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Original article

Rare-male mating advantage in *Drosophila ananassae*

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Summary – Minority-male mating advantage is a phenomenon whereby genetic variability can be maintained in populations without genetic load at equilibrium. Rare-male mating advantage has been shown to be a widespread phenomenon in *Drosophila*, although it is seen to be absent in some species. During the present investigation, experiments were conducted to study the mating success of wild type and mutant males at different ratios in the Elens-Wattiaux mating chamber using sepia and cardinal mutant stocks and a wild-type stock in order to detect a rare male effect in *D. ananassae*. In all the experiments the female-choice technique was employed. Red eye males were tested separately with sepia and cardinal mutants. Experiments were conducted at 9 different ratios. The results show that although both types of males are equally successful in mating when present in the same ratio, they are more successful in mating when in a minority. This advantage disappears when the males become common. Thus the present results provide evidence for the existence of a minority-male mating advantage in *D. ananassae*.

Drosophila ananassae **– sepia and cardinal mutants – rare male mating advantage**

Résumé – L'avantage au mâle rare chez *Drosophila ananassae*. *L'avantage donné aux mâles de génotypes minoritaires, à l'occasion des appariements, est un phénomène susceptible de maintenir une variabilité génétique sans induire de fardeau génétique dans une population en équilibre. C'est un phénomène très répandu chez les Dorsophiles, mais absent dans certaines espèces. Le succès à l'appariement de mâles de type sauvage et de mutants a été étudié en utilisant la technique du choix par la femelle, avec neuf proportions différentes de mâles mutants ou sauvages. Des stocks de type sauvage, et de mutants* sepia *et* cardinal, *chez D. ananassae ont été utilisés. Les mâles à yeux rouges étaient testés séparément avec les deux types de mutants. Les résultats indiquent que les deux types de mâles ont des succès équivalents quand ils sont présents dans la même proportion, et qu'ils sont favorisés quand ils sont minoritaires. L'avantage d'un type disparaît quand il devient commun.*

Drosophila ananassae – ***mutants*** **sepia** ***et*** **cardinal** – ***avantage au mâle rare***

INTRODUCTION

Frequency-dependent selection occurs when the selective value of a given genotype depends on its frequency. Frequency-dependent selection may be positive in favour of the common type, or negative in favour of the rare type. One of the most interesting and best studied examples of this frequency-dependent sexual selection is the rare-male mating advantage. When 2 strains of the same species are present together, the rare type is more successful in mating than the common. Petit (1951) was the first to report the occurrence of rare-male mating advantage in multiple-choice matings between Bar and wild-type *Drosophila melanogaster.* It was also demonstrated that this phenomenon was able to maintain polymorphism and was influenced by genetic background, temperature, age and nutrition (Petit, 1954, 1958). In 1964, Spiess (in Ehrman, 1966) observed a minority advantage for mating propensities of WT vs. KL chromosome arrangements in *D. persimilis* at different frequencies of 2 karyotypes among males. Ehrman (1966) demonstrated frequency-dependent selection between populations of different geographic origin in *D. pseudoobscura* and *D. paulistorum.* More recently, a number of reports have been documented which suggest that rare-male mating advantage is of common occurrence in *Drosophila* (for references see the reviews by Petit and Ehrman, 1969; Ehrman and Probber, 1978; Ehrman and Parsons, 1981; Spiess, 1982; Knoppien, 1985a).

The phenomenon of rare-male mating advantage is of considerable evolutionary significance, as from the view point of population genetics it may represent a way of maintaining high levels of genetic variability in natural populations without genetic load at equilibrium. Minority-male mating advantage also promotes outbreeding and exchange of genes among different populations (Averhoff and Richardson, 1974; Lewontin, 1974). Minority-male mating advantages have so far been reported in 7 species of *Drosophila: melanogaster, pseudoobscura, persimilis, willistoni, tropicalis, equinoxialis* and *funebris.* It has been shown to occur for mutants, inversion karyotypes, isozyme variants, and strains reared at different temperatures and having behavioural differences. This phenomenon has subsequently been shown in other animals also; *e.g.*, flour beetles *Tribolium castaneum* (Sinnock, 1970), a parasitic wasp *Mormoniella vtripennis* (Grant *et al.*, 1974, 1980) and some sex-linked colour mutants of the guppy, *Poecilia reticulata* (Farr, 1977, 1980). The generality of this phenomenon where rare males are favoured in mating has become accepted but it has given rise to much discussion and controversy with regard to its causes. According to the controversy, more analysis of the phenomenon might be of interest. It has been suggested that the rare-male effect might be an artifact due to bias as a result of marking the flies for identification during experiments (Bryant *et al.,* 1980). It has been shown that the fluctuations in mean sexual vigour of the males caused by sampling errors might result in spurious rare-male advantage (Markow, 1980). Differential storage conditions can also bias the outcome of a rare-male experiment (Knoppien, 1985b). A number of explanations have been proposed to account for the minority-male mating advantage (see the reviews by Spiess, 1982; Knoppien, 1985a).

