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Female remating in Drosophila: Comparison of duration of copulation between first and second matings in six species

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Female remating is the fundamental to evolutionary biology, as it determines the pattern of sexual selection and sexual conflict. During the course of the present study, female remating behaviour of six species of Drosophila, D. ananassae, D. nasuta, D. eugracilis, D. melanogaster, D. simulans and D. pseudoananasae was observed and compared. Periodic confinement design (2 h daily observation) was used for female remating experiments. The frequency of female remating ranges from 16 (D. eugracilis) to 82% (D. melanogaster) in different species of Drosophila, and the differences among different species are statistically significant. Remating latency also varies from 5.51 days (D. melanogaster) to 9.85 days (D. pseudoananasae) in different species, and variation among different species is statistically significant. Duration of copulation in first (virgin mating) and second (remating) matings was observed and compared in each of the six species. Among all the species tested, females of D. ananassae, D. nasuta, D. eugracilis, and D. pseudoananasae show significantly shorter duration of copulation in the second mating compared to the first mating, while D. simulans females show shorter duration of copulation in the second mating compared to the first mating, but the difference is statistically not significant. However, D. melanogaster females show significantly longer duration of copulation in the second mating compared to first mating. Based on these findings, it may be suggested that different species of Drosophila may vary in the incidence of remating and duration of copulation due to differences in their reproductive biology and adaptation.

MATING by animals is an important component of sexual behaviour, and transfer of sperm to females is the primary function of mating in sexually reproducing animals. Since each mating provides an opportunity to produce offspring, males can generally increase their fitness by mating with many females1. However, females intensify their reproductive success by increasing the number of viable eggs produced. This basic asymmetry between the sexes results in sexual conflict over remating, which suggests that male fitness increases monotonically with increased mating rate, while single or a few matings are sufficient for females to maximize their reproductive success. However, females of a majority of animal species mate several times, most often with different males (polyandry–multiple mating or remating), but also with the same male (repeated mating)2.

Female remating is an important component of Drosophila mating systems because after mating, the females store a large number of sperms in the paired spherical spermathecae and a single elongate tubular seminal receptacle3 and utilize them to fertilize eggs as they are laid. Once a virgin female Drosophila has mated, she is usually unwilling to accept another male for some time because after mating, behavioural and physiological changes occur, including decrease in attractiveness to males, decreased receptivity to further mating2, increasing of oogenesis, ovulation and oviposition rates3, storage and utilization of sperm5 and decreased lifespan1. These behavioural and physiological alterations after mating in females have both short and long-term effects. The short-term effect, also called the ‘copulation effect’ in Drosophila is due to seminal fluid components transferred during mating by males, which cause the initial decrease in receptivity4, thus maximizing sperm usage and minimizing the chance
of occurrence of sperm competition. However, the long-term suppression of female remating in Drosophila has been indirectly linked to sperm load and is termed as the ‘sperm effect’.5,8

Female remating is common in many species of Drosophila under both natural and laboratory conditions.2,10-22 Parker23 used the term ‘sperm competition’ and defined it as the ‘competition within a single female between the sperm from two or more males for the fertilization of the ova’. Sperm competition should, therefore, exert a strong evolutionary pressure on characteristics that allow males to pre-empt the sperm stored by females from previous matings, and/or to avoid sperm pre-emption by males that encounter a female after mating.25 The total impact of sperm competition on male fitness and significant effect of remating on female fitness, combine to make an excellent example of sexual selection.24

