

SHORT COMMUNICATION

Genetic and Sensory Aspects of Mating Success of Phototactic Strains of *Drosophila melanogaster*

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In female choice experiments, Drosophila melanogaster males from a selected photonegative strain show a highly significant mating advantage over males from a photopositive strain. While photonegative behavior is sex linked in this species, the X chromosome is not involved in the mating advantage seen for this strain. The degree of the photonegative male advantage changes when tests are conducted with females mutant for olfaction and auditory perception but not when females are blind.

KEY WORDS: *Drosophila*; phototactic behavior; mating advantage; vision; olfaction; auditory stimuli.

INTRODUCTION

It is not uncommon in the *Drosophila* literature to find that males of various wild-type genotypes exhibit differential mating success (Spiess, 1970). In most cases the underlying behavioral basis for increased success is unknown. Female *Drosophila* are thought to be responsible for determining if a mating will take place and with which male. Presumably certain male courtship components will be more important than others in influencing the outcome of a courtship. Visual, olfactory, and auditory factors have all been shown to be of importance, but it has been difficult to show the relative importance of each (Connolly *et al.*, 1969; Averhoff and Richardson, 1974; Bennet-Clark and Ewing, 1969).

Our laboratory has observed that in female choice experiments, males from laboratory strains selected for negative phototaxis are significantly

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more successful than males from the photopositive strain. Since negative phototactic behavior is known to be completely sex linked in this species, we have carried out a genetic analysis of the mating advantage in order to see if it is also under the control of the X chromosome. In addition we attempted to assess the roles of visual, olfactory, and auditory cues for female discrimination. The results of these experiments are the subject of this paper.

MATERIALS AND METHODS

Drosophila Stocks. Highly photopositive and photonegative strains of flies were created by selection with Hadler phototaxis mazes (Hadler, 1964). The mazes and selection procedures are described extensively elsewhere (Markow, 1975a). When the phototactic behavior of populations of flies is characterized using mazes, the most photopositive flies have a phototactic score of 16 and the most photonegative flies have a score of 1. Photoneutrality is characterized by a score of 8.5. At the time of the present study the strains used had been undergoing selection for 8 years. The photopositive strain had a phototactic score of 13.89 ± 0.10 ($N = 401$) and the photonegative strain had a score of 2.03 ± 0.06 ($N = 374$).

A mutant strain of flies characterized by complete blindness and designated *norpA*^{p24} ("no receptor potential," 1-6.5), was obtained from Dr. William Pak at Purdue University. Flies with the *norpA*^{p24} phenotype fail to show any behavioral or neurophysiological responses to light (Pak *et al.*, 1976). Another mutant strain of flies designated *smbB* ("smell-blind B") was provided by Dr. Jeff Hall at Brandeis University and Dr. William Quinn at Princeton University. While not physiologically characterized, *smbB* is inherited as a sex-linked recessive and fails to give any responses in standard tests of olfaction. The mutant *al* ("aristaleless" 2-0.01) and the wild-type strain Canton-S were both obtained from the *Drosophila* stock center at the California Institute of Technology.

Culturing Procedures. All flies were reared in half-pint bottles on a standard cornmeal molasses agar medium at 24 ± 1 C. Virgin flies were sexed under light ether anesthesia and stored in vials until use in experiments at 4 days of age. A small clip was made in the distal portion of the wing during collection. Although no effect of clipping (Markow *et al.*, 1978) on the outcome of female choice experiments has been found, the type of male clipped was alternated between replications of each experiment.

Experiments. Female choice experiments were conducted by placing 4-day-old virgin males of contrasting genotype into an 8-dram shell

vial. A single virgin female was then added. The time until mating and the genotype of the successful male were recorded. All transferring of flies was accomplished by aspiration in order to avoid further anesthesia. Several replications of each experimental situation were conducted with a usual sample size of $N = 20$ vials per replication.

RESULTS AND DISCUSSION

In the first series of experiments females were offered a "choice" between males of the photonegative and photopositive strains. The data in Table I show that photonegative males were significantly more successful than photopositive males. When the females were from the photopositive strain, negative males were 39% more successful than expected and 35% more successful than expected when photonegative females were used. With Canton-S females, photonegative males were 39% more successful than expected.

We were curious about the relative importance of visual, olfactory, and auditory cues for the success of photonegative males. Presumably if visual cues are important, male mating success would depend upon the female being able to see what the male is doing. To test this, pairs of males, one from each photostain, were supplied with "blind dates," females homozygous for *norpA*^{P24}. From Table I it is apparent that photonegative males are still at a significant advantage with *norpA* females and are 36% more successful than expected. The inability of females to perceive visual cues is not essential to the mating advantage of photonegative males. Another

Table I. Outcome of Female Choice Experiments Using Photopositive and Photonegative Males

Female genotype	N mating	Successful male				χ^2 (1:1)
		Photopositive		Photonegative		
		O	E	O	E	
Photopositive	198/201	60	99	138	99	30.7272**
Photonegative	208/219	68	104	140	104	24.9231**
Canton-S	98/103	31	49	67	49	13.224**
<i>norpA</i> ^{P24}	94/94	30	47	64	47	12.297**
<i>smbB</i>	106/122	41	53	65	53	5.4340*
<i>al</i>	96/98	20	48	76	48	32.667**

* $P < 0.05$.

** $P < 0.001$.

series of tests was run using females homozygous for *smbB*. While photonegative males still enjoyed a significant advantage, they were only 23% more successful than expected. This result may suggest that females with an altered ability to use olfactory cues also differ in their ability to discriminate photonegative from photopositive males. Evidence exists in the literature that the arista of *D. melanogaster* females are important in perceiving auditory information from males (Manning, 1967a; Burnet *et al.*, 1977). Females homozygous for the autosomal recessive *al* were tested for the ability to discriminate between photopositive and photonegative males. Not only were photonegative males more successful than photopositive ones but they were 58% more successful than expected.

