




MONOHYBRID AND DYHYBRID CROSS ON FRUIT FLIES (*DROSOPHILA MELANOGASTER*)

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<p>Info Article</p> <p>Received : 01 Agustus 2023</p> <p>Revised : 05 September 2024</p> <p>Accepted : 03 Oktober 2024</p> <p>Publication : 30 Oktober 2024</p>	<p>Abstract : <i>Drosophila melanogaster</i> is a member of the insect class that is used to prove the truth of Mendel's monohybrid and dihybrid crossing theory. Fruit flies were chosen as the object of the experiment because they are easy to find, have many offspring, have various phenotypes, have many mutants, and male flies do not crossbreed. This experiment aims to practice making monohybrid and dihybrid crosses and calculate the phenotype ratio of F1 and F2 offspring. The method used in this experiment is to make a medium for the fruit flies, inoculate for transferring fruit flies, and conduct monohybrid and dihybrid crossing methods. The results obtained will interpret the phenotype ratio in F1 and F2 offspring. This experiment concludes that the factors that control each character will separate at the time of gamete formation, and the factors that determine different characters are entered freely from each other.</p>
<p>Keywords: Monohybrid, Dihybrid, <i>Drosophila</i> <i>Melanogaster</i></p> <p>Kata Kunci: Monohybrid, Dihybrid, <i>Drosophila</i> <i>Melanogaster</i></p>	<p>Abstrak : <i>Drosophila melanogaster</i> merupakan anggota kelas serangga yang digunakan untuk membuktikan kebenaran teori persilangan monohibrida dan dihibrida dari Mendel. Lalat buah dipilih sebagai objek percobaan karena mudah ditemukan, memiliki banyak keturunan, memiliki fenotipe yang beragam, memiliki banyak mutan, dan lalat jantan tidak melakukan perkawinan silang. Percobaan ini bertujuan untuk mempraktekkan pembuatan persilangan monohibrid dan dihibrid serta menghitung perbandingan fenotipe keturunan F1 dan F2. Susunan metode dalam percobaan ini adalah, membuat media untuk lalat buah, inokulasi untuk memindahkan lalat buah, dan melakukan metode persilangan monohibrid dan dihibrid. Hasil yang diperoleh akan menginterpretasikan rasio fenotipe pada keturunan F1 dan F2. Kesimpulan dalam percobaan ini adalah faktor-faktor yang mengendalikan setiap karakter akan terpisah pada saat pembentukan gamet, dan faktor-faktor yang menentukan karakter yang berbeda masuk secara bebas satu sama lain.</p>
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INTRODUCTION

Living things can reproduce to preserve their offspring. Breeding can occur through the stages of marriage. Offspring that inherit parental traits from that marriage can be produced (Aurora & Ika, 2020). Parental traits can be passed on to offspring because of a genetic material called Deoxyribo Nucleic Acid (DNA), which can express information about decreasing traits. DNA plays a vital role in expressing genetic information by translating the information it carries to be transmitted to offspring (Irawan, 2010). To prove Mendel's theory of segregation and independent assortment, monohybrid and dihybrid crossovers can be performed using *Drosophila melanogaster*. Crossover is a series of processes carried out in a planned manner, with strict arrangements, to be able to see the offspring resulting from the crossover of two individuals (Bhagat *et al.*, 2004).

Drosophila melanogaster crosses have been done many times before. Schweitzer (1935) analyzed crossing over in *Drosophila melanogaster* and concluded that crossing over does not start at a specific chromosome location. In addition, Wu *et al.* (2020) have also used *Drosophila melanogaster* as a genetic model to determine inheritance patterns for the categories of body color, wing shape, and eye color. Rasyid *et al.* (2024) crossed *Drosophila melanogaster* to analyze the phenomenon of sex linkage in fruit flies of different strains. In addition, Choi (2024) also conducted research using *Drosophila* to analyze the relationship between genetic factors and chromosomes and prove the chromosome theory using *Drosophila*.