D. ananassae, a cosmopolitan and domestic species, belongs to the ananassae species complex of the melanogaster species group. This species occupies a unique status in the whole of the genus Drosophila, owing to certain peculiarities in its genetic behaviour.25 Recently, female remating and male remating in D. ananassae have been studied by the present authors.1,25 The results have shown that: (i) male remating occurs more frequently than female remating; (ii) strain variation for remating time exists in both males and females, and (iii) duration of copulation is shorter in the second mating compared to the first mating. Evidence has been presented for sperm displacement and greater productivity of remated females in D. ananassae.25 Density also increases the female remating frequency in D. ananassae.25 Positive response to directional selection for female remating speed demonstrated that it is under polygenic control in D. ananassae.25 D. nasuta belongs to the nasuta complex of the nasuta subgroup of the immigrans species group of the subgenus Drosophila. Mating behaviour of this species has been studied.29,30 D. eugracilis belongs to the eugracilis subgroup of the melanogaster species group of the subgenus Sophophora. Almost no work on mating behaviour of D. eugracilis has been reported yet. D. melanogaster, a cosmopolitan and domestic species belongs to the melanogaster species complex of the melanogaster species group of the subgenus Sophophora. Remating behaviour in this species has been extensively studied by many workers.2,3,8,24 D. simulans is a sibling species of D. melanogaster. Some work on female remating, sperm competition and sexual selection in this species has been reported.31 D. pseudoanansassae belongs to the D. bipectinata species complex of the ananassae subgroup of the melanogaster species group of the subgenus Sophophora. This species hybridizes with other members in the D. bipectinata complex.32 Among these species only D. ananassae, D. melanogaster and D. simulans were previously tested for female remating and other species such as D. nasuta, D. eugracilis, and D. pseudoanansassae have never been tested for female remating behaviour. In view of this, we conducted experiments to test female remating in these six species of Drosophila, with particular reference to the frequency of mating and remating, remating latency and duration of copulation in the first and second matings. The results are reported in the present communication.

To study female remating behaviour, flies of six species of Drosophila were used. These species are D. ananassae, D. nasuta, D. eugracilis, D. melanogaster, D. simulans, and D. pseudoanansassae. The data on the female remating behaviour of D. ananassae given in the present study are based on the data reported by Singh and Singh.20 The D. nasuta stock was obtained from the Drosophila stock centre, Mysore, India. The stocks of D. eugracilis (isofemale line), D. melanogaster and D. simulans (mass culture) used in the present study are being maintained in our laboratory. The D. pseudoanansassae stock (14024-0421.0) used in this study was obtained from the National Drosophila Species Resource Center, Bowling Green, OH, USA. All the stocks are being maintained under uncrowded conditions on a simple culture medium containing agar-agar, dried yeast, maize powder, brown sugar (crude sugar), nipagin, propionic acid in the laboratory in a 12 : 12 light–dark cycle at 24 ± 1°C. From each species virgin flies (males and females) were collected on the day of eclosion, anaesthetized with ether to facilitate sorting of the sexes, and stored in food vials. Flies were aged for seven days in food vials for sexual maturity. The males and females were then paired according to the protocols described below, without being anaesthetized.

In each species, to obtain once-mated females, a single seven-day-old virgin female was placed individually in a fresh food vial (3"length x 1"diameter) with a single seven-day-old virgin male and the pair was observed for 60 min. When mating occurred, courtship time and duration of copulation were recorded for each mated pair. Observation was continued until 50 females had mated, usually within 60 min. Females failing to mate during a 60 min observation period were discarded. Following completion of copulation, males were removed by aspiration, usually within 30 min. The next morning, 50 once-mated females were individually paired with virgin males in fresh food vials and were observed continuously for 2 h. After 2 h observation, the males were discarded from the vials and the same procedure was repeated on twelve consecutive mornings with fresh males.20 When remating occurred, the duration of copulation was noted for each pair in each species. The number of remated females per day was also noted in each species from 1 to 12 days after the first mating. Remating days (the number of days spent after the first mating until the female accepts to copulate again within 12 days) were also noted for each remated female in each species. Females that remated on any one of the 12 testing days were no longer given the opportunity to remate. In this way 50 once-mated females were observed in spe-
cies for remating frequency, remating days and the duration of copulation in first and second matings (remating).

An analysis of variance (one-way ANOVA) was applied to test the variation in courtship time among different species. The number of virgin females mating in different species was compared by $\chi^2$-test. The $\chi^2$-test was used to test the hypothesis that the frequency of remating is equal in all species. An analysis of variance (one-way ANOVA) was applied to test the variation in mean remating days among different species. To test the differences in the mean duration of copulation between the first and second matings, the Student’s ‘t’-test (paired, for dependent samples) was used. The SPSS package was used for these statistical analyses.