In order to look for quantitative courtship differences between males of the two strains, courtship latency (time until courtship initiation) and courtship duration were measured in matings between Canton-S testor females and males from both the photopositive and the photonegative strains. From Table II it can be seen that photonegative males begin courting sooner and show shorter (though not significantly) overall courtship durations. In all experiments males from both strains were observed to actively court females.

From earlier experiments (Markow, 1975b) photonegative behavior is known to be strongly sex linked in *D. melanogaster*. If the mating advantage of negative males is genetically related to their photonegativity, the mating advantage should also show sex linkage. Reciprocal crosses between the two photostains gave F_1 males receiving X chromosomes from either the photonegative or the photopositive strain. Females were given a choice of the two types of F_1 males (Table III). No difference in mating success was observed, indicating that the photonegative male advantage is not a function of the same locus or loci giving photonegative behavior.

In the experiments described above, males from a strain of flies selected for negative photomaze behavior showed a significant mating advantage over males from a photopositive strain. This mating advantage was expressed with females from a number of wild-type and mutant strains. There was no evidence of reproductive isolation between the

Table II. Mean Courtship Latency and Duration

Character	Photopositive	Photonegative	<i>t</i>
Courtship latency (sec)	125.043 ± 19.94	67.948 ± 12.89	2.442*
Courtship duration (min)	7.095 ± 1.399	4.337 ± 0.54	1.838

* $P < 0.05$.

Table III. Outcome of Female Choice Experiments Using F₁ Males from Reciprocal Crosses Between Photopositive and Photonegative Strains^a

Female genotype	N mating	Successful male				χ^2 (1:1)
		X ⁽⁺⁾ Y		X ⁽⁻⁾ Y		
		O	E	O	E	
X ⁽⁺⁾ X ⁽⁻⁾	224/225	95	94	93	94	0.0021
Canton-S	99/102	53	51	49	51	0.078

^a X⁽⁺⁾ designates X chromosomes from the photopositive strain. X⁽⁻⁾ designates X chromosomes from the photonegative strain.

photopositive and the photonegative strains. In *D. melanogaster* photonegative behavior is strongly sex linked, but genetic analysis showed no evidence of sex linkage for the mating advantage of photonegative males. Apparently the photonegative advantage is a function of other genetic factors less directly related or even unrelated to selection for phototaxis itself.

What is it about photonegative males that accounts for their increased success? For one thing, they begin courting sooner and their time until mating tends to be shorter than observed for photopositive males. The total time spent actually courting and the average bout length of courtship were not measured, but superficially do not appear to differ between males from the two strains. Some behavioral element or elements being performed during courtship are probably more important than others in stimulating the females to accept photonegative males. The question is, is it merely persistence and the quantity of overall courtship that is important, or is there a particular element or sensory mode that assumes a dominant role in determining mating success?

While a *D. melanogaster* male does engage in courtship displays in front of as well as in back of a female, the visual aspects of the males behavior may not be critical to the success of photonegative males. The advantage for photonegative males was a similar magnitude greater than expected whether females were wild type or blind. An intact visual system is absolutely necessary for males to locate females and direct courtship toward them (Connolly *et al.*, 1969), but the above findings would imply that females may not be relying too heavily upon visual cues to discriminate between males. In experiments with females from the *smbB* strain the mating advantage of photonegative males was not as great as with other females (although still significantly greater than random). This observation would be consistent with the idea of olfactory attributes being

important for male success (Averhoff and Richardson, 1974). It is also possible, however, that since the *smbB* mutant has not been characterized physiologically, other phenotypic effects of the genotype are responsible for the slight reduction in mating advantage observed for photonegative males. Curiously, photonegative males are at their greatest advantage with *al* females. Studies from other laboratories have shown that females with surgically or genetically modified aristae are unable to perceive auditory signals from males (Manning, 1967a; Burnet *et al.*, 1977). That photonegative females show the highest proportion of success with *al* females could be suggesting that the aristae are involved but perhaps in a more complex way than originally anticipated.

It would be tempting to conclude that, in addition to the importance of early courtship initiation, visual cues are secondary to olfactory and auditory factors, the latter two of which might interact in some unknown way in the courtship success of photonegative males. Even if it were certain that each of the three mutants, *norpA*, *smbB*, and *al*, affected only visual, olfactory, and auditory reception, any generalizations from these findings to other examples of differential courtship success should be made with great caution. Analysis of determinants of courtship success within a species may in some ways be a more complex problem than finding the key behavioral factors involved in reproductive isolation between two different species. The former is apt to be more variable. There have been several approaches to the problem of determinants of intraspecific courtship success in *Drosophila*. One has been to try to define differential importance for the roles of particular sensory modes (Averhoff and Richardson, 1974; Bennet-Clark and Ewing, 1969; Grossfield, 1971). Another approach has been the quantification of various courtship components (Connolly *et al.*, 1974).

It is clear from the literature, however, that males showing a mating advantage usually show the shortest courtship latency and mating speed, just as we see for the photonegative males in this study. The sooner a male begins courting and presenting sensory stimuli to a female, the sooner courtship summation (Manning, 1967b) should occur. But the relative weights carried by pattern and intensity of particular components and sensory modes toward the summation may vary between males and females. In the case of the present study, females deprived of certain sensory information might have weighed the quality and quantity of other sensory information differently. In natural populations interindividual variability in both male courtship behavior and female thresholds for different stimuli would not be surprising and would provide the raw material for selection during evolution of ethological isolation.

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