

Genetic and Sensory Basis of Sexual Selection in *Drosophila*

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Introduction

Years of research on mating behavior have revealed that matings rarely occur at random between individuals in the same population. Why are some individuals more successful than others when competing for mates? What factors account for the particular combinations of types we observe in mating pairs? Current evolutionary theory explains mating patterns on the basis of maximization of fitness on the part of each sex and most species that have been investigated provide at least indirect support for this hypothesis. With *Drosophila*, it is possible to design controlled experiments that can answer directly questions about the relationship between mating behavior and fitness, and discover those factors that produce the nonrandom associations we observe.

Recent investigations in our laboratory have uncovered a number of examples in which male mating advantage is associated with superior fertility. Markow et al. (1979) reported that among *Drosophila melanogaster* Meigen males of the same strain and age, individuals that were temporarily less fertile due to previous copulations were less successful than virgin males when competing for females. When enough time had elapsed to restore fertility, this disadvantage disappeared. Since both types of males appear to court with equal vigor, it could be argued that females perceive them to be different qualitatively and actually choose the virgin male, thereby increasing their own productivity from a given mating. In another study, 4-day-old *D. melanogaster* males were found to give more progeny on a single mating than 2-day-old males of the same strain (Long et al. 1980). Despite the apparent absence of differences in courtship vigor, 4-day-old males had an advantage over 2-day-old males when competing for females. Also, in this situation it is tempting to conclude that female choice is operating.

The explanation of female choice in the above examples assumes that the competing males are providing different sensory cues. It follows that in the absence of such information, it should be impossible for females to make their choices properly and observed mating patterns should approach randomness with respect to male fertility. On the other hand, if male courtship vigor, rather than female choice, is the determinant of success, the absence of any contrasting sensory cues should make little difference to the success of the more fertile males under competitive conditions.

These predictions have been tested in my laboratory with *D. melanogaster* using sensory deficient mutants and males that differ in fertility due to known and controlled factors. We have conducted a series of experiments in which males of contrasting fertility competed for mates. Females either were wild type or had various sensory deficiencies.

Methods

Fly strains

Two wild type strains, Oregon-R (OR) and Canton-S (CS), were obtained from the *Drosophila* Stock Center at the California Institute of Technology. Another wild type strain, TM4, was collected in Tempe, AZ in May 1980 by the author. The mutant *norpA*^{P24} was obtained from Dr. William Pak, Purdue University, *sbl* from Dr. Jeffrey Hall, Brandeis University, and the double mutant *al th* (aristaless, thread) was constructed from strains from the California Institute of Technology and Bowling Green State University stock center. All strains were reared in half pint milk bottles containing standard cornmeal molasses agar medium at $24 \pm 1^\circ\text{C}$ on 12 h L/12 h D cycle.

Male competition experiments

A single female was placed in a vial with two males. The female was either TM4 wild type, *norpA*^{P24} (a blind mutant), *sbl* (smell blind, olfactory deficient), or *al th* (impairment of sound perception). The vials were observed and a record was kept of the first male to begin courting, courtship latency of each male, courtship intensity and successful male ("winner"). After mating, the thorax lengths of both the winner and loser were measured in order to compare body size. In competition experiments conducted with TM4 females, all females mating with winner males were saved, transferred individually to fresh vials daily, and their progeny counted. The unpreferred "loser" male was immediately mated with another TM4 female (no choice or competition involved) which was also saved to determine progeny numbers.

Four competition experiments were performed. Females used in the first three were TM4; in the last, mutant. In experiment one, 4-day-old males of the Canton-S strain and 4-day-old Oregon-R males were used. In the second, 4-day-old CS males were in competition with 2-day-old CS males. In experiment three, single females were placed with CS virgin males and CS males that had mated twice in the hour immediately preceding the choice test. Both males were 4 days old. Finally, in the fourth experiment, two males were chosen at random from a pool of virgin 4-day-old TM4 males. Each of the four competition experiments was conducted with females that were blind (*norpA*^{P24}), olfactory deficient (*sbl*) or auditory defective (*al th*) as well as with wild type TM4 females.

Results

In Table 1, we report the outcome of the first three competition experiments in which TM4 females were used. Canton-S males were twice as successful as OR males. In similar experiments using females from other wild type strains, CS males consistently were more successful than OR (unpublished observation). The data on virgin vs. mated males and 4- vs. 2-day-old males confirmed earlier findings from this laboratory (Markow et al. 1979, Long et al. 1980). When the number of progeny was compared (Table 2), the winner males were seen to produce more offspring than the losers.

In view of the finding that successful males were more fertile than unsuccessful males, we determined whether the successful male of two taken at random from an outbred strain showed a higher fertility than the loser. Among competing TM4 males of the same age and mating status, the winner was significantly more fertile than the loser (Table 2).

