Mating propensity in *Drosophila bipectinata* under different sex-ratios and choice situations

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To test the effect of sepia eye colour mutation on mating activity in *D. bipectinata*, experiments were conducted by using mutant and wild-type stocks and employing multiple-, female-, male- and no-choice techniques. Mating success in different experiments was observed in Elens–Wattiaux mating chamber for one hour. The values of isolation estimate in different experiments are close to one and the difference between the numbers of homogamic and heterogamic matings is not significant, indicating random mating between wild-type and mutant flies. The comparison of sexual activity of two types of flies clearly indicates that wild-type males are more successful in mating than mutant males. However, there is no difference in the sexual activity of two types of females. Thus sepia eye colour mutation diminishes the sexual activity of males in *D. bipectinata* which provides evidence for sexual selection. The results of mating propensity tests obtained in the experiments employing multiple-, female- and male-choice techniques are similar showing significant difference in the number of matings of the two types of males. However, the difference in the number of matings of sepia mutant and wild-type males is not significant in no-choice experiments. Thus, sexual activity is influenced by different choice situations. However, sex-ratio has no effect on the mating success in *D. bipectinata*.

THE Indian subcontinent harbours rich *Drosophila* fauna and certain species are of common occurrence. Behavioural studies on certain Indian species such as *D. ananassae, D. bipectinata, D. malerkotliana* and *D. biarmipes* have been carried out by Indian workers (see refs 1 and 2). *Drosophila bipectinata* is a member of the *bipectinata* complex of the *ananassae* subgroup of the *melanogaster* species group. It has wide geographical distribution and is of common occurrence in India. It is characterized by genetic polymorphism in its natural populations[2] and shows incomplete sexual isolation with the closely related members of the *bipectinata* complex[3]. Incipient sexual isolation among certain geographic strains of *D. bipectinata* has also been reported[4]. Evidence for genetic control of sexual activity and existence of sexual selection in *D. bipectinata* has been presented on the basis of mating propensity tests carried out on geographic strains, their hybrids and diallel crosses[5,6]. Evidence for rare male mating advantage and positive correlation between duration of copulation and fertility has also been presented in *D. bipectinata*[7,8]. The effects of mutations on mating propensity and selective mating have been widely tested in various species of *Drosophila*[9–13]. In general, it has been shown that mutation diminishes the sexual activity of males. However, certain mutations may affect the receptivity of females. Although a lack of assortative mating between wild-type and mutant *Drosophila* has been reported by several authors, selective mating has also been found in some cases.

Male mating success in *Drosophila* has been extensively studied and appears to be related to male size in a number of species[14–16]. Interestingly in *D. ananassae*, flies possessing high number of sternopleural bristles are more successful in mating than those with low number of bristles[17]. There are substantial genetic variations for female receptivity in *Drosophila*[18]. Relative courtship success of mutant *D. melanogaster* male have been found to vary in different sex-ratios[19]. An influence of sex-ratio on the frequency dependence of mating success in *D. melanogaster* has been reported[20]. Aspi and Hoikkala[21] investigated the importance of male song and morphological characters to the male mating success in a two-year field study in natural populations of *D. littoralis* and *D. montana*. These findings indicate the possible balance between force of sexual and natural selection.

To test the effect of mutations on mating behaviour and propensity, different experimental techniques have been employed by different investigators. These experimental techniques are multiple-choice, female-choice, male-choice and no-choice. In the experiment employing multiple-choice technique, males and females of two types are confined together and this is a situation perhaps most closely corresponding to natural condition. Both males as well as females have choice. In no-choice experiments, one type of male is placed with one type of female and it does not represent a choice situation. In male-choice experiments, one type of male is placed with both types of females. Thus males can choose one of the two types of females. In female-choice experiments, one type of female is placed with both types of males and thus females can choose one of two males[10]. These experimental techniques have been used to score mating success in various *Drosophila* species. Variation in the results due to different choice situations and sex-ratios has been found in some cases with respect to the pattern of mating and mating propensity[13,22–25]. We detected a spontaneous mutation, i.e. sepia eye colour (autosomal recessive) in a wild laboratory stock of *D. bipectinata*[26]. To test the effect of mutation and different experimental methods on mating propensity in *D. bipectinata*, experiments were conducted and the results are represented in this communication.

In order to study the effect of mutation on mating propensity in *D. bipectinata*, two stocks used were: (1)

wild type (geographic origin Trivandrum) and (2) sepia eye colour mutant stock. Sepia eye colour is an autosomal recessive mutation which is the first report of spontaneous mutation in *D. bipectinata*[26]. These two stocks were crossed with each other for many generations for randomization of genetic background so that the wild stock becomes isogenic to the sepia stock except at the sepia locus. All the experiments were carried out by direct observation in an Elens–Wattiaux mating chamber kept in a room maintained at approximately 24°C temperature under normal laboratory light condition. All the experiments were conducted between 7.00 and 11.00 A.M. After the strains were made isogenic they were cultured separately in food bottles. Virgin females and males were collected and aged for seven days. 15 virgin females and 15 virgin males were introduced into a mating chamber, females introduced first. When a pair commenced mating it was taken out with an aspirator and the type of mating was recorded. Mating was observed for 60 min. In all the experiments 15 flies of each sex were used and 5 trials were carried out for each experimental set. Different experimental techniques were used to study the effect of different sex ratios and choice situations on mating behaviour of *D. bipectinata*.

