

Relative Male Age, Fertility, and Competitive Mating Success in *Drosophila melanogaster*

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Female choice experiments were used to investigate the effect of relative male age on mating success in D. melanogaster. Experiments were conducted with a Canton-S (CS) strain, in which two virgin males of different ages (2, 4, or 8 days old) were offered to virgin females. Older males were found to be more successful under competitive conditions. In another group of experiments, vermilion (v) males of different ages competed with CS males of different ages. The competitive success of v males was found to increase with their relative age. Male fertility at 2, 4, and 8 days of age was documented for both male genotypes mated with CS females. CS males fathered more offspring per copulation than v males, and the fertility of all males was found to increase with age. Discussion focuses on the changes in male mating success and fertility with age and genotype.

KEY WORDS: *Drosophila melanogaster*; mating success; male fertility; male age; female choice experiments.

INTRODUCTION

Reports of genotype-dependent mating success are numerous in the *Drosophila* literature (Spiess, 1970). However, nongenetic factors may also influence mating success. For example, when *D. pseudoobscura* is reared at low temperatures, males are larger and are more successful in mating than males of the same strain reared at higher temperatures (Ehrman, 1972). Also, when males of a given strain make up a high proportion of the

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number of individuals in an experimental population they are less successful than are males of the same strain when they occur in the minority (see review by Ehrman and Propper, 1978). While thought to be dependent on olfactory cues, the exact mechanism by which females discriminate minority males is still unclear.

Another unresolved situation for which there may be female discrimination was reported by Markow *et al.* (1978). *D. melanogaster* females were given a choice of mating with virgin males or males that had mated before and were less fertile. In these experiments virgin males were much more successful in mating, although no behavioral differences could be found to account for their differential mating success.

The effect of age on fertility of *D. melanogaster* males is well known (Strömnaes and Kvelland, 1962; Kvelland, 1965; Lefevre and Johnsson, 1962), very young males being less fertile than older males of the same strain. We were curious if females, when provided with a choice between males of different ages, would selectively mate with older, more fertile males. In the present study we found the relative ages of males to be a very important factor in determining their competitive mating success, although examining certain courtship parameters revealed no immediate explanation for the age-dependent success.

MATERIALS AND METHODS

Drosophila Stocks

Flies used in the present study were derived from stocks obtained from the California Institute of Technology in Pasadena, California. Two strains of flies were used, Canton-S wild-type (CS) and the sex-linked recessive vermilion (*v*). After a receipt at Arizona State University, vermilion was inserted into the Canton-S genetic background by means of a multiply marked X chromosome and autosomal inversions (Markow and Scavarda 1977). Flies were reared at $24 \pm 1^\circ\text{C}$ in population cages, each containing 12 cups of standard cornmeal-molasses-agar medium. Virgin males and females were separated under light ether anesthesia and stored in 8-dram vials with food until being used in experiments. Females were 4 days old when used. Males were tested when either 2, 4, or 8 days of age.

Experimental Procedures

Female choice experiments were conducted by aspirating a single female into a vial containing two males. The two males differed from each other in age, genotype, or both. In the first series of experiments both virgin

males were from the CS strain and differed from each other only with respect to age. The age combinations used were 2 and 4 days old, 2 and 8 days old, and 4 and 8 days old. Males were distinguished from each other by small clips in the wings made at the time of collection. Even though in previous studies small clips did not appear to affect the outcome of previous female choice experiments (Markow *et al.*, 1978), the age of the male clipped was varied between replications of each age combination in the present study. Six replications of approximately 20 observations per replication were made for each age combination, and the six replications were tested for homogeneity by a χ^2 test prior to being pooled. Matings were scored for the age of the successful male and for the time until copulation.

In the second series of experiments, a single 4-day-old virgin female was placed in a vial with two males, one CS and one v . The relative ages of the wild-type and the mutant males included the following combinations: 2-day-old CS with 2-, 4-, or 8-day-old v males, 4-day-old CS with 2-, 4-, or 8-day-old v , and 8-day-old CS with 2-, 4-, or 8-day-old v males. These combinations were tested using CS females as well as v/v females.

The fertility of 2-, 4-, and 8-day-old virgin CS and v males was measured by placing single males with single 4-day-old virgin CS females in order to count offspring. Females that had not mated within 45 min were discarded. Pairs in the series testing CS males were scored for courtship latency (time between introduction of flies and first courtship in minutes), duration of courtship (time between initiation of first courtship and initiation of copulation, in minutes), and copulation duration (in minutes). Mated females were then transferred to fresh vials every few days until no fertile eggs were deposited, and the number of adult offspring was counted. Use of single males and single females in this study may appear objectionable, but one of us (C. L.) in unpublished experiments, working with the same strain, found no significant differences with respect to courtship latency or duration of courtship between courtships with one or two males present.

