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RESEARCH ARTICLE

UNUSUAL MALE REMOUNTING AND ITS FITNESS BENEFIT IN FEW SPECIES OF MONTIUM SUBGROUP OF *DROSOPHILA*

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ABSTRACT

Male mating a second female after the first (male remating) is a common feature particularly in mass culture of *Drosophila*. Female remating is not frequent as that of male remating, but yet it is reported in few species here we report that males of *D.gangotri and D.nagarholensis,* remounting the same female for second and subsequent times immediately after first mount. There was no relation between courtship latency and mating latency or copulation duration (first mount). However, the copulation duration of second and subsequent mounts gradually decreased in both the species. *D.gangotri and D.nagarholensis* also showed female remating. Even during female remating, the repeated male mounting occurred. Only the copulation duration of first mating and second mating of *D.gangotri* was not significant. Males of *D.nagarholensis* remounted the same female for three times, whereas *D.gangotri* mounted the same female twice, immediately after first mount. The male remounting is absent in *D.kikkawaii* and *D.bipectinata*. Fitness traits (Fecundity, hatchability, viability, fertility and sex ratio) were studied and result showed significant variation between first, second, third mounts for *D.nagarholensis*. In case of *D.gangotrii* except hatchability and sex ratio all other fitness traits showed significant variation between first and second mount.

INTRODUCTION

Mating is the most important and fundamental process to select the best partner and to produce progeny. It is an important component of sexual behaviour (Bernasconi and Keller, 2001; Hoenigsberg, 1960) and transfer of sperm to female is the primary function of mating in sexually reproducing animals. Since each mating provides an opportunity to produce offspring, male can generally increase their fitness by mating with many females (Singh and Singh, 2000). Once a virgin female *Drosophila* has mated, she is usually unwilling to accept another male for some time. Because the female is usually the discriminating or limiting partner in the mating act, her activity of accepting or rejecting a courting male decides the fate of the second male in mounting the same female. Thus there would be a refractory period between the first and second mating. The length of the refractory period varies between species and in different strains of the same species (Manning, 1962). After mating, behavioural and physiological changes are seen in *Drosophila* females, which include; decrease in attractiveness to male (Tram and Wolfner, 1998), less movement in response to males (Tompkin *et al*., 1982), decreased receptivity to further mating (Fuyama, 1995; Ringo, 1996), increasing of oogenesis, ovulation and oviposition rates (Heifetz *et al*., 2001), storage and utilization of sperm (Gromko *et al*., 1984), and decreased life span (Chapman *et al*., 1995; Sgro and Partridge, 1999). These behavioural and physiological alternations in female after mating have short and long term effect. The short term effect, called the “Copulation effect” in *Drosophila* is due to seminal fluid components (Proteins), transferred during mating by males, causing an initial decrease in receptivity (Wolfner, 1997; Neubaum and Wolfner, 1999), thus maximizing sperm usages and minimizing the sperm competition. However, the long term suppression of female remating in *Drosophila* has been indirectly linked to sperm load and termed the “sperm effect” (Manning, 1962; Gromko *et al*., 1984; Scott, 1987). Copulation and sperm effects would usually obscure any sperm effects within a day.

Drosophila females also mate again before the sperm from a previous mating have been exhausted (Lefevre and Johnson, 1962). Although female re-mating is possible, the second mating or subsequent mating occurs after lapse of considerable time of the first mate. This female remating occurs after the performance of all the activities and rituals of the sexual behaviour of the male followed by mounting and copulation. There are also instances of second mount only if the first mount is unsuccessful. We have noticed that in few species of montium subgroup of *Drosophila*, the male performs all the courtship rituals, then mounts a female and copulates. After successful mating for considerable length of copulation duration the male dismounts and immediately remounts and mates the same female (Sowmya and Hegde, 2009). This

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remounting of female by the same male immediately after first mating is an unusual phenomenon in *Drosophila*. The reason for male remating another female, or female remated by another male is already been known. But the reason for male remounting the same female immediately after the first mating has not been analysed. Therefore it is hypothesised that the male remounting and mating the same female immediately after the first mating should have some fitness advantage. Otherwise this phenomenon would not have been evolved only in one group of *Drosophila*. The present study therefore has been taken up to verify this hypothesis in two species of *Drosophila montium* subgroup.

MATERIALS AND METHODS

The three species of *montium* subgroup of *Drosophila melanogaster* species group *D.gangotrii, D.nagarholensis* and *D.kikkawaii* along with one species of *bipectinata* complex viz. *D.bipectinata*, were obtained from Drosophila Stock Centre, Department of Zoology, University of Mysore, Mysore. The flies were cultured in a standard laboratory food (wheat cream agar yeast medium) at 22±2°C. The virgin females and naive males were sorted by sex and aged for 7 days in separate food vials. One bachelor male and one virgin female were allowed to mate in an Elens Wattiaux Mating Chamber (Observation chamber). The pair was observed for 2 hours. When mating occurred the pair was allowed to complete copulation. The sexual behaviour such as courtship latency, mating latency and copulation duration was studied following the procedure described by Hegde and Krishna (1997). The pair was allowed to stay together even after completion of mating. The second and subsequent mounting and mating whenever occurred was noted. After each mating the pairs were separated and subjected for fitness analysis. A minimum of twenty pairs were observed in each species.
The following fitness parameters of these flies were studied.

