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A test for the rare male mating advantage in coisogenic strains of *Drosophila melanogaster**

BY THERESE A. MARKOW

Department of Zoology, Arizona State University, Tempe, Arizona 85281, U.S.A.

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SUMMARY

Experiments were carried out to see if rare male mating advantage could be detected when males differ at only one locus. The eye colour mutant sepia was inserted into a strain of *Drosophila melanogaster* homozygous for a first, second, and third chromosome from the Canton-S strain. Tests for the rare male advantage were conducted using ratios of 4:1, 1:1, and 1:4 of the coisogenic wild-type and sepia males. No deviations from expected types of matings were observed. The results are discussed with respect to possible conditions necessary for the rare male effect to occur.

1. INTRODUCTION

The well known rare male mating advantage occurs when two types of males are present in unequal proportions and the number of females inseminated by minority males is significantly greater than expected on the basis of their relative frequency. This very interesting minority male advantage and some of its evolutionary implications have been most widely discussed in *Drosophila*. The effect has been reported when *Drosophila* males differ at single loci affecting external somatic traits (Petit, 1958, 1959; Ehrman, 1970), when males come from different laboratory strains (Tardif & Murnick, 1975) or are of differing karyotypes (Ehrman, 1966, 1968; Ehrman & Spiess, 1969), or if they carry different isozyme variants (Ehrman *et al.* 1977).

Investigations of factors important in the rare male advantage have shown that the relative proportions of types of females present is of little influence (Petit, 1958; Ehrman & Spiess, 1969). The importance of olfactory cues in the rare male effect associated with gene arrangements in *D. pseudoobscura* has been reported reported by Leonard, Ehrman & Pruzan (1974) and Leonard, Ehrman & Schorsch (1974), although the loci responsible for the differences in olfactory stimuli have not been identified. It is also unknown how differences at single loci, whether external somatic markers or isozymes, function at sensory levels to bring about the rare male effect.

With *Drosophila*, it is possible to control genetic background almost entirely and in this way determine the effects of 'rarity' with respect to a single locus

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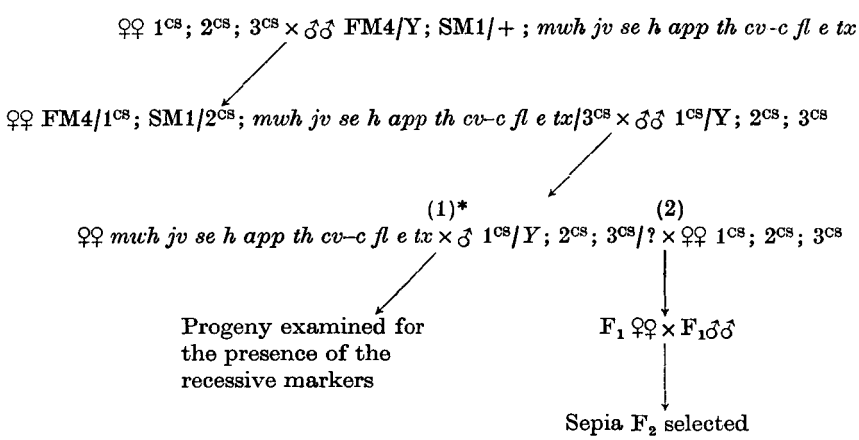
alone. One way to control genetic background is to repeatedly cross a single mutant into a wild-type strain and thereby 'randomize' the differences between mutant and wild-type flies which exist at other loci. However, by using this procedure it is not possible to know if alleles linked to the mutant locus in question are also being randomized. An alternative approach would be to create coisogenic strains in which the only genetic difference would be at a single locus. By using coisogenic strains one could ask if an allelic substitution at just one locus can be associated with a rare male advantage. The rare male phenomenon has never been examined in exactly this way.

Our laboratory had synthesized virtually coisogenic strains of *D. melanogaster* for another series of experiments and the availability of these strains was taken advantage of to conduct some rare male tests. The mutant gene used was the recessive third chromosome marker sepia (*se*) which causes a build-up of sepiapterin producing a very dark eye colour in mature adult flies.

2. MATERIALS AND METHODS

Sepia (*se*) was inserted into a strain of flies homozygous for a first, second, and third chromosome from the Canton-S wild type strain. The allelic substitution procedure is shown in Fig. 1. Map locations of the ten third chromosome markers

Fig. 1. Synthesis of coisogenic strains of sepia and wild-type (CS) *D. melanogaster*.



