

The Effect of Climate Change on the Sex Outcome of *Drosophila Melanogaster*

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Abstract

Many insects and amphibians use temperature-dependent sex determination, which is based on temperature to determine if the offspring is male or female. In the face of climate change, this method of sex determination can be threatening to populations, as the increasing temperature can cause there to be more of one sex. *Drosophila melanogaster*, or fruit flies, are a model organism that have been used for years to study how certain environments may impact a greater organism population. Research on the temperature's effect on their size, viability, and genetics has been conducted, but not on how temperature may impact their sex outcome. This research project looked at how higher and lower temperatures affect the sex outcome of *Drosophila melanogaster*. This was done by housing groups of the fruit flies in three different incubation temperatures, one set at 13 C, another set at 25 C (the control), and another one set at 30 C. Once the adult fruit flies had eggs, they were separated, and once the eggs hatched, the number of males and females were counted. This was done for each generation. It was found that there was a higher number of females than males overall in each incubator. Additionally, the fruit flies in the 30 C incubator were the smallest, and fruit flies incubated at 13 C were the biggest. This shows that it is harder to produce males when the environment is not optimal, and that temperature variation has a major impact on the size of *Drosophila melanogaster*.

Insects and Climate Change

65% of the insect population could potentially go extinct over the next century (Center, F. Nasa. A. R., 2022, November 10). Dr. Kate Duffy, a former postdoctoral researcher at NASA's Ames Research Center in California's Silicon Valley, conducted a study examining how different species of insects would be affected by dramatic temperature swings in their natural environment (Center, F. Nasa. A. R., 2022, November 10). They found that 25 of 38 insect species they studied could face increased extinction risk over the next century (Center, F. Nasa. A. R., 2022, November 10). Since many insects are ectotherms, meaning that

they cannot regulate their internal body temperature and that they are very dependent on the conditions of their environment's climate, they might respond to climate change in an intense way compared to other animals (University of Maryland., n.d.). A way that ectotherms could cope would be by shifting to areas that have cooler climates, but that could potentially lead to the places they are leaving not having enough pollinators (University of Maryland., n.d.). This drastic species change could also potentially risk the genetic diversity of that insect species, which limits their ability to adapt to changes (University of Maryland., n.d.). Climate change is not just affecting insects' bodies, but also their fertility, hatching rate, food supply, and behavior (Scientists.,2022, December 9). Some insects can only survive up to certain temperatures, meaning if it gets too hot, an insect could die, or the heat could sterilize them, rendering the insect unable to reproduce (Scientists.,2022, December 9). There have been several studies done to inspect the effect of heat on insects, and quite a few have found that heat can damage insect sperm (Scientists.,2022, December 9). In a 2018 study, researchers used flour beetles as a model and found that male reproductive organs were damaged at heatwave conditions of 5 to 7 C above optimum, which was tested over five days, but did not damage female reproductive organs (Sales, K...., 2018). When it comes to hatching, insects must hatch at the right time in order to have a decent food supply, and enough time to mature so they are prepared for temperature change. Increase in temperature can cause insects to hatch too early, so they are then not lined up with the correct amount of food supply, or they can be born too late, which does not give them enough time to mature (Sales, K...., 2018). This could potentially impact species of birds, who may hatch before insects hatch, making them miss out on food (Sales, K...., 2018). On another hand, climate change could potentially lead to generalist insect species being pervading, such as disease carrying insects (University of Maryland.,n.d.). Climate change could also lead to an increase in heat loving insects or abnormal insect migration, which could lead to invasive species in that specific area.

Drosophila Melanogaster and Climate Change

Drosophila melanogaster is beneficial in insect related experiments because they are domesticated for laboratory studies, but still exist in the wild (Markow, T. A., 2015). They have been introduced to every area of the earth besides Antarctica, which shows adaptability (Drosophila Melanogaster). They have short generation times, which allows for multiple generations of data, and they are easy to raise (Markow, T. A., 2015). Drosophila melanogaster can serve as an

example for how climate change affects insects. They wake up from hibernation when their food supply is right at peak (September 7, 2023). They then mate and eat for a couple of weeks, then go back to being dormant. As climate change has been shifting the growing seasons, *drosophila melanogaster* has found a way to keep up, likely because of polygenic traits, which allow for faster adaptation (September 7, 2023). With these adaptations, different factors such as reproduction rate, fertility, genetics, morphology, and viability can be examined based changes in environmental conditions.