(i) Multiple-choice: Females and males of sepia and red eye were used in equal ratio, i.e. 15 flies of each type and of each sex. The total number of flies in each replicate was 60 and sex-ratio was 1 female : 1 male.
(ii) Female-choice: 15 females of one type were placed with 15 males of each of two types. The total number of flies in each replicate was 45 and sex-ratio was 1 female : 2 males.
(iii) Male-choice: Males of one type were kept with females of both types, i.e. 15 males of one type with 15 females of each of the two types. The total number of flies in each trial was 45 and sex-ratio was 1 male : 2 females.
(iv) No-choice: The flies were not given a choice and one type of male was confined with one type of female. Thus a total of four different combinations were carried out in no-choice experiments. The total number of flies in each replicate was 30 and sex-ratio was 1 female : 1 male.

The numbers of different mating combinations between sepia and red eye *D. bipectinata* are presented in Table 1. To test selective mating, isolation estimate was calculated for each experiment separately using the formula suggested by Merrell[27]. The values of isolation estimate for different experiments are also given in Table 1. Isolation estimate ranges from 0.70 to 0.95, suggesting that there is no selective mating between sepia and red eye *D. bipectinata*. Furthermore, different experimental techniques had no effect on the pattern of matings as isolation estimate was close to one in all the experiments. The χ^2 values were calculated to measure the differences between homogamic and heterogamic matings under the assumption of random mating. The difference between the homogamic and heterogamic matings is insignificant which provides no evidence for preferential mating between females and males of same type. The χ^2 values calculated for 1:1 ratio on marginal totals to assess the relative mating propensity of the two sexes of both strains are presented in Table 2. In all the experiments, except the no-choice method, wild-type males are more successful in mating than mutant males. Differences are significant for males in multiple-female and male-choice experiments but not significant for females in all the experiments. In no-choice experiments, both types of males are equally successful in mating. Thus the results of no-choice experiments are different from those of the other experiments with respect to male mating propensity.

It is known that male activity and female receptivity are the main factors responsible for successful mating in *Drosophila*[28]. The relative mating propensity (success) varies within the species and has been found to be associated with several genetic factors[9–12]. It is evident from the results of these studies that the efficiency of mating varies for different genotypes. This provides evidence for sexual selection as well as for a genetic determination of sexual behaviour. Contribution of males to the variation in mating propensity is greater than that of females and thus males are inherently more subject to intrasexual selection[29–31]. The males which inseminate more females in a limited time will contribute more progeny[32]. Thus male mating propensity is an important component of fitness. A positive correlation between mating activity and fertility has also been found[31]. During the course of the present study, it has been found that the sepia eye colour mutation diminishes the sexual activity of males and sepia males are less successful in mating as compared to wild-type (red eye) males of *D. bipectinata*. However, this gene has no effect on the receptivity of females. This provides evidence that males are inherently more subject to intrasexual selection than females as it has been demonstrated in other species of *Drosophila*[29–31]. The effects of sepia mutation on mating success also lend support to the genetic control of sexual behaviour in *Drosophila*. The sepia eye mutation in *D. melanogaster*[33] and in *D. ananassae*[34] has been utilized to test the rare-male mating advantage. In *D. ananassae*, rare male-mating advantage was observed by Singh and Chatterjee[34] when sepia males were tested with red males, although Markow[33] did not find a rare-male effect for the mutant sepia competing with a wild type in *D. melanogaster*. Thus the results may vary in different species with respect to the effect of mutation on mating success. Evidence for polygenic control of sexual activity with substantial amount of genetic

Table 1. Number of matings in 60 min in a mating chamber containing 15 flies of each sex from sepia eye and red eye *D. bipectinata* in multiple-, female-, male- and no-choice experiments

Experiment	Red ♀ × sepia ♂	Red ♀ × red ♂	Sepia ♀ × red ♂	Sepia ♀ × sepia ♂	Isolation estimate	χ^2
Multiple-choice	19	30	23	14	0.95	0.02
Female-choice red ♀	14	33	–	–	0.88	0.40
Female-choice sepia ♀	–	–	30	17	–	–
Male-choice red ♂	–	34	21	–	0.70	2.64
Male-choice sepia ♂	14	–	–	16	–	–
No-choice red ♀ × sepia ♂	35	–	–	–	0.93	0.18
No-choice red ♀ × red ♂	–	38	–	–	–	–
No-choice sepia ♀ × red ♂	–	–	32	–	–	–
No-choice sepia ♀ × sepia ♂	–	–	–	34	–	–

Data based on 5 replicates.