In seeking the biological and behavioral basis for the apparent correlation between success and fertility, these competition experiments were repeated and the courtship latency of each male as well as his thorax length were measured. Table 3 reports the type of male that

Table 1. Results of three competition experiments. (1) Four-day-old males from the CS and OR wild type laboratory strains competed for TM4 wild type females. (2) Four-day-old CS virgin males and 4-day-old males having mated twice previously competed for TM4 virgin females. In all experiments, a single female was placed in a vial with the two males. (3) Four-day-old virgin and 2-day-old virgin males from the CS laboratory strain competed for TM4 females.

Kinds of males		Male mating		χ^2 (1:1)	p
A	B	A	B		
Canton-S	Oregon-R	85	49	9.672	<0.01
Virgin	Mated (1 h)	115	65	13.88	<0.01
4-Day	2-Day	42	24	4.91	<0.05

Table 2. Average number of offspring produced by successful and unsuccessful males. In experiments 1, 2, and 3, average progeny produced by each class are compared. In experiment 4, average progeny of successful and unsuccessful individuals are compared.

Experiment number	Males		Average Number of Progeny		p
	A	B	A	B	
1	Canton-S	Oregon-R	342.11 ± 16.99	267.2 ± 18.5	<.01
2	4-Day	2-Day	202.85 ± 9.98	128.13 ± 5.23	<.001
3	Virgin	Mated (1 h)	288.0 ± 17.4	206.0 ± 21.0	<.01
4	TM4 (W)†	TM4 (L)†	438.0 ± 22.1	361.0 ± 19.2	<.01

†W = winner, L = loser.

courted first, size of males of both types and type of male that was successful. First to court (or shortest latency to courtship) and the amount of time spent courting were positively correlated in each case, therefore only first to court is reported in the table. When TM4 females were used, there was no difference between CS and OR males for being the first to initiate courtship. Thorax lengths of CS and OR males were similar and yet CS males were significantly more successful in gaining matings. Four-day-old males were more successful than 2-day-old males but there were no significant differences between them in which male was the first to court or for the size of thorax. The same was true of virgin and mated males. Virgin males were significantly more successful than mated males but were, as was expected, the same size and also were equal in courtship initiations. Only with TM4 males were there differences between the "winners" and "losers" in courtship latency and body size. Winners tended to court first and were larger. The data show that successful males in the first three competitions

Table 3. Four male competition experiments conducted with females from the wild type strain TM4 or with sensory deficient females having *norpA*^{P24} (blind), *sbl* (smell-blind), and *al th* (auditory interference). In experiment number 1, Canton-S and Oregon-R males competed for females. In experiment 2, 4- and 2-day-old males were in competition. Virgin and mated males competed for females in experiment 3 and in experiment 4, pairs of virgin males were selected at random from the TM4 wild type strain and competed for females. The data on thorax lengths are presented to give the average sizes of the actual winners, regardless of their strain or treatment. The thorax lengths for flies of each treatment (A or B) appear one time in parenthesis under the values for winners and losers in the experiments using TM4 females. The average thorax length for each strain and treatment did not change over the course of the experiments.

Exp. no.	Type male and <i>n</i> of each type mating per total		First to court		Thorax length	
	A	B	A	B	W (A)	L (B)
<i>TM4</i> Females						
1	CS (34/46)	*OR (12/46)	22	24	26.41 (26.13)	26.32 (26.03)
2	4-Day (24/30)	*2-Day (6/30)	18	12	25.95 (26.17)	26.11 (25.90)
3	V (33/51)	*M (17/51)	23	29	25.78 (26.02)	25.93 (25.84)
4	TM4-W	TM4-L	41 *	8	28.69 *	27.83
<i>norpA</i> Females						
1	CS (23/39)	OR (16/39)	23	16	25.56	25.97
2	4-Day (24/40)	2-Day (16/40)	22	18	26.43	26.01
3	V (29/43)	*M (14/43)	20	23	25.86 *	26.00
4	TM4	TM4	23 *	12	28.73 *	27.66
<i>sbl</i> Females						
1	CS (16/32)	OR (16/32)	16	16	26.96 *	25.69
2	4-Day (19/41)	2-Day (22/41)	21	20	27.01 *	25.88
3	V (20/37)	M (17/37)	19	18	26.89 *	25.81
4	TM4	TM4	29 *	16	28.66 *	27.29
<i>al th</i> Females						
1	CS (26/33)	*OR (7/33)	16	17	26.18	25.99
2	4-Day (29/38)	*2-Day (9/38)	21	17	26.19	26.07
3	V (28/40)	*M (12/40)	19	21	26.29	26.01
4	TM4-W (37)	TM4-L (37)	27 *	10	28.51 *	27.14

**p* < .05; comparison of A and B

were no larger than unsuccessful males nor did they court sooner or more intensely. While body size and courtship intensity were associated with success for TM4 wild type, random-selected males, there is nothing in the experiments with TM4 females that points to the sensory basis of male success.

