

Dynamics of Mating Success in Experimental Groups of *Drosophila melanogaster* (Diptera: Drosophilidae)

Therese Ann Markow^{1,2} and Susan Sawka¹

Accepted October 28, 1991; revised December 21, 1991

*Determinants of male courtship success in *Drosophila melanogaster* were examined in groups of five males sequentially presented with five individual females. Thirty-three percent of males never mated, while approximately half of the males mated two or three times. Rapid courtship initiation was associated with male success in early matings only. Male size was important for courtship outcome, but the size distributions of mating and nonmating males and their progeny numbers indicate balancing rather than directional selection on size-dependent courtship success.*

KEY WORDS: mating success; male courtship; male size; *Drosophila melanogaster*; sexual selection.

INTRODUCTION

How closely do laboratory studies of *Drosophila* mating behavior approximate the conditions under which sexual selection takes place in natural populations? Experimental approaches to studying sexual selection in *Drosophila* have typically employed "choice" designs originating with Sturtevant (1915). A "female-choice" test consists of placing females with males of two contrasting types and scoring the type of male which is successful. "Male choice" tests are done by placing males of one type with two types of females. Data from choice tests are then used to calculate isolation indices (Stalker, 1942), which

¹Department of Zoology, Arizona State University, Tempe, Arizona 85287.

²To whom correspondence should be addressed.

provide a valuable means of testing the significance of observed deviations from random mating (Malagowolkin-Cohen *et al.*, 1965) and detecting the action of sexual selection. These empirical procedures have been highly effective in quantifying intra- and interpopulational differences in mating success in numerous *Drosophila* species. While the overwhelming majority of choice experiments has been undertaken in the laboratory to measure sexual selection against flies carrying visible genetic variants, discrete visible variants are rarely seen in natural populations. If sexual selection is acting in natural populations or laboratory populations that include only wild-type individuals, its detection will require a different experimental approach.

In our laboratory we have attempted to elucidate the basis of mating success among wild-type flies under conditions similar to those encountered in nature. In laboratory experiments in which one wild-type *D. melanogaster* female is placed with two virgin wild-type males of the same age, the successful competitor is typically larger, courts sooner, and is more fertile than the unsuccessful male (Markow, 1987, 1988). However, in nature, females are not likely to be courted by males of the same age and mating status. Laboratory experiments have also shown that when females are competed for by a virgin and a recently mated, less fertile male of the same age and strain, the virgin male is successful a significantly greater proportion of the time (Markow *et al.*, 1978), despite the fact that no differences have been observed in the latency or vigor of courtship by the two males. The same is true of virgin males of the same wild-type strain that differ in age (Long *et al.*, 1980). In this case older males were relatively more fertile and more successful in gaining matings, although at the ages studied, they did not differ in courtship vigor compared to younger males.

It is tempting to generalize from these experiments in evaluating the roles of male and female sexual selection in natural populations of *Drosophila*. However, in nature, females of this species are usually courted by more than two males at a time (Markow, 1988). Mating takes place at the feeding site, where arriving females are greeted by the courtship of an average of five wild-type males. This scenario is repeated a number of times as new females are courted and mated during the course of a morning or evening activity period. The mating status of males in the pool changes over time. The dynamics of male reproductive success in such groups have not been studied. While the outcome of female choice tests predicts that large males that initiate courtship quickly should experience greater mating success, other observations suggest that their advantage may decrease once they mate. Below we describe experiments in which single virgin females were serially presented to the same group of five wild-type males, each male distinguishable by individual wing markings. In each experiment we recorded the following information: sizes of all males, first male to court, second male to court, male successfully copulating with the female, and number of progeny sired by each male on each mating. The information was then used to

elucidate the relationship among male size, male courtship latency, male fertility, and courtship success in a group of wild-type males over a time period similar to the activity period of flies in a natural population.

MATERIALS AND METHODS

All flies were from the TM4 strain of *D. melanogaster*, which was derived from a multifemale collection made in Tempe, Arizona, in 1981. The strain had been maintained in a large population cage for 2 years when these experiments were conducted. Virgins were separated within 4 h of eclosion under light ether anesthesia. Males were marked at that time for future identification by small clips in the wings. The five clip patterns employed are depicted in Fig. 1. The number of matings observed for males of each type was tested for deviation from expected numbers. The χ^2 value of 2.91 ($df = 4$) is not significant.

