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The Influence of Strain Type and Female Age on the Receptivity of Female *Drosophila melanogaster* in Homogamous Crosses

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Abstract

Drosophila melanogaster is a widely used model organism in genetic research due to its short life cycle and the presence of many genes homologous to those found in humans. It plays an important role in studies related to genetic inheritance, gene interaction, sex determination, and developmental genetics. One key aspect of its reproductive biology is female receptivity—the willingness of a female to accept mating—which is influenced by various internal and external factors. External factors include environmental conditions such as temperature and humidity, as well as male courtship signals like wing vibrations and chemical cues. Internal factors are mainly related to the female's sexual maturity and mating status. Generally, female receptivity increases with age after eclosion, reaches a peak, and gradually declines. This study investigates the impact of strain type and female age on receptivity in homogamous crosses of *D. melanogaster*, using two mutant strains: *dp* (with a wing mutation) and wa (with an eye color mutation). Receptivity was measured by the presence or absence of F1 offspring following crosses between males and females of the same strain, with females tested at different ages ranging from 10 to 60 hours after eclosion. The results indicate that strain type does not have a significant effect on female receptivity. In contrast, female age plays a clear role: individuals aged 30 years and older were more receptive and more likely to produce offspring. No significant interaction was found between strain type and age. These findings suggest that female receptivity in *D. melanogaster* is more strongly influenced by sexual maturity (as indicated by age) than by genetic differences between strains.

Keywords: *Drosophila melanogaster*; female receptivity; strain type; sexual maturity; homogamous crosses.

Abbreviations: Analysis of Variance (ANOVA); First Filial Generation (F1); Randomized Block Design (RBD); White Apricot (eye color mutant strain of *Drosophila melanogaster*) (w^a); Drop (wing mutant strain of Drosophila melanogaster) (*dp*).

INTRODUCTION

D. melanogaster is a type of fruit fly commonly found in our surroundings due to its ability to reproduce rapidly. According to Parvati et al. (2009), many *Drosophila* genes are homologous to human genes. This makes *D. melanogaster* a widely used organism in genetic research. It is beneficial for studying several genetic mechanisms, including general principles of gene transmission, sex determination, gene interaction, as well as molecular, biochemical, and developmental genetics.

D. melanogaster reproduces sexually. Mating occurs when both male and female individuals have reached maturity (Karmana, 2010). However, the female plays the most critical role in determining whether mating takes place. This is due to various influencing factors on the female, both internal and external. External factors include environmental conditions such as temperature and humidity, and more importantly, the signals emitted by the male during courtship. Internal factors include the female's sexual maturity and mating status. A female's receptivity increases with age after emergence and then declines after reaching its peak (Feng, 2010).

In this study, the *D. melanogaster* strains used are the *dp* strain, which has a wing mutation, and the w^a strain, which has a mutation affecting eye color. In line with previous research, including that of Karmana (2010), it was found that both strain type and female age affect the number of offspring produced. However, these two factors exert their influence independently, with no interaction between them.

MATERIALS AND METHODS

Research Design

This study employs a quantitative descriptive design, aiming to determine the effect of strain type and female age on the receptivity of female *D. melanogaster*. the presence or absence of F1 offspring indicates receptivity. If F1 offspring appear, their number is counted over a 7-day period. Crosses are performed within the same strain

(homogamous crosses: *dp*♂ × *dp*♀ and w^a♂ × w^a♀), with three replications, using media labeled A, B, C, and D to obtain F1 offspring.

Population and Sample

The population in this study consists of *D. melanogaster* of the *dp* and w^a strains, bred in the Genetics Laboratory, Faculty of Mathematics and Natural Sciences, State University of Malang. The samples used are individual flies from both strains taken from the laboratory cultures and used as stock for the study.

Procedure

Medium Preparation

For one batch of medium, the ingredients used are 700 grams of *pisang rajamala* (a local banana variety), 200 grams of cassava tape (fermented cassava), and 100 grams of palm sugar, with a 7:2:1 ratio. First, the bananas are chopped, and the palm sugar is boiled until it melts. Then, the bananas and cassava tape are blended with enough water until smooth. The blended mixture is transferred to a pot and cooked until it comes to a boil. Once boiling, the melted sugar is added, and the mixture is cooked for 45 minutes for one batch, or 60 minutes for two batches. While still hot, the medium is poured into jam jars, sealed with sponge lids, and allowed to cool. Once cooled, 3–5 grains of yeast (fermipan) are added. The inside of the jar is wiped to remove condensation, and a pupation paper is placed inside.

Stock Renewal and Pupal Isolation

Begin by preparing jam jars filled with the prepared medium. Introduce 3–7 pairs of *D. melanogaster* from each strain into separate jars, and label each jar with the strain and the date of introduction. When darkened pupae appear, isolate them by transferring them with a brush into a plastic ampule tube that has a slice of banana placed in the middle. Both ends of the tube are sealed with sponge to prevent newly emerged flies from escaping.