The hypothesis that differential success depends upon differences in the particular sensory information that males present to females was tested by depriving females of specific sensory information during competition experiments. Females homozygous for the mutant *norpA^{P24}* are blind and are, therefore, deprived of visual information about courting males. Canton-S males were still more successful than OR males but the proportion of the time they "won" was no longer significant as it was with wild type females. The same was true for 2- and 4-day-old males but not for virgin vs. mated males. However, with TM4 males, the winners were still the first to court and were larger.

When competition experiments were conducted with olfactory deficient females (homozygous for *sbl*), a significant change was observed in the outcome of the first three competition experiments. The advantage that one type had over the other disappeared completely. There was still no difference between types for first to court but winners, regardless of type, were those males that courted soonest and courted most intensely. Winners also were larger when females were *sbl*. When females were deprived of olfactory information, differential success of particular types of males disappeared. In the absence of olfactory information, a new system of success determination occurs in which success is associated with short courtship latency, greater courtship intensity, and larger body size.

Finally, these experiments were repeated with *al th* females whose ability to perceive courtship songs was obstructed. Here the results of all four competition experiments resemble the findings when TM4 females were used. When females' song perception is altered, CS males are more successful than OR, older males more successful than young males, virgins more successful than mated males. In the case of TM4 males, the larger males are still the winners.

Discussion

In nature, courtship and mating occur in aggregates of flies found on rotting fruit. Females are exposed to a large number of males that vary in almost every way imaginable: genotype, age, mating status, environmental conditions during development, etc. Much of this variability is correlated with both fertility and the ability to obtain mates. In the experiments described here, males that because of their age, mating status, or genotype were more fertile were most successful also in gaining matings with wild-type females. An important question facing evolutionary biologists is whether sexual selection operates by female choice or simply by differences in male vigor. These experiments suggest that mechanisms of sexual selection may be heterogeneous and depend upon the nature of the differences between competing males in a given situation. In the first three competition experiments, male differences were highly contrived. Using wild type females, however, there was a strong and consistent advantage for males of a given treatment, whether it was strain, age or mating status. That these advantages were dependent upon different olfactory cues is suggested by the change that occurs when males of various treatments competed for *sbl* females. Canton-S males no longer had an advantage over OR, 4-day-old males were no longer more successful than 2-day-old males, and virgin males lost their advantage over nonvirgins. In the absence of the female's ability to detect olfactory cues, male success appears to be dependent upon two correlated traits, larger body size and greater courtship propensity. Therefore, CS and OR males must be providing different olfactory cues to females. The same can be said for old and young males and virgin and mated males. However, when females were blind, we saw also that

CS males lost their significant advantage and so did older males. Although CS and OR males court on the average with equal vigor, and so do 4- and 2-day-old males, a fine structure analysis of their courtship behavior (Markow and Hanson 1981) reveals differences in the sequence of courtship components, and in the stationary probability of occurrence of specific components, some of which have a visual basis.

It is impossible to determine whether wild type females are "preferring" CS males over OR males because of differences in their odors and courtship structure. The olfactory cues and other courtship signals of OR males simply may not be adequate to evoke a receptive state in females. In such a situation, it could appear as though females are choosing CS males when CS males may be winning by default. The same could be true of 4-day-old males and virgin males.

When no age or mating status difference exists among males of a genetically heterogeneous population, male success is correlated with body size and courtship propensity. The question about choice can be asked again here. Are females choosing larger males or are the large vigorous males winning the contests? These two alternatives may not be different when viewed from the standpoint of the females' central nervous system. Certain features of a courting male, whether they be specific sensory cues or overall courtship intensity, may bring a female into a receptive state. In certain contests described above, it was possible to distinguish between the importance of particular sensory modes, for example olfaction *vs.* overall courtship vigor, in producing differential success of certain types of males.

Several evolutionary consequences of these processes are obvious. A system in which female receptivity is stimulated only by a particular constellation of courtship components provides a mechanism for conserving a species specific mate recognition system. At the same time, the tendency for larger males to be more successful may reflect the action of balancing selection. There is some evidence suggesting that within the TM4 strain, larger males may be more heterozygous. When the number of sternopleural bristles on the right and left sides of body were counted, winner males (symmetry index, $L/R = 0.96 \pm 0.02$) showed a significantly higher degree of symmetry than did loser males (symmetry index, $L/R = 0.87 \pm 0.03$, $p < .001$). According to Lerner (1954) and Waddington (1957), bilateral asymmetry results from a poor capacity for developmental homeostasis associated with homozygosity at a large number of loci. If, in fact, larger males are more successful at inseminating females and are relatively more fertile than smaller males, and if both are a function of increased heterozygosity, success might act to maintain genetic variability in a population.

A question that appears to be more interesting than whether or not "female-choice" is operating is concerned with the apparent association between success and fertility. Is it simply a fortuitous observation in all four experiments that the males that win contests are also the most fertile? This laboratory is in the process of comparing the relative courtship success and fertility of males from additional inbred strains, varied rearing conditions and additional, more complex treatments.

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