When the flies were 4 days old, five males, one from each marking category ($n = 5$), were placed in an observation chamber. A virgin female of the same age was introduced. The identification pattern of both the first and the second male to court was recorded, as was the pattern for the male that eventually mated. If no courtship occurred within 10 min, the female was replaced with another. If no copulation occurred within 30 min, the female was replaced. Females rarely had to be replaced for either reason. Following the termination of copulation, the mated female was removed and placed in an 8-dr culture vial for oviposition. Oviposition vials were changed daily. All vials were saved in order to count the adult progeny produced by each mating. The entire experimental sequence was repeated with a new virgin female 5 min after termination of the previous copulation until five matings were obtained. After each group of five males had completed five copulations, all five males were anesthetized and their thorax lengths were measured. Thorax length (TL) has been demonstrated to be a reliable estimate of body size in *Drosophila* (Reeve, 1950; Robertson and Reeve, 1952). Measurements were made under a Bausch and Lomb dissecting scope to which a micrometer had been installed in an eyepiece. Micrometer readings were converted to millimeters. Due to the scale on the micrometer, and to rounding during conversion, converted measurements resulted

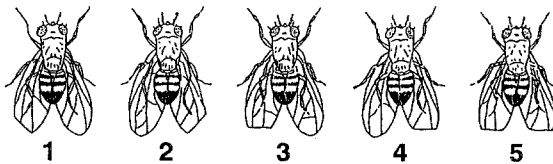


Fig. 1. Wing markings used to distinguish the identity of the five males in each experimental group.

in flies being assigned sizes of 0.83, 0.86, 0.90, 0.93, 0.96, and 1.0 mm, with a mean size of 0.8924 for the 100 males in the entire experimental population.

All flies were reared on standard cornmeal-molasses-agar medium at $24 \pm 1^\circ\text{C}$ on a 12L:12D cycle. Mating experiments began at 0700, 30 min after lights went on.

RESULTS

Male Size and Courtship Success

Male thorax lengths ranged from 0.83 to 1.0 mm. Males from each group were not measured until after all five matings had taken place. In three of the experiments males were present in only two sizes. None of the experiments contained five males of identical size. In Table I the mean thorax lengths of males mating once, twice, and three times are compared to the mean thorax length of males that did not mate. While it was theoretically possible for one male in a group to mate with all five females, no single male mated more than four times. Males that mated ($\bar{X} = 0.8961 \pm 0.004$, $n = 67$) were significantly larger ($t = 10.92$, $P < 0.001$) than males that did not mate ($\bar{X} = 0.8851 \pm 0.003$, $n = 33$). The one male that mated four times came from a group in which he was one of three males having a thorax length of 0.86 m. The fourth and fifth males had TLs of 0.83, making the male mating four times one of the larger males. However, a regression of the number of matings on thorax length was not significant ($F = 0.86$, $df 1,97$, $P = 0.3546$). The mean size of successful males remained the same across five matings (Table II). Of the males that never mated, 7 were the largest in their set of five males, 10 were of intermediate size compared to other members of their set, and 16 were the smallest. When nonmating males from all sets were pooled, their size distribution was not significantly different from that predicted by chance ($\chi^2 = 5.037$, $df 5$, $0.50 > P > 0.30$).

Table I. Average Thorax Lengths (mm) of Males Mating Once, Twice, and Three Times and Males Never Having Mated

Number of times male mated	$\bar{X} \pm \text{SE}$ thorax length (n)
0	0.884 ± 0.005 (33)
1	0.896 ± 0.009 (38)
2	0.899 ± 0.005 (24)
3	0.908 ± 0.006 (4)

Table II. Mean Thorax Length (mm) of Males Mating the First Through the Fifth Times ($n = 20$ Males/Mating)

Mating No.	$\bar{X} \pm SE$
1	0.895 \pm 0.032
2	0.898 \pm 0.044
3	0.886 \pm 0.037
4	0.899 \pm 0.049
5	0.891 \pm 0.034