Drosophila ananassae, a cosmopolitan and domestic species, belongs to the *ananassae* subgroup of the *melanogaster* species group. This species is unique as it possesses many genetic peculiarities. Certain aspects of behaviour genetics in *D. ananassae* have been investigated by the present authors (Singh *et al.*, 1985; Chatterjee and Singh, 1987; Singh and Chatterjee, 1985a, b, 1986, 1987, 1988a, b). In order to test the rare-male effect in *D. ananassae*, we studied the mating success of wild-type and mutant males at different ratios in a mating chamber using sepia and cardinal mutants. The results of the present investigation reveal the occurrence of rare-male mating advantage in *D. ananassae* and this is the subject of this communication.

MATERIALS AND METHODS

Three stains of *D. ananassae*, were employed in the experiments to test for the rare male effect:

(i) VN-ST: a wild-type (red eye) stock;

(ii) sepia: a recessive mutation on the second chromosome; eye colour sepia;

(iii) cardinal: a second chromosome recessive mutation; eye colour yellowish vermillion, ocelli white.

The 2 mutants, sepia and cardinal, were tested separately with the red eye males for the rare male effect.

To test for minority-male mating advantage with red eye and sepia flies, virgin females and males of both the strains were separated under ether anaesthesia and stored separately in food vials, 10 flies per vial, until use in experiments at 7 days of age. One day before the experiment, the males were kept in the ratios to be tested in vials. Female-choice tests were used. Flies were introduced into the mating chamber, females (20) followed by males (20). The Elens-Wattiaux (1964) mating chamber was used for the direct observation of flies. The matings were recorded at 5-min intervals for a period of 30 min. All copulating pairs were aspirated out in order to prevent males from mating a second time. Five replicates were run with each of the 9 ratios, with red eye and sepia females separately. Following the same procedure, the rare-male effect was tested for cardinal flies with red eye flies at 9 different ratios.

All the tests were performed from 7 to 9.30 am in a temperature controlled room (24 °C approximately) under normal laboratory light conditions (Lux-lux range 10 000).

RESULTS AND DISCUSSION

The numbers of matings of red and sepia males in the first 10 matings in female-choice experiments at different ratios are shown in Table I. For each of the 9 ratios, 5 replicates were run. Thus 100 males in total (red + sepia) were tested for each ratio. The expected numbers were calculated on the basis of the ratio between 2 types of males introduced into the mating chamber with equal mating ability. The red and sepia males were equally successful in mating when present in an equal ratio (10:10). This shows that both kinds of males have equal mating ability ($\chi^2 = 0.08$ with both females). Both types of males were more successful in mating when present in a minority. The χ^2 values show a significant difference in 6 cases in which males in minority were more successful. The same males were less successful in mating when they were common.

The mean mating percentages of both red and sepia males were calculated by combining the data of both females. The mean mating percentages of red and sepia males as their ratios varied are given in Table II, and illustrated in Fig. 1. It is apparent that the mating percentage of both males remained high when they were

Table I. Number of matings of red (R) and sepia (se) males with both types of females in mating chamber at different ratios (data based on 5 replicates).

Female type	*Male ratio*		*Observed frequency*		*Expected frequency*		χ^2
	R	*se*	*R*	*se*	*R*	*se*	
R	18	2	41	9	45	5	3.55
R	16	4	37	13	40	10	1.13
R	14	6	32	18	35	15	0.86
R	12	8	27	23	30	20	0.75
R	10	10	26	24	25	25	0.08
R	8	12	25	25	20	30	2.08
R	6	14	24	26	15	35	7.70*
R	4	16	14	36	10	40	2.00
R	2	18	10	40	5	45	5.55*
se	18	2	40	10	45	5	5.55*
se	16	4	33	17	40	10	6.13*
se	14	6	28	22	35	15	4.67*
se	12	8	31	19	30	20	0.08
se	10	10	24	26	25	25	0.08
se	8	12	14	36	20	30	3.00
se	6	14	17	33	15	35	0.38
se	4	16	17	33	10	40	6.10*
se	2	18	8	42	5	45	2.00

* $P < 0.05$.