The choice of *Drosophila melanogaster* as a genetic model is certainly with reason. Thomas Hunt Morgan was the first to use *Drosophila* as a model for controlled mating in 1865. From there, the trend of using *Drosophila* as a genetic model system continues today (Nath *et al.*, 2021). *Drosophila melanogaster* has many advantages that can facilitate and support the proof of Mendel's law. They have a fast life cycle (Snustad & Simmons, 2015). They are easy to find and have phenotypes that are easy to distinguish (Miguel-Aliaga *et al.*, 2018). *Drosophila melanogaster* is a quickly reproducing insect (Nainu, 2018). It has a short mating time and only takes two weeks to mature (Coronado-Zamora *et al.*, 2019). In a single mating, they can produce hundreds of offspring. These characteristics favor fruit flies to be used as model organisms suitable for genetic research (Campbell *et al.*, 2021).

In the history of the development of genetics, Gregor Mendel is known as the first person to introduce a simple system to analyze the genetic traits of a living body. The

principle used by Mendel is quite simple, that is by making crosses between *Pisum sativum* flowers that have different phenotypes. The color of the flower and the appearance of the seeds that appear from the cross are then used as the basis for mathematical analysis. Mendel's law was first applied to crosses of ercis plants. The results of the cross can indicate the ratio of the resulting offspring. In addition, crossing the ercis beans with round and wrinkled marker phenotypes can detect the birth of Mendel's Law I. These markers can be seen from the results of F1 and F2 crosses. Likewise, the birth of Mendel's Law II can be detected through the results of F1 and F2 crosses (Irawan, 2010).

Mendel conceptualized the principle of segregation. This principle says that only one allele of a gene is passed down from the parent cell to the offspring cell. The second principle proposed by Mendel is the principle of separation and independent assortment. This principle states that the segregation of a pair of traits takes place independently of each other. The law of separation explains the existence of a pair of factors that control how each character will separate at the time of gamete formation. The law of independent assortment explains that the factors that determine different characters are inherited freely. The term factor described by Mendel is later known as the gene (Arumingtyas, 2016).

The transmission of characters or genetic information from one generation to another is termed heredity or inheritance, which is the transmission of characters from parents to the offspring (Galton, 2018). Inheritance was studied even before the chromosomes or genes were known. Gregor J. Mendel was the first to correctly explain the inheritance mechanism with the help of hybridization techniques. Mendel considered seven characters in the ercis plant and proposed the Laws of Inheritance. The characters were studied individually, one at a time, or combined with two or three at a time, and the data was statistically analyzed. Variation is the dissimilarity among the individuals of the same species. For example, we are different from our brothers and sisters even though our origins are the same (Arumingtyas, 2016).

DNA, as a genetic material carrying genetic information, can undergo changes or disconnection of information so that it can cause mutation (Hartl & Maryellen, 2012). With its wide range of experimental tools, the terminalia of *Drosophila melanogaster* has recently become the focus of increased interest in development, evolution, and behavior (Rice *et al.*, 2019). Normal *Drosophila melanogaster* has morphological characteristics: it has compound eyes that are slightly elliptical with a red color, ocelli

eyes on the top of the head, a forked snout, an elliptical head, and a hairy thorax with a white base. The abdomen of *Drosophila melanogaster* has five segments, and the color of the segments is black. The wings of normal *Drosophila melanogaster* are transparent and long. The ebony mutant of *Drosophila melanogaster* has a shiny black body color (Massey *et al.*, 2019; Okada *et al.*, 2016).