Mating relies heavily on temperature (Miwa, Y., Koganezawa, M., & Yamamoto, D., 2018). Most female, virgin, *Drosophila melanogaster* will mate between 20-25 C, but if the temperature gets higher, mating will decline (Dauwalder, B., 2008). A likely cause of this could be that increased heat leads to a decrease in courtship by males, and this decrease could be caused by male fertility (Dauwalder, B., 2008). Male *drosophila melanogaster* fertility decreases as temperature becomes higher, and becomes infertile at 29 C (Dauwalder, B., 2008). However, adults cannot withstand colder temperatures, which affects their offspring production due to there being a decrease in fertility (Mockett, R. J., & Matsumoto, Y., 2014). There has been much research done over the genetics of *drosophila melanogaster*. Temperature can contribute to how genes function in many ways, including how animals breed. A study was done on how temperature can line up with inbreeding (Torsten Nygaard Kristensen, Barker, F., Pedersen, K., & Volker Loeschcke., 2008). It found that there were higher levels of inbreeding among the flies that were kept in extremely warm temperatures, or extremely cold temperatures (Torsten Nygaard Kristensen, Barker, F., Pedersen, K., & Volker Loeschcke., 2008). This indicates that under harsh environmental conditions, there could be a loss of genetic diversity, which would lead to an increase in inbreeding (Torsten Nygaard Kristensen, Barker, F., Pedersen, K., & Volker Loeschcke., 2008).

Along with genetics, temperature can also have an impact on the anatomy and physiology of *drosophila melanogaster*. A study was done to observe how different temperature ranges could affect the development of various parts on a fruit fly (Crill, W. E., Huey, R. B., & Gilchrist, G. W., 1996). In this experiment, they started out in two parental groups, one being kept at a temperature of 18 C, and the other at 25 C (Crill, W. E., Huey, R. B., & Gilchrist, G. W., 1996). Once they laid eggs, the eggs were developed in a different areas (Crill, W. E., Huey, R. B., & Gilchrist, G. W., 1996). The parental group that was kept at 18 C then had eggs develop at 25 C, and the parental group kept at 25 C had eggs develop at 18 C (Crill, W. E., Huey, R. B., & Gilchrist, G. W., 1996). It was found then that the temperature the

parental group was at had little impact on the anatomical and physiological outcome of the eggs, but the temperature the eggs were raised at did (Crill, W. E., Huey, R. B., & Gilchrist, G. W., 1996). Eggs that developed at 25 C had a larger body and wings, increased maximal speed, and smaller eggs (Crill, W. E., Huey, R. B., & Gilchrist, G. W., 1996).

This then leads to the question of how temperature can have an effect on the viability and lifespan of *Drosophila melanogaster*. The life of *Drosophila melanogaster* is dependent on its metabolic rate, meaning that if they have a higher metabolic rate, they have a shorter lifespan. This is because higher metabolic rates increase aging (Mołoń, M., Dampc, J., Kula-Maximenko, M., Zebrowski, J., Mołoń, A., Dobler, R., ... Skoczowski, A., 2020). In a meta-analysis that was done, it was found that higher temperatures increase metabolic rate (Mołoń, M., Dampc, J., Kula-Maximenko, M., Zebrowski, J., Mołoń, A., Dobler, R., ... Skoczowski, A., 2020). It can be concluded then that higher temperatures can decrease the lifespan of *Drosophila melanogaster* (Mołoń, M., Dampc, J., Kula-Maximenko, M., Zebrowski, J., Mołoń, A., Dobler, R., ... Skoczowski, A., 2020).

