ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 14, Issue, 12, pp.23171-23173, December, 2022
DOI: https://doi.org/10.24941/ijcr.43820.12.2022

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EFFECT OF EXPOSURE TO MALE FLIES ON THE LIFESPAN OF FEMALE ADULT *DROSOPHILA MELANOGASTER* (DIPTERA: DROSOPHILIDAE)

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ARTICLE INFO

Article History:
Received 09th September, 2022
Received in revised form
25th October, 2022
Accepted 18th November, 2022
Published online 30th December, 2022

Key words:
Drosophila melanogaster, fruit flies, mate exposure, Competition, Mortality, Survivorship.

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ABSTRACT

Energy is a limited resource that is used in reproduction, growth, and maintenance. For organisms to reproduce, energy may be transferred from maintenance to reproduction. We studied the effect of a single mating period on adult lifespan in *Drosophila melanogaster* to examine whether exposing female *D. melanogaster* to 0, 1, 2, or 6 male(s) for a 48-hour time interval would impact female lifespan while also examining how male competition during these intervals would impact male lifespan. We assigned females (n=20) to one of the four above treatments and exposed them to the designated number of males for a 48-hr time period, and we then isolated both males and females and monitored them daily for survival. There were no significant differences in the number of days survived between treatments for either males or females when comparing 0 versus 1 male or when comparing 2 versus 6 males. All four treatments could not be statistically compared because they were not conducted at the same time due to the number of virgins required for trials These results suggest that lifespan is not affected by the number of mates a female is exposed to or the number of competitors a male is exposed to. Further research could investigate whether 1 versus 6 males results in statistical significance.

Citation: ***Elexis A. Richardson and Dr. Marianne Robertson,* 2023.** "Effect of exposure to male flies on the lifespan of female adult Drosophila melanogaster (Diptera: Drosophilidae)*". International Journal of Current Research*, 15, (01), 23171-23173

INTRODUCTION

There are three primary factors that energy is allocated to in an organism's life: growth, maintenance, and reproduction (Travers *et al*., 2015). Since energy is limited, an organism must take energy from one category to divert more energy towards another. For an organism to reproduce more, it must take that energy from growth or maintenance. If it is at reproductive age, it is most likely no longer growing, so the energy would come from maintenance (Travers *et al*., 2015). In *Drosophila melanogaster*, males are likely to copulate with previously mated females as well as with the same female many times if given the chance because a single copulation event is often not enough to fertilize all eggs (Castrezana *et al*., 2017). Travers *et al*. (2015) studied the tradeoff between reproduction and longevity in *D. melanogaster* by examining the interactions between lifespan and early life mating frequency. They varied the number of mating opportunities over the lifetime by exposing *D. melanogaster* females to different *D. melanogaster* males one at a time and found that females that had a genetic tendency to mate multiply had a shorter lifespan than females without this genetic tendency. Bretman *et al*. (2013) studied similar effects; however, they focused on the interactions between the males. Experimental *D. melanogaster* males were exposed to rival conspecific males while controls were not. The lifespan of males with exposure to rivals decreased compared to the males without rivals (Bretman *et al*., 2013). The purpose of this study is to determine whether female lifespan is affected by the number of mates a female is exposed to and whether male lifespan is affected by the number of rivals a male is exposed to in *D. melanogaster.*

We hypothesized that virgin females would have a longer lifespan lifespan than females exposed to one male. We also hypothesized that females exposed to two males would have a longer lifespan than females exposed to six males. The Disposable Soma Theory states that there are energy trade-offs between growth, maintenance, and reproduction (Travers *et al*., 2015). This theory proposes that the females who are mating may have to divert energy from growth or maintenance to put it towards reproduction. Females exposed to more males are also at risk of injury from male coercion/harassment (Castrezana *et al*., 2017). For the males, we hypothesized that males with the least competition would have the longest lifespan and that males with the most competition would have the shortest lifespan. Males with rivals present may use more energy on pre-mating interactions to secure an opportunity to mate and on post-copulatory mechanisms to increase the chance of successful fertilization (Bretman *et al*., 2013). Since virgin males and males only exposed to one female have no competition, they should expend less energy on pre-mating interaction and post-copulatory mechanisms.

