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Male Effects on Fecundity in *Drosophila melanogaster*

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Male Effects on Fecundity in *Drosophila melanogaster*

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Abstract

Effects of male *Drosophila melanogaster* on female fecundity and productivity were examined, considering both females held in containers with males and females exposed to male effects not involving contact. Females were more fecund when male effects were present, and the largest increase was recorded when vials were previously conditioned by males. This effect was probably due to the growth of transmitted microorganisms, which were observed on the laying surface, as further experiments with vials conditioned by virgin females showed a similar increase in fecundity. A male-specific effect was isolated by conditioning bottles containing only agar with males and virgin females. The observation of a male factor that stimulates oviposition without mating is novel and suggests complicated fertility interactions between the sexes.

Fecundity as a fitness component is usually considered only in terms of the female, although it may also be affected by the presence of males. For example, Serradilla and Ayala (1983) kept different female and male genotypes in vials and found that fecundity depended on the male as well as the female genotype. Theoretical work (Hadeler and Liberman, 1975) indicates that such interactions between the sexes can give rise to stable equilibria for genetic polymorphisms not predicted by independent fertility estimates for the sexes.

One effect of males on female fecundity or productivity which has been studied extensively is that resulting from multiple mating. Recently, Turner and Anderson (1983) examined the productivities (number of progeny emerging per female) of *Drosophila pseudoobscura* females held in containers with or without males. They found that the presence of males increased female productivity, and that this effect was accentuated with an impoverished food resource. These workers argued that multiple mating is responsible for the

increased productivity, and suggested that transfer of nutrients with the ejaculate may be involved. The phenomenon of copulatory feeding by males has been demonstrated directly with radioactively labeled probes in *D. mojavensis* (Markow and Ankney, 1984) and various *Lepidoptera* (Boggs and Gilbert, 1979). Sperm replenishment may also contribute to the increase in productivity from remating in *D. melanogaster* (Gilbert et al., 1981; Gromko et al., 1985).

Males may influence female behaviors, especially those related to mating. Averhoff and Richardson (1976) described evidence for an airborne male pheromone which elicited courtship behaviors in female *D. melanogaster*. Spence et al. (1984) found that female *D. melanogaster* and *D. simulans* were slightly attracted by residual odors of males left on glass cylinders. In experiments on the oviposition site preference of *D. melanogaster* for food vials, Mainardi (1968, 1969) found that females preferred to oviposit in vials exposed previously to males. Mainardi argues for a male pheromone inducing oviposition, although this interpretation has been questioned by David (1970) and Atkinson (1983) on the grounds that surface texture was not controlled.

In this study, we explore male influence on fecundity in *D. melanogaster*. In order to examine mating and non-mating effects we have undertaken experiments in which inseminated females were held in containers with males or exposed to the influence of other flies without contact.

Materials and Methods

The *D. melanogaster* population used in these experiments was initiated with the progeny of 100 females collected in November 1983 at Putah Creek, near Davis, California. Flies were maintained in 0.4 pint bottles by mass transfer, on a laboratory medium consisting of cornmeal (6.2%), semolina (3.1%), sucrose (3.6%), dextrose (7.1%), agar (1.1%), and dead yeast (1.5%), with propionic acid (0.5%) as a preservative. Experiments were undertaken when stocks were 4–8 generations removed from the field. Progeny were collected over 2 days and flies were sexed under CO_2 anesthesia. Virgin males and females collected from 60 culture bottles were pooled and redistributed into fresh bottles, where they were aged for two days. Medium without live yeast was used throughout, as this treatment accentuated male effects in the Turner and Anderson (1983) experiment. After aging, flies were allowed to mate for 2 days by combining 100 virgins of each sex into fresh bottles. Anesthesia was used only in the initial sorting of the sexes.

We undertook an initial experiment to investigate male influence on female fecundity and productivity and a second experiment to isolate a specific non-mating male effect. The testing apparatus consisted of a 30 ml glass scintillation bottle with its opening inserted into a 40 ml glass vial. The two containers were separated by gauze and held together with elastic bands. The apparatus permitted the separation of flies contained in the scintillation bottles from three inseminated females held in the vials. The scintillation bottles contained 10 ml of medium (laboratory or agar), while vials were filled approximately half full with laboratory medium.

Experiments were carried out at room temperature (22–24°C), with a dark period of 7–10 hours. The assembled vials with bottles were placed horizontally in a shaded area (32

lux), and flies were transferred to fresh vials every day. Eggs were counted directly, and progeny were counted after eclosion from the vials.