Climate change can have an impact on a multitude of factors that factor into *Drosophila melanogaster* life, but there has been miniscule research done over climate change effect on the sex of *Drosophila melanogaster*, which leaves the gap of how climate change may affect the sex of *Drosophila melanogaster*. *Drosophila melanogaster* uses temperature-dependent sex determination, meaning that the temperature of the environment outside the eggs will determine the sex. With there being warmer temperatures, and less variation, it could lead to there being more of one sex (Gilbert, S. F., 2015).

Objectives

The main objectives of this research project are to identify how higher and lower temperatures may affect the sex outcome of *Drosophila melanogaster*. Secondary objectives include looking at viability, reproduction, size, and development of these fruit flies. In order to reach this goal, there would be requirements necessary:

- Looking at reproduction rate in the three different incubators that would be used (at three different temperatures) for *Drosophila melanogaster*.
- Examining connections between temperature and sex.
- Comparing how long it takes for *Drosophila melanogaster* to reach maturity in their varying habitats.
- Looking at how morphology is different between the three incubators.

Methodology and Approach

For my methodology, my plan is to have 3 incubators set up for *Drosophila melanogaster*, they will be set at 13 C, 25 C, and 30 C. 13 C is the lowest temperature these fruit flies can survive, 25 C is their normal temperature range, and 30 C is the highest temperature they can withstand. Initially, there were trays of water set in each incubator, which acted as the authentic humidity of *Drosophila melanogaster* natural habitat. However, flies were getting too much precipitation, so the tray was completely taken out in all incubators. Each incubator initially started out with 6 sample tubes, 5 of them containing 4 or more *Drosophila melanogaster* inside. Due to technical difficulties, all but 3 of the tubes had all females instead of a mix of females and males, but 3 of the tubes had a mix of males and females. They would have 12 hours of light and 12 hours of darkness. Over the next few weeks, the flies from the tubes that came with both males and females were separated and mixed with the other tubes as they reproduced. It was then decided that another order of flies would be made, and from there, there was no mixing between tubes. After 7 days of collecting data, it was decided to use a different way of collecting data. A different batch of flies were ordered, and both the 13 C and 25 C incubators started out with 63 fruit flies per incubator, but the 30 C started out with 17. This was due to a shipping error, where the 30 C fruit flies arrived later, which meant generation 2 was already produced, and most of generation 1 had passed away. It was decided that each generation would be labeled as G1, G2, G3, etc. G1 was the initial group of flies, and they were first put in what was labeled tube 1. Once G1 has their offspring, the offspring would be left in tube 1, and the adults would be moved to a different container, which would be called tube 2. The offspring left in tube 1 would be called G2, and once they mated and reproduced, they would be moved to tube 3, and their offspring would be called G3. In order to count the flies, they were examined under a microscope using the drug triethylamine to put the flies to sleep. To differentiate between the sex of *drosophila melanogaster*, it was looked at which flies had black striped pointed ends (females) and which had fully black rounded ends (males). Testing the amount of females and males in each generation will help answer the question of how temperature variation might have an effect on sex. Some technical issues that could occur would be other factors causing harm to the fruit flies, or them potentially escaping. Constraints could consist of identifying some of the bugs wrong, for example, mistaking a female for a male, or vice versa.

Results and Analysis

After conducting the methodology listed above, there were two different quantitative test types available for my results: the independent T test and the paired samples T test. For the independent T test, temperature was put under the grouping variables, and its defined groups were 25 C and 13 C the first time the test was run, and 25 C and 30 C for the second time it was run. The tested variables were generation 3 male and female Drosophila. This test was run for all the temperature numbers put together, and for each individual temperature. There was a connection found between temperature and generation 3 Drosophila when the independent T test was done on all the temperatures combined with 25 C and 13 C as the defined groups. For the paired samples T test, two pairs were tested, which was the male fruit flies in generations 1 to 3, and female fruit flies in generations 1 to 3. This test was also conducted on all the temperatures and on the individual temperatures, and a connection was found between generation 1 males and females to generation 3 males and females when this test was done on the 30 C fruit fly numbers.

Table 1.

