

# Female Remating in *Drosophila ananassae*: Evidence for Sperm Displacement and Greater Productivity after Remating

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**ABSTRACT**—In *Drosophila ananassae*, female remating with respect to productivity and sperm displacement was studied by employing different mutant strains and a wild type strain. In all the experiments, the continuous confinement technique was used. The comparison of productivity between once-mated (control) and remated females reveals that the productivity of remated females is significantly higher than that of once-mated ones in all the crosses. The  $P2'$  values (the proportion of second male progeny produced after remating) were calculated to test sperm displacement in each cross of remated females. In all the crosses, high  $P2'$  values (0.91–0.94) were found which indicate sperm precedence of second male to mate suggesting the existence of sperm displacement in *D. ananassae*. Furthermore, female productivity is increased after remating in *D. ananassae*.

## INTRODUCTION

*Drosophila* females, like those of many other insects, store the sperm received from a male, which are then gradually released for fertilizing eggs as these mature and are laid. But *Drosophila* females may also remate before the sperm from a previous mating have been exhausted. This mating system provides opportunities for sperm competition, and for the evolution of mechanism by which males may inhibit remating and reduce the impact of sperm competition on their reproductive success (Parker, 1970; Smith, 1984; Birkhead and Hunter, 1990; Harshman and Prout, 1994; Harshman and Clark, 1998). After female remating, it has been observed that the eggs laid are fertilized by sperm from the second male, so more progeny of second male, a phenomenon known as sperm precedence. The process has been studied in *D. melanogaster* starting with Nonidez (1920), and perhaps most thoroughly by Lefevre and Jonsson (1962). Lefevre and Jonsson (1962) directly observed sperm displacement in the female reproductive organs of *D. melanogaster* and suggested that circulation current or movements of the female tract achieve displacement. Parker *et al.* (1990) proposed a sperm displacement model, in which sperm displacement is viewed as a process in which one unit of sperm introduced displaces one unit of sperm from the female's sperm stores.

The phenomenon of sperm displacement has been studied by numerous investigators in both natural and laboratory populations of different *Drosophila* species (Loukas *et al.*, 1981; Griffiths *et al.*, 1982; Turner and Anderson, 1984; Gromko *et al.*, 1984a; Barbadilla *et al.*, 1991; Joly *et al.* 1991;

Gilchrist and Partridge, 1995; Price, 1997; Clark and Begun, 1998). Based on preliminary tests, a statistic  $P2$  was suggested for sperm displacement test (Boorman and Parker, 1976; Gromko *et al.*, 1984b). However, Clark and Begun (1998) suggested that  $P2$  measure the fraction of offspring sired by the second male when the matings are observed. They gave new statistics  $P2'$  in which matings are inferred to occur from the offspring counts. Clark *et al.* (1995) measured genetic variation in the male component of sperm displacement by scoring progeny from large number of females doubly mated with a tester male and males from a series of 152 chromosome replacement lines and they found that  $P2'$ , the fraction of progeny sired by the second male, varied widely ranging from 1.0 (complete second male precedence) to 0.5. Productivity is a major component of overall fitness, and in many circumstances it is the single most important ones (Turner and Anderson, 1983). Lefevre and Jonsson (1962) and Prout and Bundgaard (1977) found no difference between the productivities of singly and multiply mated females in *D. melanogaster*. However, Pyle and Gromko (1978) and Clark *et al.* (1995) in *D. melanogaster*; Turner and Anderson (1983) in *D. pseudoobscura* and Barbadilla *et al.* (1991) in *D. buzzatti* have indicated a productivity advantage to multiply mated females over singly mated ones.

*D. ananassae* is a cosmopolitan and domestic species and is of common occurrence in India. This species is genetically unique, as it possesses several unusual genetic features (Singh, 2000). *D. ananassae* has been extensively used for genetical studies particularly population genetics, behaviour genetics and crossing over by Singh and coworkers (for references see the review by Singh, 1996). We have conducted experiments on female remating as well as male remating by employing several geographic strains of *D. ananassae*

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derived from different geographic localities (Singh, SR and Singh, BN, 2000; Singh, BN and Singh, SR, 1999). The results have shown that: (i) male remating occurs more frequently than female remating, (ii) there is strain variation for remating time for males as well as for females and (iii) there is shorter duration of copulation in second mating as compared to first mating. In *D. melanogaster* and other species, there is variation in the results reported by different investigators concerning female remating with respect to sperm displacement and productivity after remating. However, the female remating with respect to sperm displacement and productivity after remating has not been studied in *D. ananassae*. In view of this, we conducted experiments to test the female remating with respect to sperm displacement and productivity after remating in *D. ananassae* by employing different mutant strains and a wild type strain and results are reported in this paper.