In Mendel's theory, there are two terms: monohybrid and dihybrid. A monohybrid is a cross representing two individuals with one distinct character, whereas a dihybrid involves two parents with two or more different characters (Awang-Kanak *et al.*, 2016). Phenotype is the external appearance of an individual for any trait. For example, in the ercis plant, purple and white are the two phenotypes for the color of the flower that are controlled by the respective genotypes. Genotype is the genetic constitution that controls the phenotype of a particular organism. For example, the purple color is controlled by 'CC' or 'Cc,' and the white color is controlled by 'cc' genotypes. Dominant is a trait or gene expressed in homozygous or heterozygous conditions, which can be written as TT or Tt. Recessive is a trait or gene whose effects are masked by a dominant trait or gene. A recessive gene is only expressed when an organism has two recessive alleles for that gene, for example, -tt (homozygous recessive). Homozygous is a genetic condition where an individual possesses identical (same alleles for a particular character on the homologous pair). Heterozygous is a condition in which an individual has two alleles for a particular trait or character, having one dominant and one recessive allele (Hartl & Maryellen, 2012).

METHOD

In this experiment, the systematics began with preparing the medium for *Drosophila melanogaster*, followed by the inoculation method, and conducting crosses.

1. The first thing to do is to make a medium to rejuvenate the fruit flies that will be crossed. The materials used to make the medium are 700 grams of banana, 200 grams of cassava tapai/tapay/tape (fermented food made from cassava), and 100 grams of brown sugar. Then, all ingredients are added to water and blended until smooth. The next step is to cook the blended ingredients until they are boiling and thick. Then, put the medium into a culture bottle, and if it is warm, sprinkle it with 7 grains of instant yeast. Wait until it cools down then place the pupation paper and close the bottle with a sponge.

2. After making the medium, the next step is to execute the inoculation method. First, prepare a small hose with the end covered with gauze and insert it into a large hose. Then, combine the two hoses and insert them into a bottle with fruit flies. Suck the flies into the large hose and then close the large hose and transfer it to the culture bottle.
3. Before making a cross, knowing the requirements for doing a crossover is necessary. Female flies that will be used for crossing must be virgins, so it is necessary to isolate virgin female flies. Isolation of virgin female flies is done by removing adult flies (imago) from the culture bottle shortly after the flies are taken from the hose. Then, after 8 hours, remove all newly hatched flies from the pupae and select the female flies. Usually, flies will not mate for at least 8-10 hours from pupation, so the selected female flies can be confirmed to be virgins. The ability to distinguish between female and male flies can be seen through their morphological characteristics. The sex comb on the tarsal of both front legs recognizes male flies. Sex comb can be seen in cocoons that are three days old because the primation of bristles only occurs one day before exiting the cocoon. The selection of female flies should be stopped after the third day, while the age of male flies does not matter because male flies can be used at any age.
4. The final step in this experiment is to commit the crossover. There are slight differences in the steps for monohybrid and dihybrid crosses. In the monohybrid crossing experiment, flies with strain N (wild type/normal) and strain e (ebony mutant) are used, while in the dihybrid crossing experiment, flies with strain N and strain ebony clot (hereafter ecl) are used. In this case, it is necessary to know the morphological characteristics that distinguish between N strain flies, e strain, and ecl strain mutants. *Drosophila melanogaster* strain N has characteristics such as yellow-brown body color, red compound eye color, the state of the wings covering the body perfectly, and smooth eye facets. As for strain ecl, it differs in body color and eye color, whereas strain ecl has a black body color and a dominant brownish-red eye color. Meanwhile, strain e only differs in body color, which is shiny black.
 - a) The first things that have to be prepared in monohybrid crosses is the culture bottle with the medium. Next, take the collection of flies from the isolation tube. In monohybrid crosses, *Drosophila melanogaster* strain N and strain e are used. After that, cross between male flies of strain e and female flies of strain N or their reciprocals in the culture bottle, and do not forget to cover the bottle with a

sponge. Next, initial the bottle with the type of cross and the date of the cross. Wait for the crossed flies to produce pupae. If the pupae have emerged, re-isolate them as the second parent to obtain F2.

- b) In the case of dihybrid crosses, the process starts with taking the male and female flies to be crossed from the isolation tube. For dihybrid crosses, flies with strain N and strain ecl are used. The next step is to put both male and female individuals to be crossed into the culture bottle. Give the date of crossing and the type of crossing. Then, wait for them to lay eggs and pupate. If the pupae have formed, isolate it again using an isolation hose to be used as the second parent to get F2.