RESULTS

In the first series of female choice experiments, with only CS males, older males were more successful in each age combination (Table I). Significant advantages were found for 4-day-old over 2-day-old males and 8-day-old over 4-day-old males.

We were curious to see if males having a mutant phenotype such as vermilion, which from unpublished observations we knew to be at a disadvantage in female choice experiments, might show increased courtship success with age. The second series of experiments was conducted with 4-day-old CS and v/v virgin females, and CS and v males in various age combina-

Table I. Competitive Courtship Success and Relative Age of Canton-S Males^a

Male age		Percent mating	Successful male		χ^2 (1:1)
male A	male B		A	B	
8	2	98	40	28	2.00
4	2	100	42	24	4.91 ^b
8	4	98	46	19	11.22 ^c

^a Clipping $\chi^2 = 0.019$, N.S.^b $p < 0.05$.^c $p < 0.01$.

tions (Table II). When the mutant and wild-type males were the same age, wild-type males were at an advantage in all cases except one. With wild-type females, when both males were 8 days old, ν males were successful in 54% of the matings, but this was not significantly different from a 1:1 ratio. The disadvantage for ν males tended to decrease with an increase in their relative age. When all experiments were pooled, the success rates of CS males

Table II. Female Choice Experiments Between CS and ν Males

Female genotype	Male age			Number mating	Successful male		χ^2 50:50
	+/+	ν	rep. s		+/+	ν	
+/+	2	2	4	57/76 (75%)	33 (58%)	24 (42%)	1.42
	2	4	4	65/74 (89%)	34 (52%)	31 (48%)	0.14
	2	8	4	47/53 (89%)	26 (55%)	21 (45%)	0.53
	4	2	4	61/65 (94%)	49 (80%)	12 (20%)	20.90 ^a
	4	4	6	87/106 (84%)	55 (63%)	32 (37%)	6.08 ^b
	4	8	3	56/59 (95%)	33 (59%)	23 (41%)	1.79
	8	2	3	53/55 (96%)	35 (66%)	18 (34%)	5.33 ^b
	8	4	3	47/50 (94%)	30 (63%)	17 (37%)	3.60
	8	8	3	52/53 (98%)	24 (46%)	28 (54%)	0.31
ν/ν	2	2	3	72/75 (96%)	45 (62%)	27 (38%)	4.50 ^c
	2	4	3	66/71 (92%)	26 (39%)	40 (61%)	2.97
	2	8	3	73/75 (97%)	22 (30%)	51 (70%)	11.52 ^a
	4	2	3	65/68 (96%)	43 (66%)	22 (34%)	6.78 ^b
	4	4	3	54/58 (93%)	32 (59%)	22 (41%)	1.85
	4	8	3	70/70 (100%)	39 (56%)	31 (44%)	0.95
	8	2	3	72/72 (98%)	56 (78%)	16 (22%)	22.22 ^a
	8	4	2	43/45 (96%)	29 (67%)	14 (33%)	5.23 ^b
	8	8	3	70/70 (100%)	50 (71%)	20 (29%)	12.86 ^a

^a $p < 0.001$.^b $p < 0.005$.^c $p < 0.05$.

Table III. Mean and Standard Error of Courtship Latency, Courtship Duration, and Copulation Duration: 2-, 4-, and 8-Day-Old CS Males Mated to CS Females

Male age	<i>n</i>	Courtship latency ^a (sec)	Courtship duration ^b (min)	Copulation duration ^c (min)
2	43	48.79 ± 8.77	3.19 ± 0.37	19.33 ± 0.43 ^(a)
4	33	51.74 ± 7.37	5.79 ± 0.84	20.51 ± 0.80 ^(b)
8	35	65.19 ± 12.10	3.51 ± 0.53	22.40 ± 0.65 ^(b)

^a Means not significantly different according to analysis of variance ($F = 0.828$, $df = 2, 108$, $p > 0.05$).

^b Mean ranks vary significantly according to the Kruskal-Wallis test ($H = 9.389$, $p < 0.001$).

^c Means vary significantly according to analysis of variance ($F = 8.929$, $df = 2, 108$, $p < 0.0001$); values superscribed with different letters are significantly different according to Duncan's multiple-range test.

were 50% at 2 days, 64% at 4 days, and 66% at 8 days. Vermilion males were successful in 32% of the matings in which they were 2 days old, 44% at 4 days, and 47% at 8 days. Courtship durations with v males tended to take slightly but not significantly longer (4.95 ± 0.41 min) than matings with CS males (4.10 ± 0.49 min) regardless of the age of either male. Courtship durations of v/v females (4.60 ± 0.33 min) were not different from those of CS females (4.70 ± 0.19 min).