## MATERIALS AND METHODS

The following stocks of *D. ananassae* were used in the present study: *ca* (claret eye colour recessive mutation- II chromosome), *rb* (ruby eye colour recessive mutation- X chromosome), *y* (yellow body colour recessive mutation- X chromosome) and a wild type stock DP (established from a large number of flies collected from Dubrajpur, West Bengal in 1994). The mating combinations used for control (single matings) as well as for rematings are given in Table 1.

### Control (single mating) group

In crosses 1–4 (Table 1) all females and males were collected as virgin and aged for seven days in food vials. A single virgin 7 days old female was placed in a fresh food vial with a single virgin 7 days old male and pair was observed for 60 minutes. When mating occurred, the pair was allowed to complete copulation and male was discarded within 30 minutes of the completion of copulation. In each cross, 50 mated females were taken for productivity analysis. For testing the productivity, each mated female was kept in an individual food vial for a period of three days and was transferred to a fresh food vial every third day. Three successive changes were made (total four set of vials) and when the offspring emerged, the total number of flies (males and females) from each vial was counted (Crosses 1–2). In crosses 3–4 involving X-linked markers only female progeny were counted. In this way, it was possible to determine the productivity of females that had mated but once (serving as control for remated females).

### Remating groups (sperm displacement test)

In crosses 5–8 (Table 1) virgin 7 days old females were first mated individually as in control groups. After the first mating, the females were placed individually in fresh food vials, where they were allowed to oviposit for 2 days (Clark and Begun, 1998). On third day, females were transferred to fresh vials, along with two virgin males. These flies were kept together for 24 hr (Hughes, 1997), and then females were transferred to fresh laying vials and the males were discarded (day four). Females were transferred to fresh food vials again on day seven. On day 10, females were again transferred to fresh food vials and on day 12, all females were discarded. In cross 5 and 6, all progeny (males and females) of first male as well as second male were counted whereas, in cross 7 and 8 involving X-linked markers only female progeny were counted. Progeny of females mated with first male were scored in vial 1 and the progeny of females mated with both males were scored in vials 2, 3 and 4. The proportion of the progeny in vials 2, 3 and 4 that were produced by the second male (female mated with both males) is designated as the statistic  $P2'$  (Clark and Begun, 1998). In each cross progeny of 50 remated females were counted.

For each cross of control and remating groups, two replicates were carried out. For maintaining the stocks and in all the experiments, simple culture medium containing Agar-Agar, dried yeast, maize powder, brown sugar, nipagin, propionic acid and plain water was used. All the experiments were conducted in a room maintained at approximately 24°C temperature under normal laboratory light conditions.

## RESULTS

Mean number of progeny produced by once-mated (control groups) and twice-mated (remating groups) females in different crosses is presented in Table 1 (data of two replicates combined- total 50+50 = 100). Productivity of both types of females are compared by using paired t-test. The t-values show significantly greater productivity for the remated females than those once mated ones (control). The mean number of progeny of first male (BF1) before second mating and first male (AFT1) and second male (AFT2) after second mating in different crosses are given in Table 2 (data of two replicates combined). Females produced more progeny from second male after second mating than first male before and after second mating. The sperm displacement statistics,  $P2'$  (Proportion of second male progeny produced after remating) are given in Table 3 (values of two replicates combined).  $P2'$  values for all crosses vary from 0.91 to 0.94, which indicates

**Table 1.** Female productivity (mean number of progeny  $\pm$  S.E.) in control and remating groups (Student's t-test compares total productivities of once –and twice-mated females)

Cross	N	Mean number of progeny ( $\pm$ S.E.)	t	p
1. <i>ca</i> $\times$ DP- control	100	123.84 $\pm$ 2.43		
5. <i>ca</i> $\times$ DP $\times$ <i>ca</i> -remating	100	167.75 $\pm$ 2.46	12.37	<0.001
2. <i>ca</i> $\times$ <i>ca</i> –control	100	82.41 $\pm$ 2.48		
6. <i>ca</i> $\times$ <i>ca</i> $\times$ DP- remating	100	180.72 $\pm$ 2.74	25.94	<0.001
3.* <i>rb</i> $\times$ <i>y</i> -control	100	61.50 $\pm$ 1.55		
7.* <i>rb</i> $\times$ <i>y</i> $\times$ <i>rb</i> –remating	100	87.57 $\pm$ 1.20	12.47	<0.001
4.* <i>rb</i> $\times$ <i>rb</i> -control	100	64.07 $\pm$ 2.11		
8.* <i>rb</i> $\times$ <i>rb</i> $\times$ <i>y</i> - remating	100	100.70 $\pm$ 1.52	14.53	<0.001

\*For crosses involving X-linked markers only female progeny were taken for analysis.