Male Courtship Latency and Courtship Success

In order to look at the *relative* courtship latency of successful males as opposed to the actual latency, the identities of the first and second male to court were recorded instead of recording the seconds elapsing until a male began courting. Forty-five percent of the males that did not court first on trial 1 never courted first on later trials. On the other hand, of those males that were first to court on trial 1, only 30% failed to court first in later trials. Therefore, the trait "first to court" appears to be relatively stable over successive trials. Of the entire 100 matings, the first male to court accounted for 34 ($\chi^2 = 12.25$, $P < 0.01$). In all 100 experiments at least two males courted, and usually three or four, so that the success of the first male is not simply a function of the female mating with the first suitor and, thereby, precluding other males from courting before mating took place. The stability of the relationship between being the first to court and eventual success in mating is shown in Table III. When the five trials were tested for homogeneity, the trials were found to be too heterogeneous to allow pooling. This significant heterogeneity is due to the trend from a 9 in the first trial to a 1 by the last trial. The association between first to court and eventual mating is significant in the first trial but not thereafter. Males that were the first to court in earlier trials lost their mating advantage by the time several matings had taken place.

Fertility and Success

The experiment yielded 13,124 progeny. Of the 67 males that mated, 39 mated once, 24 mated twice, 3 mated three times, and 1 mated four times. Forty-two percent of the mating males mated more than once and these multiply mating males sired 63.9% of the progeny. The average number of progeny produced across the five matings for all sets of males is shown in Table IV. Progeny production remained stable over time, as different males were involved in the later matings than in the earlier ones.

Table III. Male Mating Success on Trials 1 Through 5 Depending upon Whether Males Were (A) First to Court or (B) Not First to Court

Mating No.	(A) First		(B) Not first		χ^2	df
	Observed	Expected	Observed	Expected		
1	9	4	11	16	7.81	1
2	6	4	14	16	1.25	1
3	3	4	17	16	0.312	1
4	7	4	13	16	2.8125	1
5	1	4	19	16	2.8125	1
Sum of χ^2 values					14.997	5
Pooled	34	20	66	80	1.85	1
		df	χ^2	<i>P</i>		
Total		5	14.997			
Pooled		1	1.85	NS		
Homogeneity		4	13.747	<0.01		

Table IV. Average Number of Progeny on Each of Five Matings

Mating No.	$\bar{X} \pm SE$
1	143.5 \pm 67.3
2	162.7 \pm 53.2
3	170.3 \pm 55.4
4	155.3 \pm 53.8
5	147.7 \pm 64.9

When the frequencies of males having each body size were compiled in a histogram, the distribution appears slightly skewed (Fig. 2). Numbers of progeny sired by males in each size class were also placed in a histogram and presented with the size distribution. The difference in the two histograms ($\chi^2 = 526.86$, $P < 0.0001$) indicates that males are leaving progeny at different frequencies than expected by their number in the experimental populations. This tendency for males of intermediate size to have a higher fitness is also evidenced by the mean progeny number of males of each size (Fig. 2). There was only one male in the largest size category, thus the progeny number (185) does not reflect a mean and has no standard error.