Table II. Mean mating percentage of red and sepia eye males at different ratios (calculated by combining the data of both red and sepia females).

Number of males per 20	*Red male*	*Sepia male*
2	90.00	95.00
4	77.50	75.00
6	68.33	66.66
8	48.75	52.50
10	50.00	50.00
12	48.33	50.83
14	42.85	42.14
16	43.75	43.12
18	44.97	45.55

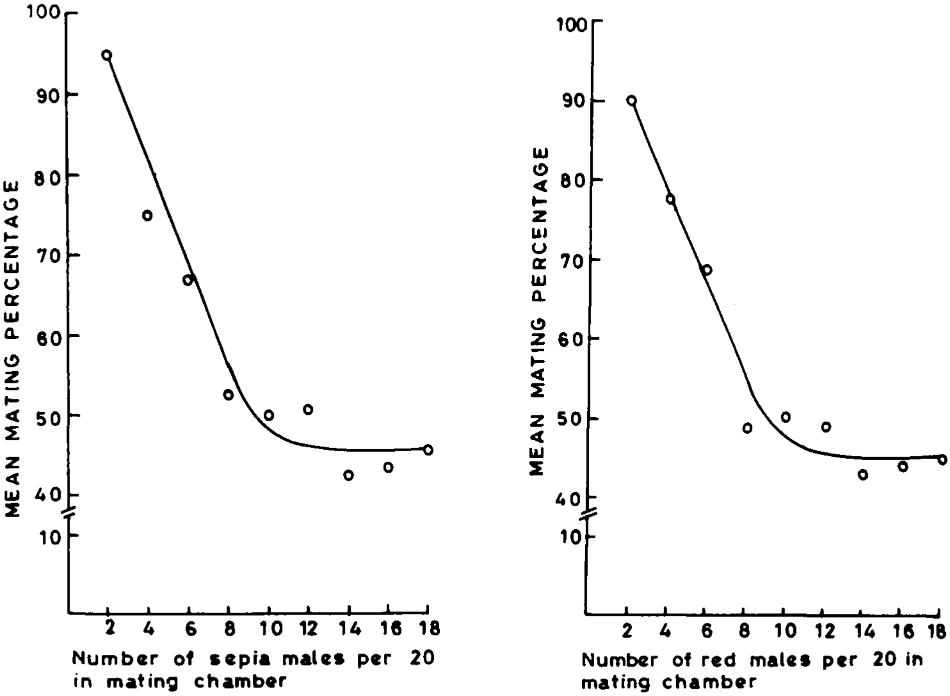

Fig. 1. Mean mating percentage of red and sepia eye males in female-choice experiments.

in a minority. However, the mating advantage of both males was lost when their ratios increased. Thus the males which show advantage when in minority remain no longer advantageous in mating when they become common in the mating chamber.

The numbers of matings of red and cardinal males in the first 10 matings at different ratios are shown in Table III. Five replicates were run at each of the 9 ratios. The red and cardinal males were equally successful in mating when they were present in equal proportion (χ^2 = 0.32 with red females; χ^2 = 0.08 with cardinal females). A significant difference in mating success was found in 1 case where cardinal males were in minority ($\chi^2 = 6.13$ with cardinal females). The mean mating percentages of red and cardinal males at different ratios are shown in Table IV and depicted in Fig. 2. It is apparent that the males were more successful in mating when they were in a minority. This advantage was lost when their number increased.

Since minority advantage has been observed when red males are tested with both sepia and cardinal males, it provides evidence for a rare-male mating advantage in *D. ananassae*. This is the first report of this phenomenon in *D. ananassae*. Furthermore, sepia eye mutant males competing with wild type show mating advantage when rare in *D. ananassae*, although Markow (1978) did not find a rare-male effect for the mutant sepia competing with a wild type in *D. melanogaster*.

Because the evolutionary implications of this phenomenon are potentially important, explanations regarding the mechanism underlying the effect have been sought.