MATERIALS AND METHODS

We had one control group and three experimental groups. Our control group consisted of virgin female and virgin male flies. Our three experimental groups consisted of 1 female with 1 male, 1 female with 2 males, and 1 female with 6 males for 48 hours. Each group consisted of20 females and the corresponding number of males (20 males for the 1 female with 1 male group, 40 males for the 1 female with 2 males group, and 120 males for the 1 female with 6 males group).Our independent variable was the number of potential mates for females and the number of potential rivals for males, and our dependent variable was the number of days survived after 48 hours of exposure. We conducted our experiment in three parts because of the large number of virgin flies necessary. We conducted the first part from March 2021 - May 2021, the second part from May 2021 - August 2021, and the third part from September 2021 - January 2022. Weonly compared virgin females and males to females and males that were exposed to one fly of the opposite sex for a 48-hour period during the first part of the experiment. In the second and third part, we compared the one female, two males group and the one female, six males group. We conducted the third part because the second part did not have a large enough sample size to make conclusions. With the exception of specified differences, we used the same procedure for all three parts. We obtained wingless *D. melanogaster* (fruit flies) from Josh's Frogs online store and housed them in room 217 in the Leighty Tabor Science Center at Millikin University in Decatur, Illinois. We received *D. melanogaster* in cultures that consisted of different ages and sexes. For the first part of the experiment, we used the flies directly from these shipped colonies. However, for parts two and three, we used flies that came from colonies being maintained in our lab. Once we had smaller colonies set up, we spent 2-5 days collecting *D. Melanogaster* within 4.0 hours of emergence from the pupae to ensure that they were virgins (Morimoto and Wigby, 2016). We prepared 237 mL, round bottom bottles with 20 mg of Formula 4-24® Instant *Drosophila* Medium, Blue mixed with 20 ml of distilled water, and we placed one virgin *D. melanogaster* in eachbottle.

To sex each fly, we anesthetized it using CO_2 and then placed it under a microscope to examine two morphological characteristics. The first characteristic was the presence or absence of sex combs. Sex combs are only found on males because they are used to hold the female in place during copulation (Hurtado-Gonzales *et al*., 2014). Under the microscope they appear as a singular dark patch on each front leg (Fig. 1).

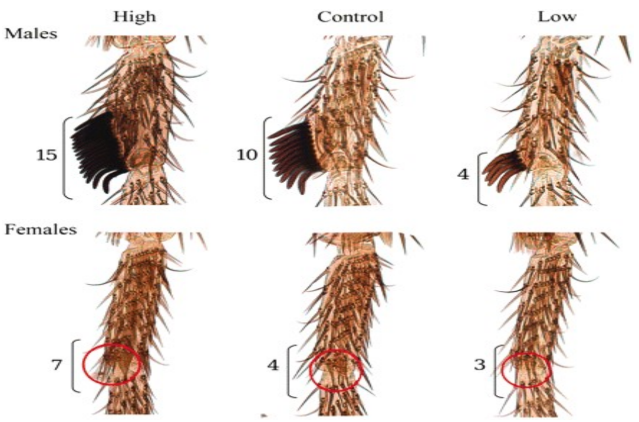

Figure 1. Sex combs seen in males (left) and the lack of sex combs in females (right) in *Drosophila melanogaster* (Ahuja and Singh, 2008)

