

The Effects of Parental Age on Egg Production, Hatchability Of the Eggs, and Survival of the Offspring in *Drosophila melanogaster*

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Introduction

The fact that the age of the parents has a definite effect on the survival of the offspring in *Drosophila melanogaster* is now well known (2, 9, 10). This investigation was undertaken to establish additional criteria in order to determine the basis for parental age effects on the offspring in this species.

Methods

For fecundity studies all cultures were maintained under conditions of constant light at 25°C., while the groups selected for survival studies were raised at the same temperature under conditions of darkness and constant light. The stocks raised under conditions of darkness were exposed to light for observation at the same time period each day, total exposure to light not exceeding one hour per week. Adults of various ages from stock cultures of *Drosophila melanogaster* Meigen (Oregon-R strain) were placed in fresh culture bottles and eggs were collected for a period of 24 hours. The adults which emerged during the first 24 hours from these eggs were young from parents of various ages. Eggs were collected from these young adults during the first 48 hours following their emergence. The adults which emerged during the first 24 hours from this collection were used to set up the parental generation. The age of the parents was calculated on the basis of zero days at the time of the first adult emergence. Eggs were collected from this parental stock when the flies were 0 to 2, 2 to 3, and 30 to 31 days old. The offspring which emerged during the first 10 hours from these egg collections were used to set up the groups offspring from parents 0 to 2, 2 to 3, and 30 to 31 days following their emergence. In this manner the virginity of the non-mated offspring was insured.

The flies were transferred to fresh culture bottles at least every five days. For studies on fecundity the initial population density consisted of 5 females and 4 males per half-pint milk bottle for a total of at least 65 females and 52 males or 13 groups per parental age selection for the mated offspring, and 8 females per half-pint milk bottle for a total of at least 120 females or 15 groups for the non-mated offspring. For survival studies the initial population density consisted of a maximum of 8 females and 8 males per half-pint milk bottle for a total of at least 52 females and 55 males per selection in the mated populations, and a maximum of 15 females or 15 males per half-pint milk bottle for a total of at least 57 females and 52 males for the non-mated populations. Egg production and hatchability was observed in the offspring at 0 to 2, 2 to 3, 5 to 6 days following their emergence, each day for the 10 to 21 day period, and every 5 days thereafter.

During this experiment a cornmeal-molasses medium was used with Dowicide® solution as a mold inhibitor. As a further precaution all

stock cultures were periodically checked for microsporidian infections (17).

Results and Discussion

The observation that parental age had no effect on the duration of the preimaginal stages of offspring in *Drosophila melanogaster* is consistent with previous work in this species (2, 9). This observation has also been confirmed in the housefly, *Musca domestica* Linnaeus, and the body louse, *Pediculus humanus humanus* Linnaeus (3 16, 4). However, the duration of the preimaginal period was slightly shorter in offspring of *Drosophila* when raised under conditions of constant light as compared to those raised in the dark. On the other hand, in the yellow mealworm, *Tenebrio molitor* Linnaeus, offspring from old parents had a shorter larval life when compared to those obtained from young parents (6, 19). In the milkweed bug, *Oncopeltus fasciatus* (Dallas), offspring obtained from the first and last eggs laid required a longer developmental time than those obtained from eggs laid by middle-aged parents (15).

In Table 1 are shown the number of eggs produced and hatched per female for the mated offspring. The mean values and the standard errors per female are based on the average for each group and the number of groups for each parental age selection. The period of egg production was 46 days for offspring from 0 to 2 day old parents, 56 days for offspring from 2 to 3 day old parents, and 51 days for offspring from parents 30 to 31 days old. The period of egg hatchability was 31 days following eclosion for offspring obtained from the first eggs laid, 51 days for offspring from eggs laid by 2 to 3 day old parents, and 41 days for offspring obtained from old parents. These results are in agreement with preliminary data reported for this species (13). Egg production and hatchability, initially high in all groups, gradually decreased from the 5th day onwards until the 11th to the 13th day of adult life, and then showed an increase until the 12th to the 15th day of adult life, gradually decreasing thereafter. With the exception of egg hatchability for offspring from 30 to 31 day old parents, this increase was significant. Egg production in offspring from 0 to 2 day old parents was consistent with that of the other two groups until the 26th day of adult life, after which except for the 40 to 46 day period there was a significant decrease. On the other hand, although egg hatchability in this selection showed a significant decrease after 18 days, when compared to offspring from 2-3 day old parents and after the 21 days with the exception of the 18-19 and 25-26 day periods, there was no significant difference when compared to offspring from 30-31 day old parents. In general, there was no significant difference in the number of eggs produced per female for offspring from parents 2 to 3 and 30 to 31 days old from the 5th to the 51st day of the egg-laying period, but with the exception of the 25-26 and 40-41 day periods egg hatchability was significantly lower for offspring from 30 to 31 day old parents after the 20th day of adult life. Previous work in *Drosophila melanogaster* has shown that there is a sharp decrease in the number of adult progeny when the female parent is 30 days or older (2). In addition,

TABLE 1
Number of Eggs Produced and Hatched Per Mated Adult Female Fly. Values Are Given With the Standard Errors.

