype (Lamb et al., 2020; Massey et al., 2021). Because the *ebony* allele is recessive, the *ebony* phenotype is expressed only in homozygous recessive individuals (ee), whereas heterozygous individuals (e^+e) retain the wild-type phenotype due to the presence of a functional dominant allele (Pool & Aquadro, 2007). This inheritance pattern explains why most F_2 individuals displayed the wild-type phenotype, while approximately one-quarter of the population exhibited the *ebony* phenotype, consistent with the expected Mendelian segregation ratio.

An exception was observed in replicate C of the reciprocal cross ♂N × ♀e, where all F_2 offspring exhibited the wild-type phenotype and no *ebony* individuals were recorded **(Figure 3)** This result deviated from the expected Mendelian segregation ratio of 3:1 and differed from the pattern observed in the other replicates. The possible explanation is the occurrence of experimental factors during culture maintenance, such as differential survival of offspring, accidental loss of individuals during handling, or incomplete recovery of all emerging adults (Assenmacher et al., 2022; Braasch & Becker, 2019). Since the *ebony* phenotype is expected to occur at a lower frequency than the dominant wild-type phenotype, the loss of only a few individuals may substantially affect the observed segregation pattern within a single replicate (Kohn & Wittkopp, 2007).

The results provide strong experimental evidence supporting Mendel's First Law. The observed segregation patterns indicate that allele pairs separate during gamete formation and recombine randomly during fertilization. Therefore, the inheritance of the *ebony* trait in *Drosophila melanogaster* represents a clear example of classical Mendelian inheritance and demonstrates the usefulness of this species as a model organism for genetics studies.

Conclusions

The results of reciprocal crosses between normal and *ebony* strains of *Drosophila melanogaster* demonstrated that body color inheritance follows Mendel's First Law of Segregation. All F_1 offspring exhibited the normal phenotype, indicating that the normal allele is dominant over the *ebony* allele. In the F_2 generation, the observed phenotypic ratios in both reciprocal crosses closely approximated the expected Mendelian ratio of 3:1. Furthermore, the similarity of phenotypic ratios obtained from both reciprocal crosses indicates that the *ebony* gene is inherited as an autosomal recessive trait and is not sex-linked. These findings confirm that body color inheritance in *D. melanogaster* is controlled by a simple dominant–recessive genetic mechanism.

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