Independent T test results comparing temperature (13 C to 25 C) on generation 3 Drosophila melanogaster

Variables	t Value	df	One-Sided p	SE of Diff.
MalesG3	1.880	13.000	.041	1.17753
FemalesG3	1.976	13.000	.035	2.74705

Temperatures at 13 C and 25 C had an effect on the sex ratio of generation 3 female and male Drosophila melanogaster. Both male and female generation 3 numbers under the one-sided p column were numbers close to .05, which means that there was a correlation between the sex ratio of the fruit fly population and what temperature that population lived at (13 C or 25 C).

Table 2.

Paired Samples T test results comparing temperature (30 C) on generation 1 to generation 3 Drosophila melanogaster

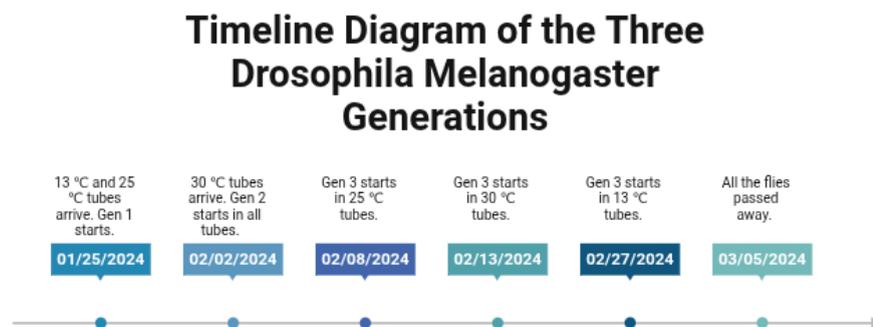
Variables	Mean	SD	SE of Mean	t Value	df	One-Sided p
MaleG1-MaleG3	-5.23077	13.84530	3.84000	-1.362	12	.099
FemaleG1-Female	-6.07692	17.35637	4.81379	-1.262	12	.115

G3						
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The results between generation 1 male fruit flies to generation 3 male fruit flies that lived in the 30 C incubator showed to have a connection based on the one-sided p column. This means that the sex ratio of the male fruit flies kept at 30 C had a change between generation 1 to generation 3, which also implies that at higher temperatures, the sex ratio of *Drosophila melanogaster* is impacted.

Chart 1.

Timeline Diagram of the Three *Drosophila Melanogaster* Generations in temperatures 13 C, 25 C, and 30 C



This shows a timeline of the arrival of the fruit flies, when each generation started, and when the fruit flies passed away.

Conclusion

More females were produced than males on average in all three temperatures. In total, three generations were tested. For the first three days, there were only flies in two of the incubators, in 13 C and 25 C, with 13 C having one extra container compared to the 25 C. The 13 C incubator ended up with 63 fruit flies from generation 1, 52 fruit flies from generation 2, and 0 from generation 3. This added up to a total of 115 fruit flies from incubator 13 C. 25 C incubator produced 63 flies from generation 1, 109 flies from generation 2, and 60 from generation 3, with a total of 232. The 30 C incubator produced 7 fruit flies in generation 1, 325 flies from generation 2, and 7 from generation 3. This was a total of 349 fruit flies from 30 C incubators. The 30 C

incubator received fruit flies on day 4 and received four containers that had flies that had already mated. This allowed for all the incubators to be at equal points in the experiment. Initially, all incubators of flies started growing and reproducing at the same rate, but by day 4, the incubator at 13 C had stopped with any type of reproduction or development. By day 8, the 13 C incubator had restarted its reproduction and development, which shows that the reproduction and development rate of *Drosophila melanogaster* could be affected by the temperature at which it grows. The 30 C incubator also experienced some changes. Initially, the fruit flies within that incubator seemed to reproduce and develop faster than the other incubators, but then development halted at day 9. By day 9, it was observed that most of the fruit flies had died off, with the exception of 1 or 2 flies. It was also observed by day 8 that the fruit flies in the 30 C incubator stopped reproducing. It was led then to believe that the higher temperature of 30 C had more of an impact on the survival rate of *Drosophila melanogaster* instead of the sex outcome. For the 25 C incubator, the fruit flies within this incubator reproduced and developed at a normal rate, and did not show any signs of randomly dying off, or randomly halting reproduction. One noticeable difference that was observed regarding the size in the fruit flies was that the ones grown at 13 C had larger bodies, whereas the ones at 30 C had smaller bodies. This could be due to the fact that the 13 C fruit flies took a longer time to develop, whereas the 30 C fruit flies took a significantly shorter time to develop, which led to a greater generation disparity between the two incubators. Looking at the habitat in which these flies grew in, it was observed that the 25 C and 30 C had a greater impact on the *Drosophila* habitat. The tubes that were held at warmer temperatures were observed to be more infected with a form of bacteria in the food, and at the 30 C incubator, it turned the food to more of a gel-like substance. In comparison, the food at 13 C was still infected, but a lot dryer, which may or may not have been more beneficial for the fruit flies. There showed no difference in sex ratio among the different temperatures for *Drosophila melanogaster*, but reproduction, development, viability, and size were affected.

