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

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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

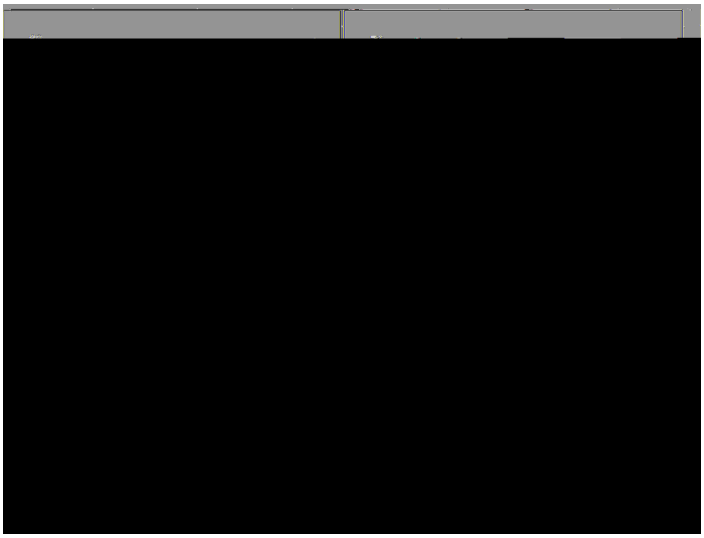
The data was graphed and analyzed using a t-test with two samples assuming equal variance in Microsoft Excel.

Results

Plate #	Maternal condition	1st plate's egg count	2nd plate's egg count	3rd plate's egg count
1	Aged	15	30	26
2	Aged	25	28	24
3	Aged	18	17	12
4	Aged	17	21	26
5	Aged	31	41	62
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 displayaid by the young flies (M = 46.4; SD = 9.96) was larger than the n



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 high