

Testing for rare male mating advantages among various *Drosophila melanogaster* genotypes¹

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SUMMARY

Experiments were conducted to look for minority male mating advantages among several *Drosophila melanogaster* genotypes. The strains of flies used were Canton-S, Oregon-R, a strain carrying the recessive mutation *vermilion*, and highly geo-positive and geo-negative populations which had been selected for over 200 generations in Hirsch-geotaxis mazes. Two series of experiments were carried out, one series using ten pairs of flies, and another series using 20 pairs. Regression analysis gave one significant slope out of seven, suggesting that in the present study, frequency-dependent advantages are not as common or as strong as reported for *D. pseudoobscura*.

1. INTRODUCTION

The influence of the genotype on *Drosophila* sexual behaviour has been the subject of extensive investigation. These studies have revealed that frequency dependent selection and the rare-male mating advantage are not unusual in *Drosophila*. The rare-male effect occurs when males of two different genotypes are present in unequal proportions and the number of matings involving minority males occurs at an unexpectedly high frequency. While the rare male advantage has been reported to occur in *D. melanogaster* (Petit, 1954, 1958; Spiess & Schwer, 1978), *D. persimilis* (Spiess, 1968), and in *D. willistoni* group species (Petit & Ehrman, 1967), the phenomenon has become best known through the work of Ehrman and her associates on *D. pseudoobscura* (see Ehrman & Propper, 1978, for the most recent review). Within a given species, rare male effects have been reported to occur between males from different wild-type strains, strains having different chromosome arrangements, strains selected for different behavioural characters, the same strain reared at different temperatures, and strains differing at loci affecting visible external traits and internal enzymes.

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Because the evolutionary implications of the rare male phenomenon are potentially important (Anderson, 1969; Ehrman, 1970) an explanation of mechanisms underlying the effect is being sought. Among *D. pseudoobscura*, chemical stimuli have been suggested to play the greatest role in producing the rare male effect (Leonard, Ehrman & Pruzan, 1974*a, b*). The extensive genetic information and analytical techniques available for *D. melanogaster* would tend to make this species more useful than *D. pseudoobscura* for in depth analyses of how the rare male effect occurs. In the present study, three different sets of contrasting genotypes were used to test for the rare male effect in *D. melanogaster*: two wild-type strains, Canton-S and Oregon R; two strains selected for geo-positive and geo-negative behaviour; and a strain carrying the recessive eye-colour mutation, *vermilion* in the wild-type Canton-S genetic background.

2. MATERIALS AND METHODS

(i) *Strains of flies*. The Canton-S strain was obtained from the *Drosophila* stock centre at the California Institute of Technology and the Oregon-R strain was obtained from the Biology Department at Yale University. Highly geo-positive and geo-negative strains were created by over 200 generations of selection using Hirsch-gcotaxis mazes and are described extensively in other reports from this laboratory (Pyle, 1978). Geotactic scores may range from 1 (highly geo-negative) to 16 (highly geo-positive) and at the time of these experiments, the geo-negative strain had a score of 2.83 ± 0.22 ($n = 864$), while the geo-positive strain had a score of 15.11 ± 0.07 ($n = 921$). The vermilion (*v*) mutant strain was obtained from the California Institute of Technology and was backcrossed to the Canton-S strain for 5 generations to have the vermilion trait in the CS genetic background prior to mating experiments with Canton-S flies.

All flies were reared at 24 ± 1 °C in population cages containing 12 cups of standard cornmeal-molasses agar medium. Virgin males and females were separated under light ether anaesthesia and then stored at densities of 25 per 8-dram vial until testing at 4 days of age. A small clip in the distal portion of a wing, made at the time of collection, served to make males of different genotypes distinguishable from each other. In the present and in previous studies, chi-square tests revealed no effect of clipping on mate selection (Markow, Quaid & Kerr, 1978).

(ii) *Mating tests*. Experiments were carried out in plexiglass observation chambers (described in Ehrman & Parsons, 1975) constructed from prototypes supplied to us by Dr Lee Ehrman. Two series of experiments were conducted with each of three different genotypic combinations of flies. In the first series, 10 females of one genotype were placed with a total of 10 males of two genotypes. One type of male was of the same strain as the female, the other was different. The proportions of the two types of males varied from 4:1, 1:1 and 1:4 and each test was replicated ten times. In the second series of tests 20 pairs of flies were observed. The genotype ratios of both sexes were varied from 9:1, 4:1, 1:1, 1:4 and 1:9. Five replications of each test were conducted.