Crossing

Once flies have emerged from the ampule, females from both *dp* and w^a strains aged 10, 20, 30, 40, 50, and 60 hours post-eclosion are crossed with males of the same strain aged 1–3 days. Each crossing jar is labeled with the strain, replication number, and date of treatment. After 3 hours of mating, the male flies are removed. When larvae begin to appear, the female flies are transferred to fresh medium, with up to three transfers (ending at jar D). Offspring (F1) are allowed to develop and emerge, and the number of F1 individuals is recorded over a 7-day period.

Data Collection Technique

Data are collected by counting the number of male and female F1 individuals from each cross and replication. The following table format is used to record F1 counts for each group.

Data Analysis Technique

The data analysis begins with chromosome reconstruction of the body. If the data set is complete, further analysis is conducted using a two-way ANOVA with a Randomized Block Design (RBD). This statistical test is used because there are two independent variables: strain type and female age. Since data collection was not conducted simultaneously, RBD is considered appropriate. However, if the data set is incomplete, descriptive analysis is used instead.

RESULTS AND DISCUSSION

Result

The *D. melanogaster* strains used in this study are *dp* and wa, with the following characteristics:

1. *dp* strain:
   - Red eye color
   - Smooth eye facets
   - Wing shape fully covers the body, with tips curving inward
   - Yellowish-brown body color

Figure 1. *D. melanogaster dp* strain

2. w^a strain:
   - Orange eye color
   - Smooth eye facets
   - Wing shape fully covers the body
   - Yellowish-brown body color

Figure 2. *D. melanogaster* w^a strain

Table 1. Number of F1 Male and Female Offspring from *dp*♂ >< *dp*♀ Crosses.

Female Age	F1 Count			Total	Average
	U1	U2	U3		
10 hours	0	-	0	0	0
20 hours	195	77	-	272	90,6
30 hours	165	0	0	165	55
40 hours	0	0	-	0	0
50 hours	143	0	0	143	47,66
60 hours	118	201	-	319	106,3

Table 2. Number of F1 Male and Female Offspring from w^a♂ >< w^a♀ Crosses.

Female Age	F1 Count			Total	Average
	U1	U2	U3		
10 hours	-	0	0	0	0
20 hours	0	0	-	0	0
30 hours	95	106	59	260	86,67
40 hours	0	64	-	64	21,3
50 hours	53	79	0	132	44
60 hours	0	62	73	135	45

In the cross between *dp*♂ × *dp*♀, the total number of male and female F1 offspring at the 10-hour female age treatment was 0, resulting in an average of 0. At the 20-hour treatment, the total F1 count was 272, with an average of 90.6. At 30 hours, the total was 165, averaging 55. At 40 hours, the total was again 0, with an average of 0. For the 50-hour treatment, the total F1 count was 143, with an average of 47.66. Finally, at 60 hours, the total was 319, with an average of 106.3. In the cross between w^a♂ × w^a♀, the total number of F1 offspring at the 10-hour and 20-hour female age treatments was 0, resulting in an average of 0 for both. At 30 hours, the total number of F1 offspring was 260, with an average of 86.67. At 40 hours, the total was 64, with an average of 21.3. For the 50-hour treatment, the total was 132, with an average of 44. At 60 hours, the total number of F1 offspring was 135, with an average of 45 (Figure 3).

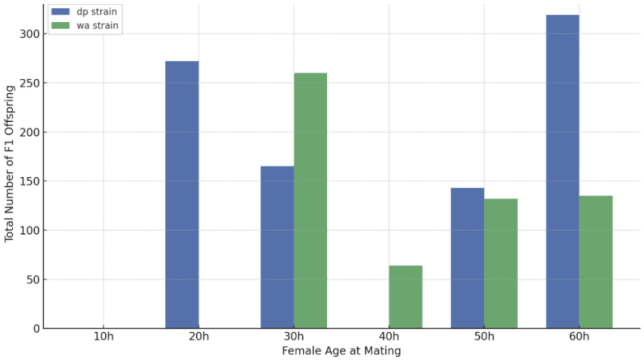

Figure 3. Total Number of Male and Female F1 Offspring.

Effect of Strain Type on Female Receptivity in *D. melanogaster*

Since the data collected are incomplete, a two-way ANOVA could not be conducted to determine the effect of strain type on the receptivity of female *D. melanogaster*. However, based on the literature review conducted by the researcher, it is understood that so far, strain type does not affect female receptivity in *D. melanogaster*. According to Muliati (2000) as cited in Karmana (2010), the number of offspring produced varies among different strains. However, Muliati further states that no existing literature clearly supports the idea that strain type affects the number of offspring. In this context, Dobzhansky (1951), as cited in Indayati (1999), states that mutations can alter the behavior of any species. Kusnawati (1996), also cited in Indayati (1999), adds that behavioral differences among mutants can influence mating success.