Table 1 presents the mean courtship time (min), number of copulations and frequency of copulation in virgin females in different species of Drosophila. The mean courtship time varies from 8.22 (D. ananassae) to 17.63 min (D. pseudoananaassae). The analysis of variance for courtship time shows significant variation among different species (Table 1). Frequency of copulation in virgin females varies from 54.35 (D. pseudoananaassae) to 96.15% (D. melanogaster). The $\chi^2$-test on the number of copulations in virgin females shows significant variation among different species (Table 1). Table 2 shows the number of remated females, remating frequency and remating latency (min) in different species of Drosophila. The female remating frequency varies from 16 (D. eugracilis) to 82% (D. melanogaster). The $\chi^2$-test for remating frequency shows significant variation among different species (Table 2). Figure 1 shows the cumulative percentage of remated females in different species of Drosophila. Female remating was observed from 1 to 12 days after first mating. The mean remating latency (in days) varies from 5.51 (D. melanogaster) to 9.85 (D. pseudoananaassae). ANOVA for mean number of remating latency (days) shows significant variation among different species (Table 2). Table 3 presents a comparison of the duration of copulation between first (DC I) and second (remating-DC II) matings in different species of Drosophila (also see Figure 2). Among all the species tested, D. ananassae, D. nasuta, D. eugracilis and D. pseudoananaassae show significantly shorter duration of copulation in the second mating compared to the first mating. D. simulans females also show shorter duration of copulation in the second mating, but the difference was not statistically significant (Table 3). However, females of D. melanogaster show significantly longer duration of copulation in the second mating compared to the first mating. The duration of copulation for the first mating varies from 4.22 (D. ananassae) to 20.06 min (D. nasuta) in different species and ANOVA for DC I shows significant variation among different species (Table 3). The duration of copulation for the second mating also varies from 3.38 (D. ananassae) to 18.03 min (D. melanogaster) in different species and ANOVA for DC II shows significant variation among different species (Table 3). A
preliminary report of these results is with the Drosophila Information Service.

During the course of the present study, six species of Drosophila were tested for female mating and remating frequency, remating latency (days) and duration of copulation in the first and second (remating) matings. It is evident from the results that there are interspecific differences in mating frequency, remating frequency, remating latency and duration of copulation during the first and second matings. Further, when the duration of copulation in the first and second matings is compared, there are interspecific differences. Out of six species tested, D. melanogaster stands out from rest of species as it shows longer duration of copulation in the second mating compared to the first matings. It also shows higher mating and remating frequency but lower remating latency when compared with other species tested during the present study.

Out of the six species tested, five belong to melanogaster species group and one species, i.e. D. nasuta belongs to the immigrans species group. D. melanogaster, a cosmopolitan and domestic species is most widespread geographically in distribution. It is also genetically most variable compared to other species.

In Drosophila, successful mating depends upon male activity and female receptivity because usually the female is the discriminating partner in the mating act. Courtship time (mating speed), the time from the beginning of the courtship to copulation is a good estimate of sexual activity in males and sexual receptivity in females. The courtship behaviour of Drosophila enables conspecifics to distinguish non-conspecifics and enables males to distinguish females, including the physiological readiness of the female to copulate. Mating activity is correlated with fitness in many species of Drosophila. The differences observed in the present study regarding courtship time and mating are due to differences in the courtship patterns in these species, which leads to fast mating in some species (D. melanogaster) and slow in other species.

In female remating studies, primarily two techniques, i.e. continuous confinement and periodic confinement, have been used by various investigators and female remating frequency varies considerably in different species.

