The effect of maternal age on fertility in *Drosophila melanogaster*

Roxana Izaguirre
Department of Biology, Lake Forest College
BIOL 342: Developmental Biology
Dr. Delventhal
Lake Forest, Illinois 60045

Purpose statement

The aim of this experiment is to determine if the age of the maternal parent affects the number of offspring *Drosophila melanogaster* can produce. This will be accomplished by counting the number of eggs produced by D. melanogaster in both young and aged conditions. Reproductive capacity is age-limited for virtually all organisms (Churchill et al., 2019). In *Drosophila*, a germ stem cell (GSC) serves as the source for the oocyte (Barresi and Gilbert, 2020, Ch. 5). These GSCs are housed within the adult ovarian stem cell niche, and reproductive senescence is partially brought on by the cellular aging of these GSCs (Barresi and Gilbert, 2020, Ch. 5; Want et al., 2011). This allows us to predict that fertility will decline as the maternal parent matures. However, there is also the possibility that fertility increases as the maternal age matures or that there is no change in fertility.

Methods

Collection of female Drosophila

We used wild-type *Drosophila melanogaster* Canton S flies to conduct all experiments and maintained them in 40 ml vials containing 7 ml of pre-prepared fly food. Males and females were kept in separate vials. The female vials contained five females in each vial, and the male vials contained approximately 40 males per vial.

First, we captured and aged female virgins (n=20) for 28 days following eclosion.

Approximately every 2-4 days throughout those 28 days, we transferred the flies from vial to vial to ensure they received clean food. Later, we utilized these flies to represent the aging maternal condition. After 21 days, virgin females (n=28) and males (n=133) were both collected once again. These new flies, which represented the young maternal condition, were aged for seven days following eclosion. Similar to the other conditions, the new flies were moved to fresh vials approximately every 2-4 days to ensure clean food. Regarding the male *Drosophila* collection, it did not matter if they were virgins or not.

Egg-laying plates

The mating cages were set up after all the aged and young female virgins had been collected. The mating cages were built of plastic bottles with holes punched around the outside and topped with an egg-laying plate that had a yeast paste in the center. Each condition had five mating cages, each with five females and five males. The flies were given 24 hours to mate. After 24 hours, the number of eggs deposited was counted and replaced with a new egg-laying plate for a second count. Due to early copulation's delay, the first 48 hours of egg laying following initial mating can often vary. Therefore, the flies from the second count were recorded and replaced with a new egg-laying plate for the final third count. Once again, 24 hours later, the number of eggs laid on the third plate was counted, and the parents were discarded.

Statistical analysis

The data was graphed and analyzed using a t-test with two samples assuming equal variance in Microsoft Excel.

Results

Plate #	Maternal age condition	1st plate's egg count (2/16)	2nd plate's egg count (2/17)	3rd plate's egg count (2/18)
1	Aged	15	30	26
2	Aged	35	39	21
3	Aged	18	17	12
4	Aged	17	21	26
5	Aged	30	40	52
6	Young	43	51	105
7	Young	52	62	100
8	Young	38	39	45
9	Young	61	57	101
10	Young	38	42	82

Table 1. The number of eggs laid on each plate for each maternal condition. Aged Drosophila represents plates 1-5 and young *Drosophila* represents plates 6-10. Egg-laying plates were replaced three times, and eggs were counted afterward.

Table 1 displays the number of eggs on each plate for all 10 plates over the three egg-laying plate swaps. The raw data demonstrates an increase in the number of eggs deposited in the old versus young condition, particularly on the 3rd plate switch. We also see that plate eight has considerably fewer eggs compared to the other young maternal conditions, especially on the third egg plate count.

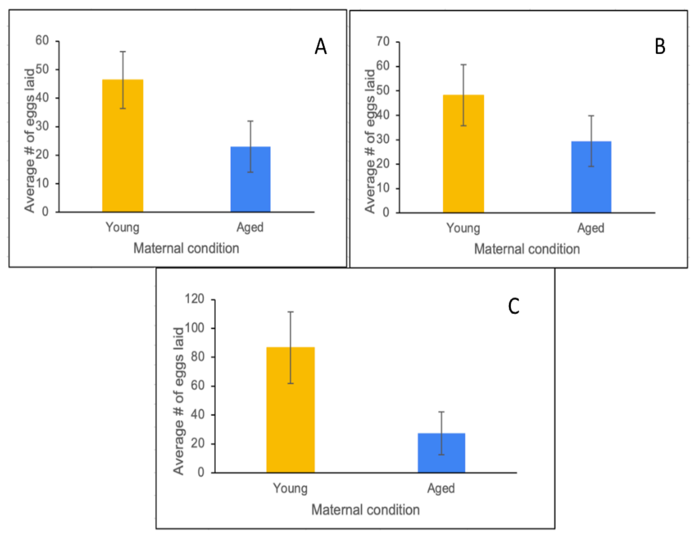

Figure 1.