Mating behavior in *D. melanogaster* is influenced by several types of stimuli, including visual, olfactory and gustatory (smell and taste), auditory, and tactile signals. Regarding auditory stimuli, Shorey (1962), as cited in Nusantari (1997), noted that males produce sounds through wing vibrations. Ewing (1964), also cited in Nusantari (1997), reported that wing size is linked to mating success. This implies that the *dp* strain, which has a wing mutation (inward-curved wing tips), may have lower mating success compared to the wa strain, which has a normal wing shape despite having an eye color mutation. The relatively "intact" wings of the wa strain may allow for stronger wing vibrations, which serve as mating stimuli and are more effectively received by females.

From the explanation above, it becomes clear that while strain type may influence mating success, it does not influence female receptivity. Female receptivity is more closely tied to sexual maturity than to the type of mutation present. Even if females come from different mutant strains, they will become receptive once they reach sexual maturity and will accept copulation from males. Therefore, mutation differences between *dp* and wa strains are not a determining factor—receptivity depends primarily on the female's ovarian or sexual development.

Effect of Female Age on Receptivity in *D. melanogaster*

Due to incomplete data, a two-way ANOVA could not be conducted to determine the effect of female age on receptivity in *D. melanogaster*. However, based on the literature reviewed by the researcher, it is evident that female age does influence receptivity. In the crosses of *dp*♂ × *dp*♀ and w^a♂ × w^a♀ using 10-hour-old females, no offspring were produced. This aligns with Manning's (1967) statement in Feng (2010), which indicates that female *D. melanogaster* become receptive at 24–40 hours after eclosion. Females at 10 hours old are not yet receptive and therefore reject mating attempts.

In the w^a♂ × w^a♀ cross using 20-hour-old females, no offspring were produced either, further supporting Manning's claim. However, in the *dp*♂ × *dp*♀ cross at the same age (20 hours), offspring were produced (272

individuals), suggesting an exception. This can be explained by O'Dell (2003), who states that while females generally become sexually mature around 24 hours post-eclosion, some strains may mature faster. In this case, the *dp* strain may experience accelerated sexual maturation, making 20-hour-old females receptive and able to accept copulation. At 30 hours, both *dp*♂ × *dp*♀ and w^a♂ × w^a♀ crosses produced offspring. This matches Manning's (1967) range of 24–40 hours for female receptivity. A receptive female will typically mate within 30 minutes when paired with a male. She will slow her movements, allowing the male to initiate copulation using his proboscis. Once copulation occurs, receptive females can produce offspring.

In the w^a♂ × w^a♀ cross with 40-hour-old females, offspring were again produced, indicating that the females were still within the receptive window. However, in the *dp*♂ × *dp*♀ cross at the same age, no offspring were observed. This may not indicate a lack of receptivity, but rather a problem with environmental conditions—specifically, the medium was dry and moldy. The researcher noted excessive fungal growth in the culture medium, which may have discouraged egg-laying. The females were subsequently transferred to a new jar (jar B) in hopes that they would lay eggs in the improved conditions and produce offspring.

For crosses using 50-hour- and 60-hour-old females, both *dp* and wa strains successfully produced offspring. According to Manning (1967), females are receptive from 24 to 40 hours post-eclosion, so these older females are technically beyond the peak receptive window. However, this does not mean they are incapable of producing offspring. Feng (2010) notes that females can still respond to male courtship songs at 2 days old, although receptivity may decline between 2–4 days. Thus, 50- and 60-hour-old females may still respond to male signals, albeit less strongly, and are still capable of mating and producing offspring (Cook, 1973, in Feng, 2010).

Interaction Between Strain Type and Female Age on Receptivity in *D. melanogaster*

As with the previous sections, the incomplete data prevented a two-way ANOVA from being conducted to test the interaction between strain type and female age on receptivity in *D. melanogaster*. However, based on the literature, there is no significant interaction between strain type and female age in influencing receptivity. As explained earlier, only female age significantly affects receptivity, while strain type does not. Therefore, the interaction between these two factors is also not significant. This is supported by Indayati (1999), who stated that mating success—an indicator of female receptivity does not differ significantly across strains at different ages.

Furthermore, a previous study by Karmana (2010), showed that both strain type and female age affected the number of offspring, with each factor acted independently, with no interaction between them. It's important to note that Karmana's study measured offspring count, whereas the current study focuses on female receptivity as the dependent variable. The reference to Karmana's findings here is intended solely to reinforce the point that no interaction effect was observed. However, to draw a more definitive conclusion, the researcher wants to complete the dataset and conduct a two-way ANOVA with a randomized block design (RBD) to test for interaction formally.

CONCLUSIONS

The results of the study show that the strain type does not affect the receptivity of female *D. melanogaster* in homogamous crosses. In contrast, female age has a significant impact on receptivity levels in these crosses. Meanwhile, the interaction between strain type and female age does not have a significant effect on female receptivity in the context of homogamous mating.

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