**Table 2.** Mean number of progeny ( $\pm$  S.E.) of first male (BF1) before second mating and of first male (AFT1) and second male (AFT2) after second mating in different crosses

Cross				N	BF1	AFT1	AFT2	Total
	$\times$	1 $\times$	2					
5. <i>ca</i> $\times$ DP $\times$ <i>ca</i>				100	32.98 $\pm$ 1.12	12.72 $\pm$ 0.80	122.05 $\pm$ 2.20	167.75 $\pm$ 2.46
6. <i>ca</i> $\times$ <i>ca</i> $\times$ DP				100	30.67 $\pm$ 1.00	9.93 $\pm$ 0.51	140.12 $\pm$ 2.68	180.72 $\pm$ 2.74
7. <i>rb</i> $\times$ <i>y</i> $\times$ <i>rb</i>				100	22.15 $\pm$ 0.64	6.27 $\pm$ 0.33	59.15 $\pm$ 0.99	87.57 $\pm$ 1.20
8. <i>rb</i> $\times$ <i>rb</i> $\times$ <i>y</i>				100	20.79 $\pm$ 0.88	5.33 $\pm$ 0.28	74.58 $\pm$ 1.12	100.70 $\pm$ 1.52

**Table 3.** P2' values (proportion of second male progeny produced after remating = AFT2/ (AFT1 + AFT2)) in different crosses

Cross	$\times$	1 $\times$	2	P2'
5.	<i>ca</i> $\times$ DP	<i>ca</i>	<i>ca</i>	0.91
6.	<i>ca</i> $\times$ <i>ca</i>	<i>ca</i>	DP	0.94
7.	<i>rb</i> $\times$ <i>y</i>	<i>rb</i>	<i>rb</i>	0.91
8.	<i>rb</i> $\times$ <i>rb</i>	<i>rb</i>	<i>y</i>	0.94

sperm precedence of second male to mate.

## DISCUSSION

During the course of present study, experiments were conducted to investigate the female remating with respect to productivity and sperm displacement by employing different mutant strains and a wild type strain of *D. ananassae*. It is evident from the results that remated females show greater productivity than once mated females and P2' values (proportion of second male progeny produced after remating) range from 0.91 to 0.94 in different crosses indicating sperm precedence of second male to mate suggesting the existence of sperm displacement in *D. ananassae* which is the first report of this interesting phenomenon in this species. Furthermore, female productivity increased after remating in *D. ananassae*.

Newport and Gromko (1984) proposed that experimental designs might affect productivity and sperm displacement in *Drosophila*. They proposed that female remating in periodic confinement design depends on amount of sperm transferred and in continuous design female remating is rapid because female is forced to remate in this experimental design with second male, hence does not exhibit sperm dependence. Males whose sperm are in storage at the time of remating lose a substantial fraction of progeny they would otherwise have produced. However, these authors failed to find evidence for the assumptions leading to a conclusion of sperm displacement in *D. melanogaster*. Two hypotheses have been proposed to account for the predominance of second male sperm and the loss of first male productivity when *D. melanogaster* females remate. According to the incomplete storage hypothesis (Lefevre and Jonsson, 1962): (i) release of first male sperm from storage after remating due to the presence of second male sperm in the uterus; a physiological effect of second male seminal fluid or the act of copulation itself and (ii) incomplete sperm storage after each mating. The first males suffer a loss of productivity because their sperm undergo two cycles of incomplete storage. In contrast to the incomplete

storage hypothesis, the inefficient use hypothesis postulates that sperm from a previous mating are not released from storage when a female remates (Newport and Gromko, 1984). Instead, the addition of second male sperm to those already in storage initiates a second round of inefficient use in which more sperm are released than would be necessary to fertilize the available eggs, so first male productivity is reduced by this second round of inefficient use.