In our comparison of maternal age on the fertility of young flies and aged flies in the first plate (Fig. 1A), we found that the number of eggs laid by the young flies (M = 46.4; SD = 9.96) was larger than the number of eggs laid by the aged flies (M = 23; SD = 8.92), with a difference of 23 flies ($p = 0.0045$). We see similar results in our second plate (Fig. 1B) as well: the number of eggs laid for the young flies (M = 48.2; SD = 12.48) was significantly larger than the number of eggs laid for the aged flies (M = 29.4; SD = 10.36), with a difference of approximately 18 flies ($p = 0.032$). As expected, the third plate (Fig. 1C) showed similar results: the number of eggs laid for the young flies (M = 86.6; SD = 24.89) was significantly larger than the number of eggs laid for the aged flies (M = 27.4; SD = 14.89), with a difference of approximately 59 flies ($p = 0.0018$). Overall, the average number of eggs deposited on all three plates (Fig. 2) by young flies (M = 60.4; SD = 15.78) was substantially greater than the number of eggs laid by aged flies (M = 26.6; SD = 11.39), with a difference of roughly 34 flies ($p = 0.013$). In other words, older flies lay fewer eggs than younger ones.

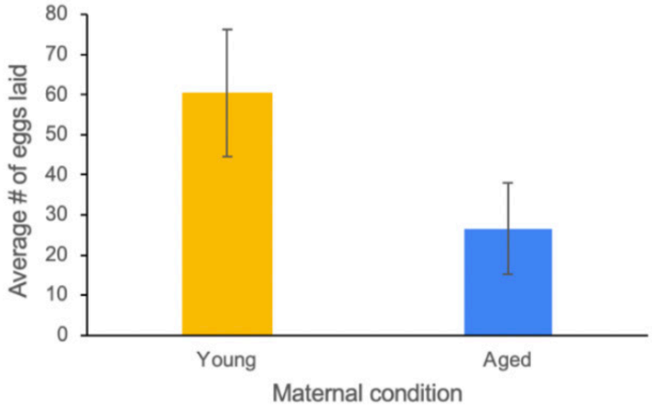

Figure 2. The average number of eggs laid on each plate for each maternal condition. The average number of eggs laid was higher for the young maternal condition compared to the old. Error bars represent the standard deviation determined in Excel.

Discussion

We found the effects of maternal age on fertility to be pronounced. In *Drosophila melanogaster*, we discovered that fertility diminishes as maternal age increases in all three egg-laying plates. It was observed that plate eight (Table 1) consistently generated fewer eggs than the other juvenile flies. One probable explanation is that one of the flies, whether male or female, was sterile, meaning that no eggs were produced. Apart from that, the results were as expected, especially given that fertility diminishes with age in many organisms (Finch et al., 2010). We also know that GSCs reside in the adult ovarian stem cell niche and that the cellular aging of these GSCs contributes to reproductive senescence (Barresi and Gilbert, 2020, Ch. 5; Want et al., 2011).

There is always the risk of error with experiments. In this scenario, the first, second, and third egg-laying plates were all counted by different members of the group, which adds to the variation. Although counting eggs on a plate may appear simple, it is easy to overlook the eggs that gather on the edge of the petri dish.

Future research could focus on understanding environmental factors that affect fertility, such as temperature. Given that this study focused on the influence of maternal age on fertility, it would be interesting to investigate the effect of paternal age on fertility in terms of sperm. Finally, it would be interesting to examine plate eight closely to see whether there was a potential for a sterile fly or to discover other factors that might have contributed to the low number of eggs compared to the other young conditions. Looking at the larger picture, we notice not just a decline in fertility because of an increase in maternal age in *Drosophila melanogaster,* but also how it compares to fertility in other species. While *D. melanogaster* is frequently employed as a model organism for humans since the great majority of its biology is preserved, it also demonstrates a lot about the fertility of humans and factors that can affect it.

References

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