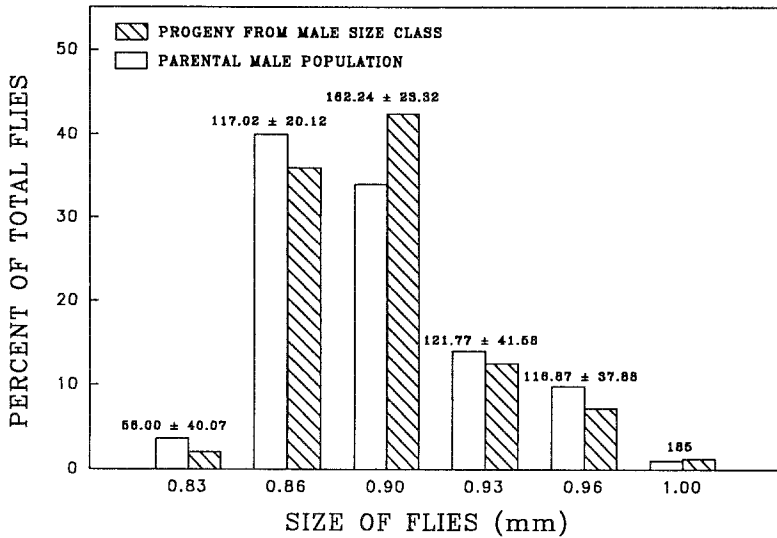


Fig. 2. Distribution of sizes of males in the experimental population of 100 flies and distribution of the proportion of progeny sired by males of the different size classes.

DISCUSSION

Under the conditions described above, the intensity of sexual selection was such that 33% of males never mated. Several variables clearly were related to male success, but the nature of these relationships changed during the observation period. Early matings were not random with respect either to size or to relative male courtship latency. This pattern is similar to the outcome of experiments in which two wild-type males compete for one female, where larger males court sooner and win more contests (Partridge *et al.*, 1987; Markow, 1987). With a larger group of males, observed over a succession of females, short *relative* courtship latencies were associated with male success only in the first contest for each group of males. While average male size and relative courtship latency themselves did not change during the experiment, the latter was no longer associated with male success in later courtships. The mean size of mating males remained stable over the five successive matings and mated males had a larger mean size than nonmated males. Males tended to contribute to progeny at different proportions than expected by their frequency in the population. The largest number of progeny came from males slightly larger than the modal category, suggesting a fitness advantage for these males. However, it is important to note that seven of the nonmated males were the largest in their sets. A decrement in male reproductive fitness at both size extremes indicates balancing selection on male size under competitive courtship conditions. Boake

(1989) reports a similar observation among males of the picture-winged *D. silvestris*.

A constant level of progeny production was maintained across all five matings. In *D. melanogaster*, male fertility declines sharply after two successive matings (Lefevre and Johnson, 1962; Stromnaes and Kvelland, 1962). While the courtship vigor of these temporarily less fertile males is indistinguishable from that of virgin males (Markow *et al.*, 1978), investigations with sensory-deficient mutants demonstrate the role of female olfaction in avoiding matings with depleted males (Markow, 1987). By avoiding males having temporarily reduced fertility, females maximized their fitness regardless of the time at which they mated during the mating period.

Comparison of progeny production from different designs underscores the complexity of the relationship among male size, fertility, and courtship success. In tests like those reported above and elsewhere (Markow, 1987) in which males compete for one female, larger (but not always the largest) males tend to have courtship advantage. However, when females are individually paired (no choice) with males of small vs large size, significantly more progeny are produced from small male matings (Pitnick, 1991). This presents a contradiction, in that if female choice were the major determinant of mating success, females should be mating with smaller males.

The present study was designed to simulate the social environment of natural populations on one of their known natural resources. Markow (1988) reported that among *D. melanogaster* breeding on fallen citrus, one receptive female is courted by an average of five males. New females continually arrive at the breeding/feeding site during an activity period of several hours. Males vary across a range of genetic and nongenetic characters including size, vigor, fertility, age, and mating status. Thus the social environment in which courtship and mating occurs in natural populations contrasts sharply with that provided in typical laboratory experiments. This contrast makes it difficult to generalize from many laboratory observations to *Drosophila* in nature.

The constantly changing nature of the characteristics of the factors influencing sexual selection within a single group of *D. melanogaster* is clearly demonstrated by these studies. No single male character can be pinpointed that, over time, consistently determines the outcome of sexual selection. Nor is it possible to single out one mechanism, i.e., male competition or female choice, that is consistently responsible for determining the outcome of courtships. Conclusions drawn from experiments with short observation periods would probably point to factors such as male courtship vigor as responsible for male success. Experiments with a long observation period would be less likely to reveal this association. The more closely an experimental design approximates conditions in nature, the more complex the basis for sexual selection.

ACKNOWLEDGMENTS

We thank Mark Gromko, John Alcock, and Scott Pitnick for comments on the manuscript. This work was supported by NIH Grant 30638 to T.A.M.

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