RESULTS AND DISCUSSION

Result

In the history of the development of genetics, Gregor Mendel is the first person to introduce a simple system for analyzing the genetic traits of organisms with different phenotypes. Mendel proposed the concept of the segregation principle. This principle says that only one gene allele is passed on from the parent cell to the offspring cell. The second principle is the principle of separation (independent assortment). This principle basically states that the segregation of a pair of traits takes place independently. The law of separation explains the existence of a pair of factors that control how each character will separate at the time of gamete formation. The law of independent assortment explains that the factors that determine different characters are inherited freely. The term factor described by Mendel was later known as the gene.

A Monohybrid cross is a cross between two individuals of the same species that have one distinct trait. The first offspring (F1 generation) will have the same trait as one of the parents, influenced by dominant and recessive alleles. Dominant monohybrid crosses are crosses between two individuals of the same species that display one distinct trait with dominant genes. Intermediate monohybrid crosses are crosses between two similar individuals that promote one trait with intermediate genes. A Dihybrid cross is a cross between two individuals of the same species that have two different traits.

The use of *Drosophila melanogaster* in this experiment is not without reason; many advantages of *Drosophila melanogaster* can support and help the continuity of monohybrid and dihybrid crossing experiments that will prove the truth of Mendel's law of genetics. *Drosophila melanogaster* is a cosmopolite insect. Therefore,

Drosophila melanogaster is easy to find. In addition, *Drosophila melanogaster* is easy to rear, usually in a bottle containing banana and cassava tapai/tapay/tape medium. However, as in microbiology experiments, sterile conditions are required to avoid fungal contamination. In addition, mold growth can be prevented by administering sodium benzoate and yeast for fermentation. The cost of maintenance of fruit flies is also categorized as cheap. Because the food medium is straightforward, maintenance is not too expensive. However, the medium must always be replaced every two weeks.

The maintenance and breeding of *Drosophila melanogaster* requires little space. Mutant stocks are usually maintained in jam bottles. One jam jar can contain about 300-500 adult flies. The bottles can be stored on a shelf. The visible size of *Drosophila melanogaster* can facilitate morphological observations to distinguish between female and male flies and between mutant and normal flies. *Drosophila melanogaster* is also harmless because it will not transmit diseases or become intermediate hosts. *Drosophila melanogaster* is capable of having many offspring. One female fly can produce 500 eggs during her lifetime. *Drosophila melanogaster* has a short life cycle. Its life cycle ranges from 10-15 days, so it does not take long to breed it. This fly has a varied phenotype and is easily distinguished in eye color, feather shape, body color, and wing shape.

Drosophila melanogaster has simple chromosomes, which only have 4 chromosome pairs, making it easier for the counting process. In addition, polytene chromosomes are giant and arrayed in the salivary glands, which help recognize gene sites. *Drosophila melanogaster* has the most mutants, making it suitable as the object used in this genetics experiment. Male flies do not crossbreed so that crossbreeding can be calculated more accurately. On the other hand, crossing over can occur in both parents of animals or plants.

The following will describe the uses of each tool and material. The main object of this experiment is to use *Drosophila melanogaster* as the most essential element and as a subject to observe. Then, a medium will be placed to support the breeding of fruit flies. It consists of bananas, cassava tapai/tapay/tape, and brown sugar. In this case, brown sugar and tapai (also tapay or tape, a fermented food made from cassava) help make the medium dough denser and act as a carbohydrate source for flies. Next is the pupation paper, which functions as a place to attach the pupa so that it is easy to observe. Instant yeast also expands the medium dough to make it denser; jam bottles are also used as a medium for crossing. There are also foam plugs that are used to cover

jam bottles to prevent flies from getting out of the bottle easily. There is also a large hose and a small hose that functions to suck up flies. There is also an isolation hose to isolate flies before crossing. Gauze is also used to seal the end of the small hose so that flies do not come out when vacuumed. Remember to use ballpoint label paper to mark the sample about the time and date.