Egg Collection in Days Following Adult Emergence	Offspring From Parents 0 to 2 Days Old				Offspring From Parents 2 to 3 Days Old				Offspring From Parents 30 to 31 Days Old			
	Eggs Produced Per Female	Number of Eggs Hatched	Percent of Eggs Hatched	Percent of Eggs Hatched	Eggs Produced Per Female	Number of Eggs Hatched	Percent of Eggs Hatched	Percent of Eggs Hatched	Eggs Produced Per Female	Number of Eggs Hatched	Percent of Eggs Hatched	Percent of Eggs Hatched
0 to 2	39.1 ± 3.46	37.4 ± 3.67	95.7	95.7	28.3 ± 1.28	27.5 ± 1.24	97.2	97.2	36.3 ± 1.96	34.2 ± 1.79	94.3	94.3
2 to 3	55.9 ± 2.51	54.1 ± 2.51	97.0	97.0	48.2 ± 1.03	47.6 ± 1.12	98.8	98.8	54.3 ± 2.20	52.7 ± 2.27	97.1	97.1
5 to 6	61.6 ± 2.93	55.9 ± 3.19	90.7	90.7	54.8 ± 3.18	53.2 ± 3.24	97.1	97.1	61.0 ± 1.15	55.2 ± 1.48	90.5	90.5
10 to 11	48.4 ± 3.93	43.1 ± 3.40	91.1	91.1	40.0 ± 2.79	37.9 ± 3.41	94.8	94.8	42.7 ± 2.29	38.7 ± 2.51	90.6	90.6
11 to 12	47.3 ± 1.71	42.4 ± 1.70	89.7	89.7	49.3 ± 1.78	46.2 ± 1.79	93.7	93.7	42.3 ± 2.34	39.2 ± 2.63	92.7	92.7
12 to 13	47.4 ± 2.55	40.5 ± 2.55	85.4	85.4	49.1 ± 2.78	44.7 ± 2.97	91.0	91.0	47.9 ± 2.09	43.1 ± 1.75	90.0	90.0
13 to 14	55.3 ± 1.70	47.3 ± 2.66	85.5	85.5	43.5 ± 1.93	39.6 ± 1.89	90.8	90.8	51.2 ± 2.25	46.0 ± 2.81	89.8	89.8
14 to 15	56.3 ± 1.75	47.9 ± 2.64	85.1	85.1	45.6 ± 1.77	42.0 ± 1.92	92.1	92.1	49.9 ± 2.18	42.8 ± 2.21	85.8	85.8
15 to 16	55.3 ± 2.21	42.0 ± 3.10	76.0	76.0	44.4 ± 1.37	39.4 ± 1.68	88.8	88.8	46.7 ± 2.96	39.6 ± 2.85	84.8	84.8
16 to 17	51.4 ± 2.24	36.5 ± 3.05	71.0	71.0	42.2 ± 1.63	37.3 ± 1.94	88.4	88.4	39.2 ± 2.54	33.0 ± 2.57	84.2	84.2
17 to 18	43.8 ± 2.17	32.8 ± 2.53	74.9	74.9	42.9 ± 1.92	37.4 ± 1.76	87.2	87.2	40.1 ± 1.53	32.3 ± 1.74	85.5	85.5
18 to 19	44.5 ± 1.95	22.9 ± 3.50	51.5	51.5	39.6 ± 2.45	33.5 ± 2.20	84.6	84.6	41.8 ± 1.96	32.4 ± 2.34	77.5	77.5
19 to 20	42.3 ± 1.68	19.6 ± 4.13	46.3	46.3	39.5 ± 1.68	31.7 ± 1.71	80.3	80.3	39.2 ± 1.16	28.0 ± 1.58	71.4	71.4
20 to 21	38.2 ± 2.42	16.6 ± 3.16	43.5	43.5	37.4 ± 1.93	29.6 ± 2.25	79.1	79.1	36.3 ± 1.91	21.7 ± 2.65	59.8	59.8
25 to 26	25.3 ± 3.36	3.4 ± 1.27	13.4	13.4	26.7 ± 1.54	16.1 ± 1.54	60.3	60.3	28.8 ± 2.58	13.1 ± 2.18	45.5	45.5
30 to 31	14.8 ± 2.28	3.4 ± 0.91	22.2	22.2	23.8 ± 1.47	12.6 ± 2.31	52.9	52.9	23.8 ± 2.59	3.3 ± 1.08	13.9	13.9
35 to 36	7.8 ± 2.08				21.8 ± 1.21	6.8 ± 1.78	31.2	31.2	19.4 ± 3.18	1.6 ± 0.51	8.2	8.2
40 to 41	3.5 ± 1.03				10.6 ± 1.15	2.5 ± 0.93	23.6	23.6	7.5 ± 2.40	0.9 ± 0.52	12.0	12.0
45 to 46	3.7 ± 1.50				9.3 ± 3.95	1.7 ± 0.82	18.3	18.3	5.7 ± 2.17			
50 to 51					6.0 ± 2.69	0.4 ± 0.19	6.7	6.7	5.8 ± 2.07			
55 to 56					0.6 ± 0.33							