Experiment 1

Productivity and fecundity were measured for the treatments listed in Table 1. The first treatment involved females held in isolation, while treatments 2 and 3 examined the effect of males held in contact with the females. Treatment 3 tested for an effect of males when they were present at a high density relative to the females. Treatments 4 and 5 tested whether adjoining males and females, separated by gauze, can influence female fecundity, while the final treatment (6) tested the importance of residual male effects. For the last treatment, ten males were placed in vials for 24 hours and removed before introducing the females. These vials were kept under high humidity to minimize drying of the food surface, and the same males were used to condition the next set of vials.

Table 1. Treatments for Experiment 1

Treatment	Vial	Scintillation bottle
1	3 ♀	empty
2	3 ♀ +3 ♂	empty
3	3 ♀ + 10 ♂	empty
4	3 ♀	3 ♂
5	3 ♀	10 ♀
6	3 ♀ + ♂ residue	empty

Scintillation bottles were changed on alternate days. Individuals lost in the course of the experiment were replaced by others of approximately the same age. Twenty replicates of each treatment were employed, and the experiment was continued for two weeks.

Experiment 2

This experiment consisted of three comparisons between the effects of virgin females and those of males on the fecundity or productivity of inseminated females held in a vial. The reproductive status of the virgin females was ensured by separating them from males soon after eclosion (within 8 hr). In the first comparison (Table 4), 20 males or 20 females in scintillation bottles were separated from inseminated females in vials and were transferred on alternate days. The second comparison was between the residual effects of 20 males and those of 20 females (1–4 days old) held in vials, as in treatment 6 of the first experiment. Since virgin females may contribute eggs to the vials, productivity rather than fecundity was scored for this comparison. (Stalker (1954) found no evidence of parthenogenetically produced *D. melanogaster* adults in a survey of 500,000 unfertilized eggs, so parthenogenetic reproduction could not have contributed to productivity in our study.) In the final comparison, scintillation bottles containing 10 ml of 0.3% agar in water were conditioned with twenty virgin males or females (1–4 days old). These flies were removed after 24 hr, and a new set of bottles with similarly aged flies was established. The scintillation bottles

were not separated by gauze from the vials, so that flies could move freely between the containers.

The handling and aging of flies in this experiment was identical to the procedure in Experiment 1, except that 2 ml of molten laboratory medium was pipetted onto the surface of the vials in order to create a smoother texture for oviposition. Twenty replicates were set up for each treatment, and the experiment was continued for one week.

Analysis

In the first experiment, three counts were obtained for the egg and progeny data: week 1, week 2, and total counts. Additionally, the mean time taken for half the eggs to be deposited (or for progeny to emerge) by each set of three females ($T_{½}$) was determined. Data were analyzed by single classification analyses of variance with orthogonal contrasts between the means of the treatments. In the second experiment, only the total counts were analyzed, using a *t* test to compare the appropriate means.

Results

Experiment 1

Means for the treatment (with standard deviations for the totals) are presented in Table 2. Females laid an average of about 63 eggs per female (treatment average divided by 3) over the 14 days, with mean fecundities ranging from 51.5 for treatment 1, to 78.3 for treatment 6. These values are low for *D. melanogaster*, suggesting that the flies may have been nutritionally stressed. The average number of progeny emerging per female was about 70, with individual fecundities ranging from 59.0 for treatment 1, to 84.7 for treatment 6. The higher values for progeny counts than for egg counts indicate that some eggs were not detected. Mean progeny and egg counts were highly correlated for the treatments ($r = 0.99$, $P < 0.01$), which indicates that females were not laying variable proportions of unfertilized eggs in the treatments. The counts for week 1 tended to be slightly higher than those for week 2 (Table 2).

Table 2. Egg and progeny counts:* Treatment averages for weeks 1 and 2, and totals

	Egg counts			Adult progeny counts		
Treatments	Week 1	Week 2	Total $\bar{x}$ ± SD	Week 1	Week 2	Total $\bar{x}$ ± SD
1—Females alone	80.8	73.5	154.4 ± 38.6	93.6	83.4	176.9 ± 43.6
2—Females with 3 males	105.8	102.6	208.4 ± 40.5	117.2	117.4	234.6 ± 39.7
3—Females with 10 males	114.4	79.1	193.5 ± 40.6	126.1	94.2	220.2 ± 43.7
4—Females separated from 3 males	99.8	78.1	177.8 ± 46.3	111.6	83.0	194.6 ± 47.0
5—Females separated from 10 females	91.4	69.6	161.0 ± 47.3	96.2	81.0	177.2 ± 46.9
6—Females with male residual effects	135.3	99.7	235.0 ± 82.0	142.6	111.4	254.0 ± 97.0

* The counts are the sum of the daily averages for each treatment. There are 20 replicates of each treatment.