The second characteristic that wee xamined was abdominal pigmentation. Males have thicker bands of dark pigmentation towards the posterior end of the abdomen(Rogers *et al*., 2014). This characteristic was harder to differentiate because there was variation with in sexes. Therefore, we used abdominal pigmentation as a second check after determining whether there were sex combs present. We labeled each bottle with the sex and a number, and we set-up more bottles to conduct the mating or isolation events in. We used an online "picker wheel" to randomize which flies were put into each of the four groups. We put the *D. melanogaster* in their assigned groups and then left them for 48 hours (Hollis and Kawecki, 2014; Hollis *et al*., 2019). After 48 hours, we transferred each of the *D. melanogaster* into an individual bottle and recorded how many days each one survived. We checked the *D. melanogaster* daily between 1600 and 1800. As the *D. melanogaster* died, we preserved them in a vial of alcohol and re-sexed flies that had been in mixed exposure bottles to double check the accuracy of our original sexing. We used an ANOVA and two t-tests for data analysis .We used an ANOVA for the first part of the experiment because it allowed us to analyze interactions between the sex of the fly and how long the fly survived. However, for the analysis of the one female, two males group and the one female, six males group, we conducted t-tests (one t-test for males and one for females) because we were no longer able to test for interactions between sex and lifespan since the males were exposed to rivals and the females were exposed to mates.

RESULTS

There was no significant effect of exposure to mates on lifespan in females ($p = 0.484$ and $p = 0.672$) and there was no significant effect of exposure to rivals on lifespan in males ($p = 0.484$ and 0.099). On average, females that were never exposed to a male survived for 2.10 more days than females that were exposed to one male (Fig. 2). This trend was not statistically significant ($p = 0.484$). Females that were exposed to 6 males survived for 2.65 more days than females that were exposed to 2 males (Fig. 2). This trend was not statistically significant ($p = 0.672$). On average, males that were not exposed to any other flies survived for 3.7 more days than males that were exposed to one female (Fig. 3). This trend was not statistically significant ($p = 0.484$). Males that were exposed to one female and one other male survived for 6.96 days more than males that were exposed to one female and 5 other males (Fig. 3). This trend was not statistically significant ($p = 0.099$).

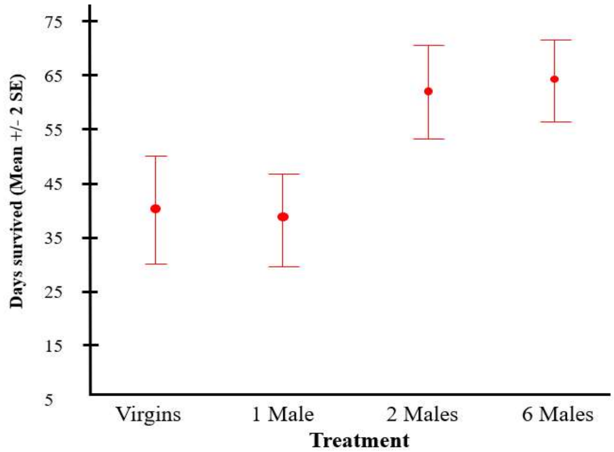

Figure 2. Average number of days survived by female *Drosophila melanogaster* after being exposed to the indicated number of males for 48 hours

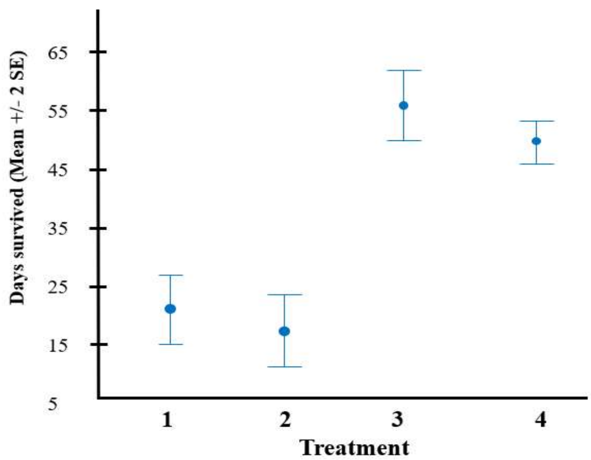

Figure 3. Average number of days survived by male *Drosophila melanogaster* after being exposed to no other flies (treatment 1), one female (treatment 2), one female and one other male (treatment 3), or one female and five other males (treatment 4) during the 48-hr exposure period