Since there was a trend toward greater mating success with increasing age, further experiments were undertaken to uncover characteristics of older males that might account for their mating advantage. In single-pair matings between 4-day-old CS females and 2-, 4-, and 8-day-old CS males, time until courtship initiation, courtship duration, copulation duration, and number of offspring for each male were recorded. Courtship initiation, courtship duration, and copulation duration for males of all three ages are shown in Table III. Although there appears to be a trend toward increased courtship latency with increased male age, no significant differences were found between ages. Since variances for courtship duration were not homogeneous, a nonparametric test, the Kruskal-Wallis H test, was used to assess intergroup differences, and significant differences were found to exist

Table IV. Mean Number of Offspring (\pm SE) from 2-, 4-, and 8-Day-Old CS Males Mated to CS Females

Male age	<i>n</i>	Number of offspring ^a
2	30	128.13 ± 5.23 ^(a)
4	20	202.85 ± 9.98 ^(b)
8	26	188.31 ± 7.74 ^(b)

^a Means vary significantly according to analysis of variance ($F = 30.005$, $df = 2.73$, $p < 0.0001$).

Table V. Mean Number of Offspring (\pm SE) from 2-, 4-, and 7-Day-Old ν Males Mated to CS Females

Male age	<i>n</i>	Number of offspring ^a
2	25	62.36 \pm 3.51 ^(a)
4	25	172.72 \pm 2.85 ^(b)
7	23	176.30 \pm 4.54 ^(b)

^a Means vary significantly according to analysis of variance ($F = 26.857$, $df = 2.71$, $p < 0.0001$).

between ages. Four-day-old males showed the longest time until mating; copulation duration increased significantly with age. A Duncan multiple-range test ($\alpha = 0.05$) gave two significantly different subsets, the first containing 2- and 4-day-old males, 8-day-old males being in the second group. Offspring number also increased significantly with male age (Table IV). A multiple-range test ($\alpha = 0.05$) placed 2-day-old virgin males in one subset and 4- and 8-day-old males in another. The average number of offspring was slightly lower than for some other wild-type laboratory strains, but this can be explained by the highly inbred nature of our CS stock. At any given age, CS males were significantly more fertile than ν males of the same age.

The fertility of ν males was measured in the same way as CS male fertility. Average progeny numbers for 2-, 4-, and 7-day-old ν males is shown in Table V. Because of the unavailability of 8-day-old ν males at the time of fertility testing, 7-day-old males were used instead. As with CS males, 2-day-old males were placed in a statistically different subset from males of the two older ages by Duncan multiple-range test ($\alpha = 0.05$).

DISCUSSION

Our results confirm earlier findings that there is an increase in fertility as males become older (Kvelland, 1965). The increased fertility of older males may be related to the longer time they copulate. Of more interest is the finding that in female choice experiments, older males had an advantage over younger males from the same strain. Mating success also increased for older males of a mutant strain when they competed with younger wild-type males.

Precisely what characteristics of the successful older males are important remains unclear. In *D. pseudoobscura* and *D. persimilis*, flies have been reported to show shorter courtship durations as they get older (Spiess, 1970). However, those observations were made on large groups of individuals in a single chamber and calculated as the proportion that had

mated by a given time. We expected that older males would begin courting sooner and mate sooner, but this expectation was not supported by the data. Possibly the proportion of time spent performing a particular courtship component or the sequence and duration of components changes in older males. The roles of auditory and olfactory cues may also be important.

While the mating success of v males also increased with age, they significantly exceeded 50% success only in tests with v/v females and only when the wild-type males were 2 days old. Interestingly, it was at 4 and 8 days of age that v males were more fertile than 2-day-old CS males. We do not know why old v males were more successful with v/v females than with CS females. It is possible that v/v females are more receptive to v males. However, the duration of courtship in experiments with v/v females was not shorter than in experiments with CS females, indicating that the overall receptivity of v/v females is probably not greater than that of wild-type females.

A significant effect of the tendency of females to mate with more fertile males is the maximization of their fitness. A similar situation was reported by Markow *et al.* (1978) where, in female choice experiments, virgin males were at a significant advantage over nonvirgin males of the same strain and age. The nonvirgin males were characterized by reduced fertility in comparison to virgin males. No differences between males were found for courtship initiation, for courtship duration, or for amount of time spent courting. In that situation as well as in the situation described here, it would be to the female's advantage to mate with the most fertile male. It is hard to tell whether the basis for the mating advantage found in this study is the same in the case of age as in the case of previous mating activity. Selection should favor any mechanisms that would result in optimally fertile matings. Furthermore, one would expect that the capacity to recognize male fertility would be very important in species in which females mate only once or twice.

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