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LETTER TO THE EDITOR

Rare Male Advantages Among *Drosophila* of the Same Laboratory Strain

Therese Ann Markow[1]

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A number of genetic and nongenetic variables have been reported to influence male mating success in *Drosophila* (Spiess, 1970; Markow *et al.*, 1978; Long *et al.*, 1980). One interesting and reportedly widespread factor bearing on mating success involves frequency-dependent sexual selection (see review by Ehrman and Probber, 1978), where males in the minority with respect to males having other genotypes or rearing conditions have been observed to mate more frequently than expected based on their low proportions in mating chambers. Although attempts have been made to elucidate the behavioral basis for this effect in *Drosophila* (Leonard *et al.*, 1974; Spiess and Kruckeberg, 1980), not all studies have been successful in demonstrating the existence of rare male advantages (Markow, 1978; Markow *et al.*, 1980; Anderson and McGuire, 1978; Childress and McDonald, 1973). In this author's laboratory, a strong rare male advantage has occasionally been observed in experiments usually showing no frequency-dependent selection whatsoever. The inconsistency with which these effects have been observed suggested some confounding environmental artifact. Subsequent investigation has revealed a factor which mimics the minority male advantage and is the subject of this letter.

Experiments were designed to test the influence of the way flies were selected for a test on the outcome of the test. All flies were *Drosophila melanogaster* from the Canton-S laboratory strain. Flies were reared under uncrowded conditions in half-pint milk bottles containing standard cornmeal–molasses–agar medium at 24 ± 1°C. Virgin males and females were separated under light ether anesthesia and stored separately in 8-dram food vials, 10 flies per vial, until use in experiments at 4 days of age. At 1 day of

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[1] Department of Zoology, Arizona State University, Tempe, Arizona 85281.

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age males were marked with either pink or blue fluorescent dust (Radiant R-103-G119). Females were not marked.

Ten pairs of flies were placed in a Plexiglas observation chamber made from a prototype kindly sent to the author by Dr. Lee Ehrman. The chambers were observed for 30 min. No flies were removed after mating and no females were observed to mate more than once. Chambers were set up with the following proportions of males: 8 blue/2 pink, 5 blue/5 pink, and 2 blue/8 pink. Females were always introduced first. Ten replications were conducted of each experiment.

Two series of experiments, differing with respect to selection of rare subjects, were carried out. In both series of tests, only flies in good physical condition were used. When a number of flies are stored in a vial, they distribute themselves in different places in the container. In the first series of experiments, the males which were to be the minority males were chosen from near the tops of the storage vials. In the second series, the minority males were chosen from the bottoms of the vials. Since there were only 10 males in any given storage vial, selection of majority males involved taking most of the males, and naturally some were found near the tops and some were found at the bottoms. When aspirating flies from storage vials, care was always taken not to disturb existing positions of males. Naturally not all vials contained the same number of males on the tops or bottoms, but in each case there were enough in each location to enable selection of the needed number of individuals. Majority and minority males were introduced into the chambers simultaneously.

The results are shown in Table I. Evidently no advantages or disadvantages were induced by marking flies with dust. At equal ratios both colors were equally successful in mating. In series one, minority males were significantly more successful than expected. In series two, when minority males came from the bottoms of storage vials, they mated much less frequently than expected. Since these observations strongly suggested a disadvantage for males coming from the bottoms of vials, experiments were set up with five males from the bottoms of vials and five males from near the tops. Ten replications consistently showed males from the tops having an advantage (matings with top males = 67, matings with bottom males = 24; $\chi^2 = 20.232$).

These observations suggest a significant behavioral difference between males chosen from different places in a storage container. Males closer to the top may be more active and therefore successful in courting. When aspirating just a few flies out of a vial as when setting up frequency-dependence tests, it is easiest to select those at the top, closest to the opening. Students and technicians who are unaware of this potential bias have been observed by this author to select minority males from the upper parts of vials. Most

Table I. Observed Numbers of Matings with Flies from the Canton-S Strain

Series	Ratio, pink:blue	Observed matings		χ^2
		Pink	Blue	
1	8:2	59	35	17.43[a]
	5:5	50	48	0.0408
	2:8	39	57	25.51[a]
2	8:2	88	9	6.96[a]
	5:5	47	49	0.042
	2:8	10	80	4.444[b]

[a] $P < 0.01$.
[b] $P < 0.05$.

investigators have wisely emphasized using flies in good physical condition but may still be introducing a bias in mating experiments. Because of the potentially important evolutionary implications of frequency-dependent selection, it is important to know if rare advantages are actually due to the genotypes of minority males or to some other factor.

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