* In cross (1) individual males carrying recombinant third chromosomes were tested for the presence of recessive markers. Males were then discarded unless they were determined to carry only sepia, in which case they were used in cross (2). Chromosomes of the Canton-S strain are labelled CS.

are as follows: *mwh*, 0·0; *jv*, 19·2; *se*, 26·0; *h*, 26·5; *app*, 37·5; *th*, 42·3; *cv*, 54·1; *fl*, 59·5; *e*, 70·7; and *tx*, 91 (Lindsley & Grell, 1968). Since *jv* and *h* are 7·3 map units apart, it is likely that some DNA around the sepia locus was incorporated in the Canton-S strain. Chromosome four is very small and was not made isogenic, but the majority of fourth chromosomes were derived from the Canton-S strain.

Flies were raised on standard cornmeal-molasses-agar medium at 24 ± 1 °C. Virgin males and females were separated under light ether anaesthetization and stored separately for 3 days, at which time the mating tests were conducted. Tests were carried out in plexiglass observation chambers (described in Ehrman & Parsons, 1975), prototypes of which had been kindly provided to our laboratory by Dr Lee Ehrman. Twenty females and twenty males were introduced into the observation chambers without anaesthetization and were observed for 2 h. The 2 h cut-off was used since preliminary tests showed no increase in the number of matings after that time. Females were always of the same genotype (either wild type or sepia), while the male ratios were 1:4, 1:1, and 4:1 (wild type:sepia). These ratios correspond to the ratios used in other investigations showing the rare male mating advantage (Ehrman, 1970; Pruzan, 1976). Five replications were conducted for each experimental situation and these replications were pooled following homogeneity tests.

3. RESULTS

The results appear in Table 1. Chi-square tests for goodness of fit reveal no increase in relative mating frequency by rare males. The only case of a significant departure from expected mating frequencies shows a minority disadvantage for wild-type males when sepia females are used. When the data for all the experiments were broken down into early and late matings, there were still no differences between observed and expected frequencies.

Table 1. *Mating success of coisogenic sepia and wild-type* D. melanogaster *males*

Female genotype	Male ratios (W.T./se)	Mating (%)	W.T. males		*se* males		χ^2
			Obs.	Exp.	Obs.	Exp.	
+/+	4:1	96	84	76·8	12	19·2	3·37
+/+	1:1	93	52	46·5	41	46·5	1·30
+/+	1:4	95	21	19·0	74	76·0	0·25
se/se	4:1	98	77	78·4	21	19·6	0·12
se/se	1:1	91	43	45·5	48	45·5	0·27
se/se	1:4	96	11	19·2	85	76·8	4·37*

* $P < 0{\cdot}05$.

4. DISCUSSION

What are the possible explanations for the absence of rare male mating advantages in the present study? In some of the earlier rare male experiments, the two strains used often had been separately cultured for years and had accumulated genetic differences as part of, or in addition to, the genotypic differences being examined for mating advantage. However, most experiments have attempted to randomize genetic backgrounds by several generations of intercrossing. In these experiments it is possible that the intercrossed strains still differ at loci linked to the ones being studied. In the present study the amount of genetic material inserted with the sepia gene was kept to a minimum by the extensive use of genetic

markers in the stock synthesis. While single mutants such as sepia are certain to be pleiotropic, there may not be enough that is 'different' about the sepia males to result in the rare male effect. Perhaps differences are required at more than one locus for the sensory cues which operate in rare male advantages to be detectable. Chemical stimuli which were found to influence the rare male effect in *D. pseudoobscura* (Leonard *et al.* 1974*b*) were isolated from strains which carried different gene arrangements and therefore probably different allelic combinations at a large number of loci.

Another possible explanation for the absence of the rare male phenomenon in the present study is related to the foregoing discussion. Perhaps rare male advantage is not associated with variations at all loci or even with all karyotype arrangements. The effect of increasing levels of eye pigmentation on male courtship success has been nicely demonstrated by Geer & Green (1964) and by Connolly, Burnet & Sewell (1969). As a rule, males with eyes which are as pigmented as wild type show courtship patterns and mating success rates which are similar to those of wild-type males. Since sepia-eyed flies have eyes which are at least as dark as wild-type eyes, it could be argued that males with sepia eyes might not be distinguishable from wild-type males by the females. Sepia males are certainly not at a disadvantage under any conditions in the present study. However, it is interesting to note that in a separate study using the same coisogenic strains, in matings involving a single female and two males, one sepia and one wild type, that sepia males are successful in only 35% of the tests ($n = 100$) (Markow & Grounds, manuscript in preparation), suggesting that females can distinguish between the two under certain conditions.

If rare male advantage does not exist for all loci or genotypes, sepia is probably not the only example. Other examples should be sought and perhaps some overall picture of the genotypic conditions necessary for rare male advantage to occur can be constructed.

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