By employing the continuous confinement technique in *D. melanogaster*, Lefevre and Jonsson (1962) and Prout and Bundgaard (1977) reported that the majority of sperm stored by the females from the first males were displaced by second male. However, they could not find any significant difference in the productivity after remating. Gilchrist and Partridge (1995) found no evidence of differential sperm displacement in *D. melanogaster*, but they found evidence that sperm displacement is caused by accessory gland fluid and also suggested that sperm displacement is derived primarily against sperm stored in the seminal vesicle rather than the spermathecae. Further, they could not find any significant difference in the productivity after remating. Clark *et al.* (1995) measured genetic variation in the male component of sperm displacement from 152 chromosome-replacement lines in *D. melanogaster* and found highly significant difference in the ability to displace sperm that is resident in the female reproductive tract. Further, they have also shown that doubly-mated females have higher fecundity than once mated ones. Clark and Begun (1998) proposed that sperm displacement is affected by genotypes of female because under certain conditions female fitness could be determined by her role in mediating use of sperm from multiple males. Hughes (1997) found differences among males in first and second male precedence among chromosome III homozygous lines but the variation was nonadditive. Barbadilla *et al.* (1991) studied sperm predominance in males and double mating in females by using two stocks of cactophilic species *D. buzzatti*. They found a positive correlation between double mating and total productivity i.e. female productivity is increased after second mating. Their results also show high values of sperm predominance (P2) after remating. Turner and Anderson (1984) measured sperm predominance after remating in *D. pseudoobscura* karyotypes carrying various combinations of four inverted gene arrangements and found P2 values varying from 0.82 to 0.90 for second male and proposed that sperm predominance depends on whether the males are good or bad "resisters" of displacement or second male predominance, rather than good or bad "displacers". Turner and Anderson (1983) measured fitness as productiv-

ity of once mated females and double mated females in *D. pseudoobscura* and found that remated females show greater productivity as compared to once-mated females. Scott and Richmond (1990) reported significantly decreased progeny production after remating in *D. melanogaster*.

There is variation in the results reported by various investigators concerning sperm displacement and productivity after remating. These variations in the results may be due in part to different morphological mutant markers or experimental designs used in these experiments. However, the results of Barbadilla *et al.* (1991) in *D. buzzatii* are entirely different from the results of Lefevre and Jonsson (1962) and of Prout and Bundgaard (1977) although these authors have used the same technique i.e. continuous confinement in their experiments. Our results show that there is high proportion of sperm displacement in *D. ananassae* which is similar to the findings of Barbadilla *et al.* (1991) in *D. buzzatii* in which it was found that there is greater proportion of sperm displacement by second male.

For continuous confinement design, an increase in the productivity after remating in *D. ananassae* observed during the present study is similar to the observation of Clark *et al.* (1995) in *D. melanogaster*, Barbadilla *et al.* (1991) in *D. buzzatii*, Turner and Anderson (1983) in *D. pseudoobscura*, and Markow (1982) in *D. mojavensis*. Thus there is evidence that females of *D. ananassae* like females of *D. melanogaster*, *D. buzzatii*, *D. pseudoobscura*, and *D. mojavensis* which multiply mate show fitness advantage. Thus repeated mating must reinforce the sexual selection favouring males which mate repeatedly, and it makes the evolution of multiple mating easier to understand. Gwynne (1984) reported that the males of those species with high predominance supply food to the female during mating. Markow and Ankey (1988) reported that males transfer nutrients to females during mating in *D. mojavensis*. Barbadilla *et al.* (1991) reported that transmission of nutrients might help to explain the increase in female productivity with the number of matings. However, according to the review of Ridley (1988), species with higher productivities are less likely to receive sufficient sperm at one mating than species with low productivity, whereas in species with a high productivity a multiple mated females will leave more progeny than a single mated ones. Clark *et al.* (1995) suggested that the increase in the total productivity among doubly mated females than mated only once may be due to a stimulation of oviposition or it may be due to a replenishment of sperm stores that would otherwise run out.

Turner and Anderson (1984) proposed three mechanisms which lead to differences in sperm predominance (high P2' value): (i) quality of ejaculate i.e., a male with a larger quantity of sperm and seminal fluid may "flush" the female's reproductive tract of more stored sperm than a male with a lesser quantity of sperm and seminal fluid, (ii) overall male vigor, may overlap the first mechanism, since a more vigorous individual may produce more sperm and seminal fluid, and (iii) the composition of ejaculate. Harshman and Prout (1994) have shown that seminal fluid from second males reduce the number of

progeny sired by the first male, even when the second males are sterile which demonstrates that a sperm incapacitation process plays a role in well documented phenomenon of sperm displacement in *Drosophila*. Recently, Price *et al.* (1999) reported that the seminal fluid of second male inhibits the use of stored sperm (first male sperm) without removing them leading to high P2 value (sperm precedence of the second male to mate).

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