The results obtained from this experiment are as follows. In monohybrid crosses, the first parental is a cross between the Normal strain (NN) and the ebony strain (ee), which produces heterozygous F1 offspring (Ne). After getting F1, the second parental is the first offspring of Ne crossed with fellow Ne. Then, F2 is obtained with a phenotype ratio of Normal (N) compared to Ebony (e) is 3:1, with details of genotype ratio 1:2:1 for NN:Ne:ee.

P1 : NN (Normal) \times ee (ebony)

G : N e

F1 : Ne

P2 : Ne \times Ne

G :

	N	E
N	NN	Ne
e	Ne	Ee

F2 :

a) Ratio genotype

NN : Ne : ee

1 : 2 : 1

b) Ratio phenotype

Normal (NN, Ne) : Ebony (ee)

3 : 1

The results obtained from dihybrid crosses are from the first parental cross, strain N with genotype NNEE and strain eel with genotype nnee produced F1 with genotype NnEe. Then, the F1 fellow crossed back into the second parental and produced F2 with a phenotype ratio of 9:3:3:1.

P1 : NNEE \times nnee

G : NE ne

F1 : NnEe

P2 : NnEe \times NnEe

G :

	NE	Ne	nE	ne
NE	NNEE	NNEe	NnEE	Nnee
Ne	NNEe	NNee	NnEe	Nnee
nE	NnEE	NnEe	nnEE	nnEe
ne	NnEe	Nnee	nnEe	nnee

F2 : Ratio phenotype (9:3:3:1)

Several factors will affect the failure of *Drosophila melanogaster* crosses; if the flies lack food availability during the breeding period, the eggs produced by the flies will decrease. In addition, adult fruit flies will produce smaller larvae. Then, the larvae will develop into small pupae and often fail to develop. Moreover, if it can develop into an adult fly, it can only produce a few eggs. The temperature of the environment also affects the survival of its life cycle. *Drosophila melanogaster* cycles for 8-11 days at an optimal temperature of 25-18⁰C. If it develops in lower temperatures, its life cycle will occur more slowly, and if it develops in temperatures of 30⁰C, the fly will become a sterile adult fly. The density of the culture bottle will also affect the failure of fruit fly development, which, if too dense, will reduce egg productivity and increase mortality in adult flies.

CONCLUSION

From this experiment, we can deduce that successful fruit fly breeding (*Drosophila melanogaster*) requires a systematic method. This involves creating a suitable medium to provide food for the flies in the culture bottle, followed by the inoculation method, and culminating in the crossing process.

In a monohybrid cross between a normal strain (NN) and an ebony strain (ee), heterozygous F1 offspring (Ne) were produced. Crosses between F1 offspring produced offspring with a phenotypic ratio of Normal:ebony is 3:1, with details of the genotype

ratio NN:Ne:ee is 1:2:1. This result proves Mendel's theory, which states that a monohybrid cross, where the cross is made between two parent plants that have only one different characteristic, produces a phenotype ratio 3:1. This result proves the correctness of Mendel's segregation theory, where allele pairs will separate during gamete formation using the meiosis cell division mechanism. As a result of this segregation, each gamete will get half of the parent chromosome and carry only one allele of each gene.

A dihybrid cross, or a cross of parent plants with two different characters, between genotypes NNEE and nnee produces an F₂ with a phenotype ratio 9:3:3:1. This result is the same as a result obtained by Mendel in his experiment of making a dihybrid cross on ercis plants. This experiment proves Mendel's theory that each pair of alleles splits independently during gamete formation, known as the independent assortment theory. This theory states that each allele in a gene will separate independently from the other gene pairs during gamete formation. The alleles of the different genes will pair freely and are independent. However, Mendel's independent assortment theory has proven that it is not always accurate in genetics.

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