Table 2. χ^2 for 1:1 ratio on marginal totals to assess the relative sexual activity of sepia eye and red eye flies of both the sexes in *D. bipectinata*

Multiple-choice	♂	Red	Sepia	Total
	Red	30	19	49
♀	Sepia	23	14	37
	Total	53	33	–
	χ^2 red, sepia ♀, 1.66 $P > 0.10$			
	χ^2 red, sepia ♂, 4.64 $P < 0.05$*			
Female-choice	Red	33	14	47
♀	Sepia	30	17	47
	Total	63	31	–
	χ^2 red, sepia ♂, 10.88 $P < 0.001$*			
Male-choice	Red	34	14	48
♀	Sepia	21	16	37
	Total	55	30	–
	χ^2 red, sepia ♀, 1.42 $P > 0.20$			
	χ^2 red, sepia ♂, 7.36 $P < 0.01$*			
No-choice	Red	38	35	73
♀	Sepia	32	34	66
	Total	70	69	
	χ^2 red, sepia ♀, 0.36 $P > 0.50$			
	χ^2 red, sepia ♂, 0.18 $P > 0.50$			

*Significant.

variation in populations of *D. bipectinata* has also been presented on the basis of work done on sexual activity of geographic strains, their hybrids and diallel crosses[5,6]. Thus sexual activity in *D. bipectinata* is under genetic control. It is clear from the present results that there is difference in the sexual activity in the two types of males. The pattern of mating is random in all the crosses as the differences between homogamic and heterogamic matings are not significant and isolation estimate remains close to one (see Table 1). Thus sepia eye gene does not affect the pattern of mating. A lack of assortative mating between wild-type and mutant *D. melanogaster* has been reported by several authors[35–37], selective mating has also been found in some cases[13,38]. In *D. subobscura*, Rendel[39] found selective mating (non-random) between yellow mutant and wild-type with yellow males. A similar situation was reported by Tan[40] in *D. pseudoobscura*. In *D. ananassae*, white eye and Beadex mutations have been found to affect the mating activity of males but there is no evidence for selective mating[25,41]. In *D. biarmipes*, purple eye colour mutation affects mating activity of both sexes but there is no evidence for preferential mating between males and females of the same type[13].

During the present study, mating success of wild-type and sepia mutant of *D. bipectinata* was directly observed in an Elens–Wattiaux mating chamber by employing different experimental techniques. When different techniques are employed, sex-ratio and choice situation are altered. It is clear from the present results that sex-ratio has no effect on mating success in *D. bipectinata* as results of multiple-, female and male-choice experiments are similar. In multiple-choice experiment, the sex-ratio is 1 female : 1 male but in female- and male-choice experiments, the sex-ratio is 1 female : 2 males and 1 male : 2 females respectively. Even if sex-ratios are different, the results are similar. However, mating propensity has been found to be influenced by sex-ratio in *D. pseudoobscura*[23] and *D. biarmipes*[13] and it has been suggested that the interference between the individuals of the same sex may delay the average time for mating.

Sharp[42] found no difference between inbred and outbred males of *D. melanogaster* competing to mate with an equal number of females. However, there was large reduction in male mating ability due to inbreeding when the receptive female : male ratio was halved. Genetic variations in male mating ability is largely due to dominance and inter-male sexual selection is a very important component of fitness in *D. melanogaster*[43].

In multiple-, female- and male-choice experiments there is significant difference in the numbers of matings

of the two types of males and wild-type males are more successful in mating than mutant males. Males and females can choose one of the two types of flies of opposite sex and the mating propensity of males varies. However, in the case of no-choice experiments both types of males are equally successful in mating during the same duration of time, i.e. one hour during which mating was observed. Thus mating propensity in *D. biptectina* is influenced by choice-situation. In *D. bipectinata*, cut wing and thoracic outgrowth mutations do not affect mating propensity although thoracic outgrowths affect the mate recognition system, leading to behavioural reproductive isolation[44,45].

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Susceptibility of *Vigna sesquipedalis* Koern (Asparagus bean) to an oncogenic strain of *Agrobacterium tumefaciens*

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A transformation procedure was developed to determine the susceptibility of the grain legume *Vigna sesquipedalis* Koern to infection by an oncogenic strain of *Agrobacterium tumefaciens*. Cotyledonary node explants were prepared from 8-day-old seedlings germinated on B5 basal medium and transformed using oncogenic strain A281 carrying the binary vector PIBGUS-INT with the *pat* gene and *npt* II gene as selectable markers and the *gus* gene as a reporter gene. About 90% of the infected plants showed tumour formation at the cut ends. The transgenic nature of the tumour was confirmed by GUS test and Southern analysis. Some axillary meristems at the nodal region developed into shoots and later into abnormal plants. Histochemical analysis of β-glucuronidase gene expression revealed high competence of the subepidermal cell layers of the cotyledonary node to transformation by *Agrobacterium*.

GRAIN legumes are, in general, difficult to regenerate *in vitro* and are relatively recalcitrant to *Agrobacterium*[1]. Hence only a few reports describe the successful transformation of *Vigna* species via *Agrobacterium*-mediated gene transfer[2]. Transformation of *V. unguiculata* leaf discs using *A. tumefaciens* led to the formation of kanamycin-resistant callus and to the expression of the mRNA of cowpea mosaic virus in these callus line[3,4]. By