DISCUSSION

There was no significant difference in lifespan between females exposed to no males versus one male or between females exposed to two males versus six males for a 48-hour mating period. There was also no significant difference in lifespan between males exposed to no males or females versus no males and one female or between males exposed to one female and one other male versus one female and five other males for a 48-hour mating period. Our hypothesis that females that are exposed to a larger number of males would have a shorter lifespan was not supported by our data, and our hypothesis that males that were exposed to a larger number of males would have a shorter lifespan was also not supported. Castrezana *et al*. (2017) found that in *D. melanogaster,* a female's lifespan only increases after a single mating event. After that event, if the female mates with a virgin male, lifespan decreases, likely due to male harassment and coercion. Males can harm females before and during mating and this injury may lead to the decreased lifespan (Castrezana *et al*., 2017). Markow (2011) studied wild *D. melanogaster* and found that females captured after reaching maturity survived longer than females captured before reaching maturity. It was assumed that those caught after reaching maturity had mated in the wild, but there was no way to verify this or to know how many times the females had mated (Markow, 2011). Branco *et al*. (2017) studied *D. melanogaster* and found that mating can lead to a change in gene expression in males that leads to a decrease in lifespan. Of the studies discussed above, Branco *et al*. (2017) used an experimental design most similar to ours. They used 48-hour exposure periods; however, they isolated their males in cohorts of 45, and the cohort was exposed to approximately 90 females during the exposure period. We believe that differences in experimental design could be why our data showed different trends.
Hoffman *et al*. (2021) found that there were no consistent differences between lifespan of female *D. melanogaster* based on exposure to mates using an experimental design similar to ours with single, timed exposure periods. Chen *et al*. (2005) did a study similar to ours, using the fly *Pseudacteontricuspis* (Phoridae). They found that lifespan was more consistently impacted by temperature and sugar availability than by mating. In our experiment, we held temperature and food availability steady which could explain why there was no significant difference between the groups.

Because this experiment was carried out in three parts, we were unable to run a statistical analysis that compared across all parts. We were not able to determine if there was a significant statistical difference between females that were exposed to 0 males or one male versus females exposed to two males or six males. We were also unable to determine if there was a significant statistical difference between males that were exposed to no females and no males or one female and no males versus males exposed to one female and one male or one female and five males. Although statistical comparisons cannot be made between these groups, we think the observed differences may be due to problems with mold from the original colonies used in the first experiment The other source of potential error was that there were slight age differences between the flies. This occurred because of the large number of flies that were needed for each part of the experiment. It was not possible to collect all of the virgin flies needed in one day, so we used a five-day window to collect emerging flies. However, since we randomized group assignment, this error should have been controlled for. In the future, it would be interesting to track the actual number of mating events. In our study we used a mating period where we assumed copulation was taking place. However, since we did not track the actual mating events, we have no way of knowing whether the flies that were exposed to more males actually mated more than the flies exposed to fewer males. Another avenue of study would be to test additional males. We chose to test two and six males because in nature females mate, on average, between two and six times (Markow, 2011). A final idea for future studies could involve only exposing the flies to flies of the same-sex. This would remove the reproduction aspect and focus more on the social environment. It would be interesting to determine whether fighting or stress from the social environment would lead to shorter life spans.

Acknowledgements

We thank Dr. Travis Wilcoxen for his assistance with the statistical analysis, Dr. Samuel Galewsky for his guidance on housing fruit flies, and Sue James for her assistance with the budgeting and ordering process. We also thank the Millikin University Department of Biology for funding the research.

Conflict of Interest: There were no conflicts of interests for this study.

Funding: Funding was provided by the Biology Department at Millikin University in Decatur, Illinois.

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