In female remating studies the 2-h periodic confinement technique has been used by Newport and Gronko, and Letsinger and Gronko in D. melanogaster and by McRobert et al. in D. biarmipes and D. melanogaster. In D. melanogaster, it was found that about 80% of the females remate when remating was observed for 12 days. McRobert et al. studied remating in females of D. melanogaster and D. biarmipes during 2-h periodic confinement for 14 days and compared the post-copulatory behaviour of D. biarmipes and D. melanogaster females. Females of both species were shown to undergo a series of behavioural changes following mating, including significant reduction in both sexual attractiveness and receptivity. However, while both attractiveness and receptivity return to 'virgin-like' levels within a few days in D. melanogaster, D. biarmipes females, which regained their sexual attractiveness within a few days, remained unresponsive to copulation for at least two weeks. They also tested remating frequency in both species and found that about 26% of D. biarmipes females mated at least twice and that the mean remating latency was 10.8 days. In contrast, in D. melanogaster 87% of the females remated and remating latency was 6.5 days. In D. ananassae, Singh and Singh used 2-h periodic confinement technique and remating was observed for 12 days. They found that the average female remating frequency was 39.4% and the mean remating latency was 7.17 days. In the present study six species of Drosophila were used for female remating.
using 2-h periodic confinement technique and remating was observed for 12 days. The differences observed in the present study for remating frequency and remating latency in different species may be due to differences in the amount of sperm and seminal fluid transferred by males during mating and also to the reproductive biology of the females in these species. In *D. melanogaster*, a longer delay of female remating is due to the transfer of a large amount of sperm. However, Service and Vosssbrink found that a longer delay in remating was associated with the slower use of stored sperm. Van-Vianen and Bijlsma showed that female remating frequency is affected by its first male, and suggested that this could be due to differences in the amount or quality of seminal fluid transferred during mating. Newport and Gromko have shown that females with less sperm in the first mating are more likely to remate.

Snoek observed female remating in *D. pseudoobscura*, *D. persimilis*, and *D. affinis* using the periodic design and found that >80% remating in *D. pseudoobscura*, >80% in *D. persimilis* and >90% in *D. affinis*. He also compared the duration of copulation between first and second matings in each species and found that invariably all species show shorter duration of copulation in the second mating. Singh and Singh reported female remating in ten strains of *D. ananassae* and compared the duration of copulation between the first and second matings. They found that invariably all the strains show significantly shorter duration of copulation in the second mating. Recently, Bundgaard and Barker also found shorter duration of copulation in the second mating compared to the first mating in *D. buzzatii* females.

There is considerable variation in copulation duration among *Drosophila* species, but causal factors influencing variation in copulation duration have been described for some species. These factors are complex and depend on the form of sperm precedence, female mating status and oviposition patterns, size of males, and age of males. In general, longer copulation leads to a higher reproductive success for males. Among the six species examined here, males that mated with non-virgin females and thus experienced sperm competition, copulated for an unexpected shorter duration than males that mated with virgin females (D. ananassae, D. nasuta, D. eugracilis, D. pseudoobscurae and D. simulans). However, in *D. melanogaster* males that mated with non-virgin females experienced sperm competition and copulated for longer duration than males that mated with virgin females. These results provide no evidence that males respond predictably to sperm competition risks through ejaculate in different species of *Drosophila* examined here, except *D. melanogaster* characterized by longer duration of copulation in the second mating. Copulation duration and ejaculate characteristics of the male may be influenced by the discerned risk of sperm competition. Longer copulation is an adaptation of males which could reduce the risk of sperm competition with future ejaculates with the help of a mating plug, which prevents the female from remating before oviposition. According to Eberhard, copulation duration may be a component of post-mating courtship, i.e., females may require some initial period of mating to evaluate the male before they permit the transfer of ejaculate.

Finally, it has been reported that copulation reduces the lifespan of *D. melanogaster* females. If this phenomenon is common in other *Drosophila* species, the females of *D. ananassae*, *D. nasuta*, *D. eugracilis*, *D. simulans* and *D. pseudoobscurae* may live longer due to their low remating frequency. However, recently Bundgaard and Barker reported that this is not true for *D. buzzatii*, because this species is known for fast remating among *Drosophila* species. Thus, it may be suggested that different *Drosophila* species may vary in the incidence of remating and duration of copulation due to differences in their reproductive biology and adaptation.

14. Aspi, J., Incidence and adaptive significance of multiple mating in...


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