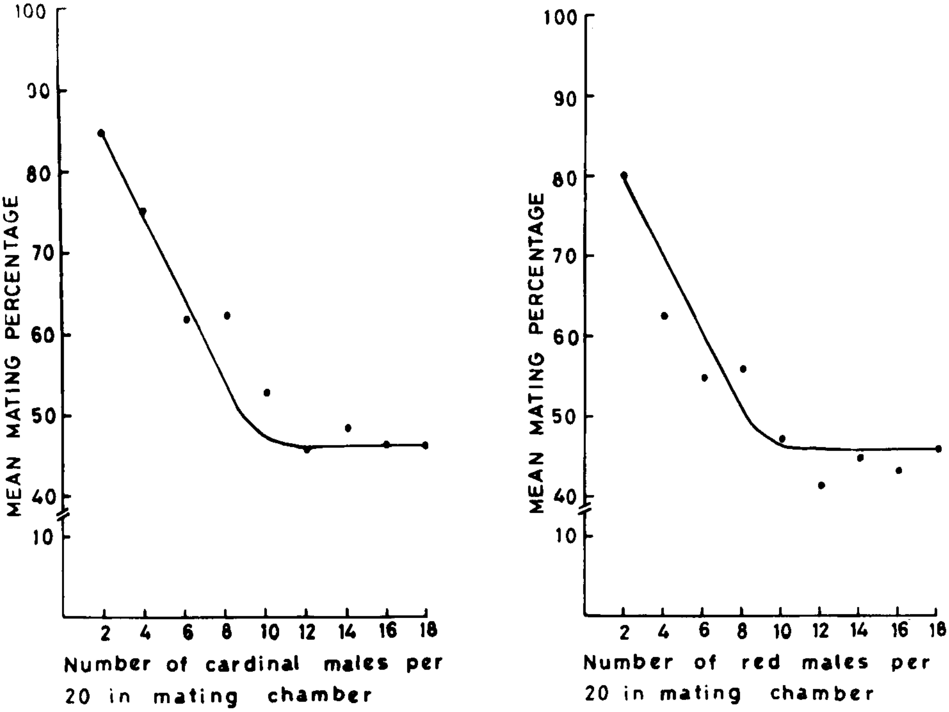

Fig. 2. Mean mating percentage of red and cardinal eye males in female-choice experiments.

According to the sampling and habituation hypothesis of Ehrman and Spiess (1969), the nature of the cue is different for different male types. The females become conditioned against mating with the males that first court them during their unreceptive period. As these males are of the more common type, the rare males are successful in mating as they are able to break through the habituation when the females become sexually active. Experiments have shown that frequency-dependent sexual selection in *D. pseudoobscura* is influenced by a number of factors; e.g. age of the females, exposure to other flies and previous mating experience (Pruzan and Ehrman, 1974; Pruzan, 1976). A minority female advantage has also been reported by some authors (Spiess and Spiess, 1969; Cereghetti *et al.*, 1987). One could even speak of a general influence of sex-ratio (Sharp, 1982). The cause of the rare-male mating advantage is not yet resolved fully. It is likely that a combination of specific mechanisms is responsible and a set of mechanisms operating in one species may be different from that in another. Experiments are being conducted to look for the behavioural basis for the rare male effect in *D. ananassae.*

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Table III. Number of matings of red (R) and cardinal (cd) eye males with both types of females in mating chamber at different ratios (data based on 5 replicates).

Female type	*Male ratio*		*Observed frequency*		*Expected frequency*		χ^2
	R	*cd*	*R*	*cd*	*R*	*cd*	
R	18	2	41	9	45	5	3.55
R	16	4	37	13	40	10	1.13
R	14	6	32	18	35	15	0.86
R	12	8	25	25	30	20	2.08
R	10	10	23	27	25	25	0.32
R	8	12	23	27	20	30	0.75
R	6	14	16	34	15	35	0.10
R	4	16	13	37	10	40	1.13
R	2	18	8	42	5	45	2.00
cd	18	2	42	8	45	5	2.00
cd	16	4	33	17	40	10	6.13*
cd	14	6	31	19	35	15	1.53
cd	12	8	25	25	30	20	2.08
cd	10	10	24	26	25	25	0.08
cd	8	12	22	28	20	30	0.33
cd	6	14	17	33	15	35	0.38
cd	4	16	12	38	10	40	0.50
cd	2	18	8	42	5	45	2.00

* $P < 0.05$.

Table IV. Mean mating percentage of red and cardinal males at different ratios (calculated by combining the data of both red and cardinal females).

Number of males per 20	*Red male*	*Cardinal male*
2	80.00	85.00
4	62.50	75.00
6	55.00	61.66
8	56.25	62.50
10	47.00	53.00
12	41.67	45.84
14	45.00	47.87
16	43.75	46.88
18	46.12	46.70

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