preliminary studies in this species indicate that there is a decrease in the duration of production of adult offspring when both parents are old, but this decrease is not observed if either parent is young (11). The observation that the duration of viable egg production is longer, and that the number of eggs hatched per female is significantly higher after the 26th day of adult life in offspring from parents 2 to 3 days old when compared to those obtained from 30 to 31 day old parents is in agreement with studies on *Musca domestica* where offspring from old parents had a reduced reproductive capacity (3). The increase in egg production and hatchability beginning with the 11th to the 13th day of adult life in the mated groups is consistent with studies on ovarian development and on the increase in the number of adult offspring when the female parent was 15 days old in *Drosophila* (18, 2). However, it must be noted that there was no increase in the percentage of eggs that hatched and that increased reproductive capacity in adults 11 to 13 days old was due only to an increase in the total number of eggs produced.

In Table 2 are shown the number of eggs produced per female for the non-mated offspring. Parental age had no appreciable effect on the duration of egg production, being 56 days for offspring from parents 0 to 2 days old, and 61 days for the other groups. Egg production was significantly higher from the 2nd to the 11th day of adult

TABLE 2
Number of Eggs Produced Per Non-mated Adult Female Fly.
Values Are Given With The Standard Errors.

Egg Collection in Days Following Adult Emergence	Offspring From Parents 0 to 2 Days Old	Offspring From Parents 2 to 3 Days Old	Offspring From parents 30 to 31 Days Old
0 to 2	25.9±1.29	20.6±2.51	30.6±3.17
2 to 3	46.3±1.15	29.0±2.48	39.2±3.33
5 to 6	45.9±1.27	32.4±1.91	37.6±3.00
10 to 11	45.0±2.16	27.8±2.30	37.8±2.40
11 to 12	41.0±2.74	30.9±1.00	39.8±2.84
12 to 13	37.9±2.23	35.6±0.74	39.9±2.35
13 to 14	37.2±1.46	36.4±1.36	41.0±2.35
14 to 15	41.0±1.14	36.5±0.91	40.8±2.23
15 to 16	40.9±1.48	35.9±1.06	39.6±3.31
16 to 17	41.0±1.32	37.0±1.80	42.0±2.58
17 to 18	37.2±1.23	36.8±2.14	42.2±2.13
18 to 19	35.9±1.29	36.8±2.03	39.0±2.22
19 to 20	36.9±1.15	36.4±1.33	38.8±2.39
20 to 21	35.6±1.36	35.6±2.03	39.9±2.53
25 to 26	31.5±1.23	27.3±1.19	33.5±2.23
30 to 31	25.8±1.94	21.2±1.32	33.0±2.35
35 to 36	25.2±1.33	20.2±1.79	25.4±1.71
40 to 41	19.8±0.51	17.0±1.25	22.1±1.71
45 to 46	16.1±1.34	12.8±2.46	15.2±1.51
50 to 51	8.6±1.73	12.6±2.02	13.0±1.77
55 to 56	2.3±1.22	10.1±2.55	9.0±2.04
60 to 61		3.5±0.71	1.2±0.78

life for offspring obtained from the first eggs laid and significantly lower after the 51st day of adult life when compared to offspring from parents 2 to 3 and 30 to 31 days old. Egg production was generally significantly higher in offspring from parents 30 to 31 days old until the 12th day and for the period of the 25th to the 45th day of adult life when compared to offspring obtained from 2 to 3 day old parents. Total egg production was highest in non-mated offspring obtained from the first and last selection of eggs and lowest for offspring from parents 2 to 3 days old.