Mean egg and progeny counts for each treatment plotted against the standard deviations indicated that no transformation was necessary. Analyses of variance indicated significant overall effects of treatments (Table 3). However, treatments did not differ for the times at which half the counts were made (eggs, $F = 1.74$, $P = 0.132$; progeny, $F = 1.11$, $P = 0.359$). Both this analysis and the similar results for week 1 and week 2 counts indicate that treatment effects did not change over time.

Table 3. Analyses of variance (mean square) and contrasts for egg and adult progeny counts

		Week 1		Week 2		Total	
	d.f.	MS	*P*	MS	*P*	MS	*P*
Eggs							
Treatments	5	7,212	<0.001	3,864	0.002	18,483	<0.001
Contrasts:*							
A. (1 + 5) vs. (2 + 3 + 4 + 6)	1	20,498	<0.001	8,933	0.003	56,494	<0.001
B. (4 + 6) vs. (2 + 3)	1	1,095	0.292	76	0.782	610	0.635
C. 2 vs. 3	1	728	0.388	5,499	0.019	2,220	0.362
D. 4 vs. 6	1	12,599	<0.001	4,666	0.030	32,661	<0.001
E. 1 vs. 5	1	1,099	0.290	150	0.693	438	0.685
Error	114	973		964		2,647	
Adult progeny							
Treatments	5	6,855	<0.001	4,994	0.004	20,215	<0.001
Contrasts:*							
A. (1 + 5) vs. (2 + 3 + 4 + 6)	1	23,207	<0.001	9,966	0.008	63,640	<0.001
B. (4 + 6) vs. (2 + 3)	1	588	0.453	1,461	0.304	200	0.805
C. 2 vs. 3	1	801	0.383	5,429	0.049	2,059	0.424
D. 4 vs. 6	1	9,641	0.003	8,037	0.017	35,283	0.001
E. 1 vs. 5	1	70	0.796	60	0.835	0	0.991
Error	114	1,046		1,377		3,199	

* The numbers refer to treatments described in Table 1.

The orthogonal contrasts tested are listed in Table 3. The first contrast (A) compares the effect of male treatments on progeny or egg counts with those treatments in which males were absent. Egg/progeny counts are significantly higher for all male treatments. In the second contrast (B), treatments in which males were in contact with females are compared to treatments without contact. This contrast is not significant. The third contrast (C) compares treatments in which the number of males held in containers with the females was varied. The contrast is significant for week 2 only. This is the only contrast in which results were not consistent for the two weeks. Means for the treatment with three males are higher than for the treatment with ten males, suggesting interference. The highest egg/progeny counts were recorded with the residual effect treatment, and these differ significantly from egg/progeny counts for the treatment in which males were held separately (Contrast D). The final contrast (E) indicates that egg/progeny counts are not increased in the treatment with females in the scintillation bottles.

These results indicate that there is a male effect on female fecundity and productivity, which is not related to contact between the sexes. In fact, residual male effects were observed to lead to the highest egg/progeny counts. These effects may be due to transmitted microorganisms as well as to a male specific factor. We noticed growth of microorganisms on the surface of male-conditioned vials, and this was probably accentuated in the high humidity conditions under which these vials were held. These microorganisms were probably transmitted by the flies, as vials held under the same conditions without flies showed no such growth, even when the surface of the vial had been scored to simulate mechanical effects of flies walking on the medium.

Experiment 2

To distinguish a specific male factor from microbial transmission, we compared the effects of males and those of an equal number of virgin females on fecundity or productivity, using three different comparisons. We found that the sexes had an equal effect on fecundity when they were separated from the test females, and an equal effect on productivity when residual effects were tested within the vials (Table 4). We tested for an increase in egg/progeny counts in male treatments relative to female treatments, and thus the tests were one-tailed. Counts were higher for the conditioned media vials, as found in the first experiment.

Table 4. Non-mating effects of males and females on fecundity (Experiment 2)

	Number of eggs or progeny*				
	♀	♂			
Comparison	$\bar{x} \pm$ SD	$\bar{x} \pm$ SD	t**	$d.f.$	P
Flies separate	142.2 ± 52.9	142.4 ± 49.5	0.04	38	0.483
Conditioned medium	199.5 ± 48.9	208.0 ± 59.6	0.48	38	0.319
Conditioned agar	96.2 ± 29.2	117.6 ± 44.5	1.80	38	0.040

* Adult progeny were counted for the conditioned medium comparison, and eggs were counted for the other comparisons. The conditioned agar comparison was monitored for five days only (see text).