Table 1. *Matings observed when all females were from a single strain and there were 10 pairs/chamber: chi-square tests reflect differences from expected ratios*

Experimental genotypes	Female genotype	Male ratios	N	Males mating				χ^2
				Observed		Expected		
CS, OR		CS:OR		CS	OR	CS	OR	
	CS	1:4	94	35	59	18.8	75.2	17.449**
	CS	1:1	100	63	37	50	50	6.760**
	CS	4:1	86	73	13	68.8	17.2	1.282
	OR	1:4	93	19	74	18.6	74.4	0.010
	OR	1:1	100	66	34	50	50	10.240**
CS, v		CS:v		CS	v	CS	v	
	CS	1:4	87	23	64	17.4	69.6	2.250
	CS	1:1	97	50	47	48.5	48.5	0.092
	CS	4:1	93	77	16	74.4	18.6	0.453
	v	1:4	90	22	68	18	72	1.111
	v	1:1	87	54	33	43.5	43.5	5.069*
GP, GN		GP:GN		GP	GN	GP	GN	
	GP	1:9	90	10	80	9	81	0.123
	GP	1:4	97	23	74	19.4	77.6	0.835
	GP	1:1	100	60	40	50	50	4.000*
	GP	4:1	100	87	13	80	20	3.062
	GP	9:1	87	80	7	78.3	8.7	0.369
	GN	1:9	96	13	83	9.6	86.4	1.338
	GN	1:4	91	12	79	18.2	72.8	2.640
	GN	1:1	100	50	50	50	50	0.000
GN	4:1	100	80	20	80	20	0.000	
GN	9:1	98	95	3	88.2	9.8	5.24*	

* $P < 0.05$. ** $P < 0.01$.

Flies were introduced into the chambers by aspiration. Males were introduced first, then the females. Chambers were observed for one hour. The time and location of each mating was recorded, along with the genotypes of the participants. Experiments were performed in the mornings to avoid any potential affects of diurnal rhythms on mate selection.

3. RESULTS

Table 1 shows the results of experiments in which only one type of female was present in a chamber and the proportions of the two kinds of males were systematically varied. Data from 10 replications at each male frequency were tested for homogeneity and then pooled. Table 2 records the results of experiments in which the proportions of both sexes were varied. The chi-square tests recorded in the tables test the significance of the observed number of matings from those expected on the basis of the proportions of males used in each experiment. These tests show, for example, that Canton-S males have an advantage over Oregon-R males regardless of the genotype of the female (Table 1). While this statistical approach

Table 2. *Matings observed when genotype ratios of both sexes were varied and there were 20 pairs/chamber: chi-square tests reflect differences from expected ratios*

Experimental genotypes	Genotype ratios	N	Males mating				χ^2
			Observed		Expected		
CS, OR	<i>CS:OR</i>		<i>CS</i>	<i>OR</i>	<i>CS</i>	<i>OR</i>	
	1:9	94	14	80	9.4	84.6	2.500
	1:4	88	15	73	17.6	70.4	0.480
	1:1	88	60	28	44	44	11.636*
	4:1	90	70	20	72	18	0.278
9:1	91	84	7	81.9	9.1	0.538	
CS, v	<i>CS:v</i>		<i>CS</i>	<i>v</i>	<i>CS</i>	<i>v</i>	
	1:9	97	13	84	9.7	87.3	1.247
	1:4	97	31	66	19.4	77.6	8.670*
	1:1	96	57	39	48	48	3.375
	4:1	95	82	13	76	19	2.368
9:1	97	91	6	87.3	9.7	2.534	
GP, GN	<i>GP:GN</i>		<i>GP</i>	<i>GN</i>	<i>GP</i>	<i>GN</i>	
	1:9	95	12	83	9.5	85.5	0.731
	1:4	89	18	71	17.8	71.2	0.003
	1:1	92	40	57	48.5	48.5	2.970
	4:1	88	71	17	70.4	17.6	0.256
9:1	92	81	11	82.8	9.2	0.391	

* $P < 0.01$.