In this study the total number of eggs produced was greater in the mated groups, and was especially pronounced in offspring from young parents when compared to that of the non-mated groups. Also, the number of eggs produced for each observational period was generally significantly higher in the mated groups until the 18th day for offspring from young parents, and until the 15th day of adult life for offspring from old parents. These results are in agreement with observations in the yellow-fever mosquito, *Aedes aegypti* (Linnaeus), where the total number of eggs produced was greater in the mated groups (5).

In Table 3 are shown the number of adult flies and the average survival time for mated and non-mated offspring from parents 0 to 2 and 2 to 3 days old under conditions of constant light and darkness.

TABLE 3
Average Survival Time (days) and Number of the Adult Flies.
Values Are Given With the Standard Errors.

	Offspring From parents 0 to 2 Days Old		Offspring from Parents 2 to 3 Days Old	
	Mated	Non-Mated	Mated	Non-Mated
Raised Under Conditions of Continual Darkness				
Male	43.1±1.05	41.5±1.40	43.8±1.65	39.2±1.15
N	68	52	71	62
Female	42.5±1.60	46.4±1.05	40.8±1.77	47.7±1.51
N	69	75	52	63
Total	42.8±0.71	44.4±0.88	42.6±1.21	43.5±0.95
N	137	127	123	125
Raised Under Conditions of Continual Light				
Male	32.9±1.05	35.4±1.42	33.0±1.33	36.2±1.43
N	63	65	55	82
Female	38.6±1.47	43.4±1.53	39.6±1.54	44.4±1.18
N	56	57	60	78
Total	35.6±0.89	39.2±0.72	36.4±0.94	40.2±0.90
N	119	122	115	160

There was no significant difference in the survivals of mated and non-mated offspring obtained from parents 0 to 2 or 2 to 3 days of age for both groups, but as would be expected, offspring raised under conditions of constant light had a shorter life span than those raised in the dark. In general, the survivals of mated and non-mated male and female offspring from young parents raised under conditions of constant

darkness were not significantly different from previously reported work (10).

The fact that selection of the first eggs may have detrimental effects on the offspring was observed in the human body louse, *Pediculus humanus humanus*, where the strains obtained from eggs laid during the first and second days of oviposition died out during the first or second generation, whereas eggs selected during the third and fourth days of oviposition yielded successive generations for the duration of the experiment, these parental age effects being more pronounced at lower temperatures. However, the reproductive capacity was not significantly affected by parental age in this species (4).

Previous studies on offspring from young and old parents in *Tenebrio molitor* have lead to the conclusion that activating ions and enzyme activity may be associated with nutrition and thus responsible for parental age effects (8). Observations reported for *Drosophila melanogaster* and the confused flour beetle, *Tribolium confusum* Jacquelin duVal, have indicated a relationship between phosphatase activity and egg production in the aging female (1, 14). Further studies in *Tenebrio* have shown a greater decrease in methionine in beetles from old parents, when compared to those from young parents, as they aged (7). Similar studies have shown that the cystine-cysteine complex was present only in homogenates of third- and fourth-instar larvae obtained from young parents in this species (20). It has been suggested that these sulfur amino acids may function in the tying up of free radicals and thus retard aging processes. Preliminary studies in *Drosophila melanogaster* have shown histological differences in the corpora allata cells of third-instar larvae obtained from young and old parents (12). This finding leads to the conclusion that a more subtle humoral influence may be involved in the effects of parental age on the offspring.

Summary

The effect of parental age on egg production and hatchability of continually mated and non-mated offspring in *Drosophila melanogaster* (Oregon-R strain) was observed. Continually mated offspring from 2 to 3 day old parents had the longest period of egg production and hatchability when compared to continually mated offspring from parents 0 to 2 and 30 to 31 days old. In this case the offspring obtained from the first egg selection had the greatest variability and the poorest performance. The secondary rise in fecundity observed in 11 to 13 day old adults was due solely to an increase in the number of eggs produced. Parental age had no pronounced effect on the duration of the egg-laying period in non-mated offspring, but egg production was highest in offspring obtained from the first and last selection of eggs. However, total egg production was higher in the mated groups when compared to that of the non-mated groups, this effect being more pronounced in offspring from young parents. There was no significant difference in the survivals of offspring obtained from parents 0 to 2 or 2 to 3 days of age. In general, although continually mated offspring obtained from the first eggs laid had a poorer performance as regards egg production and hatchability when compared to those obtained

from eggs laid 24 hours later, this selection had no effect on the survivals between these two groups.

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