** One-tailed tests for increase in male treatments.

In our third comparison, we used scintillation bottles containing only agar, which could not support the growth of microorganisms. This experiment was discontinued after 5 days, due to molten agar flooding the vials. Nevertheless, the pooled counts indicate a significant difference between the sexes for the agar-conditioned bottles, with the females laying 22% more eggs in vials exposed to males. We repeated this part of the experiment and included a control, in which the scintillation vials contained unconditioned agar. Thirty replicates of each treatment were set up. The male treatment produced a five-day cumulative average of 135.5 (SD 41.02) eggs, the female treatment produced 111.2 eggs (SD 31.12), and the control 118.1 (SD 40.93). Only the male treatment was significantly different from the control ($F = 3.12$, $P < 0.025$).

Discussion

We have found evidence of a male factor affecting female fecundity in *D. melanogaster*, which does not require mating. This factor acts like a short-range or contact pheromone, which is persistent over time. Short-range pheromones have been described previously in *Drosophila* (Ewing, 1977), including the non-volatile cuticular hydrocarbons eliciting male courtship behavior (Antony and Jallon, 1982). The stimulation of oviposition by proteins transferred from males has also been described (Chen, 1984). However, the novel aspect of the present observation is that male residual effects stimulate egg laying in the absence of mating, and we know of no other case in *Drosophila* or other insects in which this has been demonstrated.

The second factor which seems to have affected fecundity in our experiments is the growth of transmitted microorganisms. This growth was visible after males or virgin females were used to condition media before introducing inseminated females, and therefore the effect is not sex specific. The microorganism effect may be substantial, as indicated by the magnitude of the differences in egg counts between treatments 1 and 6 in the first experiment (Table 2). The transmission of microorganisms by *D. melanogaster* has been reported in a number of studies (Agrios, 1980; Hanson et al., 1980; Gilbert, 1980), and Begon (1974) has documented the transmission of yeasts and bacteria onto laboratory medium. It is well known that live yeast added to the surface of medium will increase oviposition, and the higher egg counts we observed may be due to an analogous cause. This increase may be related to nutrition provided by the microorganisms; the importance of yeasts and bacteria to *Drosophila* has been well established for the cactophilic species (Vacek, 1982). The microorganism factor may contribute substantially to the "male" effect observed by Mainardi (1968, 1969).

One factor which may reduce female fecundity is male interference. This factor was only evident in the second week (Table 3, contrast D), perhaps because of the cumulative effects of interactions with males. Atkinson and Shorrocks (1984) found that aggregation of females was reduced when males were present, although the total number of eggs deposited was similar. These authors provided a number of alternative oviposition sites, whereas, in the present study, females were confined to one site.

The microbial and residual-male effects have not previously been considered in experiments designed to test the consequences of multiple mating (e.g., Gromko and Pyle, 1978; Turner and Anderson, 1983). In our study, there was an indication that females kept with males (such that remating could occur) were more fecund than females alone (Table 2). However, females kept with males were not more fecund than those indirectly exposed to the influence of males (Table 3, contrast B), due to the high counts in treatment 6. The nonmating factors described here need to be considered in remating experiments, although they are less likely to be important in designs where males are exposed only periodically to females (e.g., Gromko and Pyle, 1978; Pruzan-Hotchkiss et al., 1981).

In our study, differences among the treatments did not tend to change over time. However, Turner and Anderson (1983) found that the fecundity of females kept with males diverged from the females kept alone, presumably as starvation was accentuated or as sperm were depleted. This difference may reflect variation in the reproductive biology of

different *Drosophila* species. For example, Markow and Ankney (1984) found that incorporation of nutrients from the male ejaculate occurs in *D. mojavensis* but not in *D. melanogaster*. If copulatory feeding also occurs in *D. pseudoobscura*, then this may account for the divergence in fecundity over time as nutrients required for vitellogenesis become limited.

Future studies should consider the possible roles of microbial and residual male effects. The role of microorganisms in *Drosophila* ecology is well recognized, especially in the cactophilic *Drosophila*, and there has been some work on the transmission of microorganisms by flies (Gilbert, 1980). The possibility that males are marking oviposition sites should be considered. Many *Drosophila* tend to mate on the breeding site, and Jacobs (1978) has observed that male *D. melanogaster* establish and defend territories on food in complex population cages. In addition, it is known that Hawaiian lek species mark mating territories by dragging their abdomens and releasing a characteristic odor (Spieth, 1978). We are extending our observations to other *Drosophila*, which will allow us to determine if the nonmating male factor is widespread and species-specific.

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Notes

1. Order of authorship was determined by the toss of a coin.

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