will detect deviations from expectation in individual experiments, significant chi-squares for one or more experiments (i.e. different frequencies) are not necessarily indicative of frequency dependence. Ayala (1972) argues convincingly that a method employing the linear regression of the logarithm of the proportion of mated males on the logarithm of the proportion of males present will serve as a statistical test for frequency dependence as well as providing estimates of the equilibrium point and the strength of the relationship. If mating occurs in direct proportion to the number of males present in a series of tests, then a regression line with a slope of 1.0 is obtained. Rare-male advantage results in a regression line with a slope less than 1.0. Thus the slopes of the regression lines obtained in tests of the rare male advantage are tested for statistical significance against a slope of 1.0. This analysis has the disadvantage of failing to account for possible differences in the number of matings which occur at each frequency. However, in the data to be analysed here, approximately equal numbers of matings were obtained at each frequency (Tables 1 and 2).

Table 3 presents the results of a regression analysis of the data summarized in Tables 1 and 2. Experiments in Table 1 employing the same kind of proportions of males but different female types were tested for homogeneity by chi-square. The CS, v and GP, GN groups are homogeneous over female type and were therefore analysed by means of a single regression. The CS, OR experiments of Table 1

Table 3. Regression analysis of the data given in Tables 1 and 2

(The values for b represent slopes obtained from linear regression of the logarithm of the proportion of mated males on the logarithm of the proportion of males present. The t tests measure the deviation of the slopes (b) from an expected slope of 1.0.)

Comparison	$b \pm \text{s.e.m.}$	t	D.F.	P
Male ratios varied				
CS/OR - CS	0.593 \pm 0.020	20.4	1	< 0.01
CS/OR - OR	1.012 \pm 0.228	0.1	1	> 0.90
CS/v	0.906 \pm 0.065	1.5	4	> 0.20
GP/GN	0.978 \pm 0.075	0.3	8	> 0.50
Ratios of both sexes varied				
CS/OR	0.915 \pm 0.132	0.6	3	> 0.50
CS/v	0.835 \pm 0.089	1.9	3	> 0.50
GP/GN	0.895 \pm 0.071	1.5	3	> 0.50

are not homogeneous over female type and were not combined in the regression analysis. Only a single regression has a slope significantly different from 1.0 - that resulting from experiments in which the ratio of CS:OR males was varied using CS females. The slope of this regression is less than one, indicating a rare male advantage. This line intercepts a line of slope 1.0 at a proportion of 91.3% CS males, indicating that no mating advantage should occur if CS and OR males were tested with CS females at a ratio of about 9:1. No evidence of significant frequency dependent mating is seen for the remaining comparisons (Table 3).

4. DISCUSSION

While the original reports of the rare male advantage dealt with *D. melanogaster* (Petit, 1954, 1958) the greatest portion of the rare male literature is comprised of work on *D. pseudoobscura* (Ehrman & Propper, 1978) where the minority effect has been most consistently observed and where rather extreme minority advantages seem to be occurring. The present study was conducted with the same type of observation chambers and genotype ratios showing clear rare-type advantages with *D. pseudoobscura*.

In only one case, CS/OR with CS, did a slope show a significant rare male effect. The fact that 5 of the other 6 slopes did differ slightly from one indicates that a rare male advantage might be operating but if so it is very weak. In many cases minority males mated less frequently than expected. This was true with vermilion males in both series of experiments and with geo-negative males in Series One. Cases where minority males mated more frequently than expected only occurred when that type of male had an advantage at other frequencies as well.

Throughout the above data there are situations where a mating advantage for a given type of male is very strong at particular genotypic ratios but disappears when other proportions are tested. For *D. pseudoobscura* these advantages are reported to be consistently associated with minority male genotypes. Results of an

earlier study with the *sepia* allele (Markow, 1978) fail to show any relationship between extreme genotypic ratios and increased mating success of minority type males. It is interesting that in other tests, where single Canton-S, Oregon-R, or vermilion females are given a choice of two males, one of which is Canton-S, vermilion males and Oregon-R males are at a strong disadvantage (in preparation). These observations, along with the results of the present study, suggest that there are interactions between male mating success, genotype ratio, and changes in the total individual density. The nature of these interactions is most likely different for each of the different pairs of genotypes studied here. While the relationship between male success and rareness appears to be rather common among various *D. pseudoobscura* genotypes, it is either not as common or strong in *D. melanogaster*, or it is not detectable under the same experimental conditions for which it was reported in *D. pseudoobscura*.

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