Discussion

Implications

Within this study, there were multiple different implications and limitations. Some implications within the research were that if temperatures continue to increase due to climate change, then the viability of insects in general will decrease. An example of this is that the fruit flies that were kept at 30 C did not live as long as the flies in

the other incubators. It was then also implied in the experiment and researched literature that the reproduction rate of insects could decline due to increasing temperatures. By day 8 of the experiment, the Drosophila at 30 C had decreased in larvae production. In addition, it was also found in a separate study that any temperature above 29 C makes male Drosophila infertile, so it can be inferred that within generation in a 30 C incubator, they became more infertile. With this information, it can be gathered that climate change can lead to population decline of insects, which would be detrimental to the environment. Regarding size of insects, it can be implied that the size of insects can be impacted by an increase or decrease in temperature. With increased temperature, the Drosophila showed to have smaller bodies, and with decreased temperature, they had larger bodies. This notion then contradicted the literature though because it was found in a different study that Drosophila melanogaster raised at 25 C had larger bodies. Overall, it implies that temperature does have a direct impact on an insect's size. Throughout the whole experiment, one thing that was constantly affected negatively was the Drosophila food. It was affected negatively in all incubators, but especially in the 30 C incubator. It would start to grow bacteria within a day, which may or may not have affected the health of the fruit flies. This implies that higher temperature can lead to a negative effect on the food of Drosophila melanogaster.

Limitations

While this study had definite results, there were many limitations within. Regardless of the temperature the containers were in, all of them got infected with different forms of bacteria. Most of the time, the bacteria infected the food, which could have led to some health problems for the fruit flies, or even death for them. As more generations were produced, an increased tolerance of triethylamine, the drug used to put them to sleep, was shown. This was a limiting factor because it then made it harder to gauge how much to give them, which could have led to death for some of the flies if they were given too much. Triethylamine is also not a natural drug that Drosophila melanogaster is introduced to in the wild, so it could have other health effects that are unknown. The fruit flies were kept in small tubes that had food and a place for them to rest inside, and a cotton ball stopper was used as a lid so that they could be kept in their container but still be able to breathe. Towards the end though, the cotton ball stopper was not able to stop some of the larvae from escaping, especially in the 25 C incubator. When they escaped, they also then proceeded to reproduce. This then skewed the number of flies present in the tubes, and the number of flies for the next generation. Additionally, the

extraction method of the flies was also a limitation. When the flies would fall asleep due to the triethylamine, some of them would get buried in the food or caught in the resting place they had, which then affected the number of flies that were counted once they were taken out.

Future Directions

Since there seemed to be a connection between temperature, development, viability, and body size of insects in this experiment, more research into how temperature impacts those factors would be necessary to improve this study. I also originally wanted to use *Pieris brassicae* as a model organism since they serve as a good model for butterflies in general, so being able to use them in the future as the model organism for a study with the same parameters as this one would be beneficial as it is another insect that could be observed. A limiting variable that would be useful to test too is how temperature affects insects' food durability, since all of the food in the plastic containers became infected. If temperature were to have a negative effect on an insect's food, it could then lead to an indirect negative effect on those insects.

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