a) Fecundity was analyzed by transferring mated female flies into individual vials containing food. The fly was transferred to fresh vial every day for next 10 days. The eggs laid in the vial were counted and the total number of eggs laid in all 10 vials was then summed up.
b) Hatchability was analyzed by counting the number of larvae hatched out from each vial after 48 hours of egg laying. The total number of larvae hatched out from all the 10 vials of each female was summed up.
c) Viability was assessed by counting total number of progeny emerged from these vials for 10 days. The percentage of adults produced out of the eggs laid was considered as viability.
d) Fertility was assessed by counting number of flies emerged from each vials everyday till the last day of emergence. The percentage of adults produced out of the hatched larvae was considered as fertility.
e) Sex ratio was calculated by estimating the ratio of males and females appeared in the progeny.

To check whether the species shows remating or not, after completion of 1st copulation the mated male was aspirated out and replaced with another bachelor male and observed for 2 hrs, remating frequency, remating time and duration of copulation were recorded.

RESULTS

Table 1 shows data on courtship latency, mating latency and copulation duration of the three species of *montium* sub group and *D.bipectinata*. *D.nagarholensis* males mount the same females thrice; *D.gangotrii* males mounted the same females twice while the males of *D.kikkawaii* and *D.bipectinata* mounted the same female only once. Whenever, there was multiple mounts, the copulation duration decreased significantly after the first mount. The copulation duration of *D.nagarholensis* was 607±60.1 sec., which decreased to 19±3.0 seconds in the second mount and to 17±0.7 seconds in the third mount. The male of *D.gangotrii* mounted only twice with a copulation duration of 231±120.6 seconds in the first mount and 23±8.0 seconds in the second mount. The courtship latency was high in *D.gangotrii* compared to *D.bipectinata*, while mating latency was high in *D.bipectinata*.

Table 1. Showing courtship latency, mating latency, and copulation duration of three species of montium subgroup of *Drosophila melanogaster* and *D.bipectinata* (values are in seconds)

Species	Courtship latency	Mating latency	Copulation duration (Seconds)		
			1st mount	2nd mount	3 rd Mount
D.nagarholensis	70±10.7	83±12.9	607±60.1	19±3.0	17±0.7
D.gangotrii	149±34.3	76±18.7	231±120.6	23±8.0	
D.kikkawaii	123±10.2	95±9.12	420±80.30		
D.bipectinata	93±8.1	240±41.2	540±3.6		

Table 2 shows the number of females of the two species studied which remated within two hours of the first mating. The parameters such as courtship latency, mating latency, copulation duration for both species have also been calculated and presented in the table. The courtship latency and mating latency of *D.gangotrii* was significantly different between the first mating and remating. In *D.nagarholensis* the mating latency was significantly different between first and second mating. Only the copulation duration of first mating and second mating of *D.gangotrii* was not significant. Even during second mating, the repeated male mounting the same female occurred in *D.gangotrii*, and *D.nagarholensis.*

Table 2. showing the remating in two species of montuim subgroup of *Drosophila* (the values are Means ± SE represented in seconds, X^2 values are given wherever significant at 0.05 levels between first and second mating)

Species	Pairs	Courtship latency	Mating latency	Copulation duration (Seconds)		
				1st mount	2nd mount	3 rd Mount
D. nagarholensis	17	67±9.5	297±30.5* X^2=120.5	290±56.5* X^2=112.86	53±7.5* X^2=16.1	
D.gangotrii	19	85±10.7* X^2=12.46	172±17.1* X^2=37.16	257±36.8	19±0.1	

Table 3 shows variation of fitness in *D.nagarholensis*, *D.kikkawaii* and *D.bipectinata*. The fecundity of first mount of *D.nagarholensis* was 94.70±11.8, while second mount and third mounts were 96.3±7.7, 167.9±6.6 respectively. The mean difference between them was significant with F=21.40 ($P<0.05$). Hatchability of first, second, and third mounts were 80.4±10.8, 78.7±7.02 and 147.1±21.3 respectively and the difference between them was significant (F=21.53; $P<0.05$). Viability of first mount was 73.2±9.6, while second mount was

74.3±6.3. The mean difference between these three mounts was also significant with an F = 20.92 (P<0.05). Fertility of first, second and third mounts were 48.50±6.4, 63.3±5.2 and 120.1±6.0 respectively with significant difference between them (F=4.58; P<0.05). Sex ratio of first mount was 1:1.13; second mount was 1:1.10 and the third mount was 1:0.89. The result thus showed that the second and subsequent mounting increases the fitness of all the parameters studied. *D.kikkawaii* and *D.bipectinata* showed only single mating.

Table 3. showing fitness of *D.nagarholensis* after first, second and third mounts and fitness of *D.kikkawaii* and *D.bipectinata* (F value by ANOVA significant at 0.05 levels)

Fitness parameter of *D.nagarholensis*	1st Mount	2nd Mount	3rd Mount	df	F	Sig	*D.kikkawaii*	*D.bipectinata*
Fecundity	94.70±11.8	96.30±7.7	167.90±6.6	29	21.40	.000	288±26.6	390.4±129.2
Hatchability	80.40±10.8	78.70±7.0	147.10±6.7	29	21.53	.000	212±11.1	342.2±41.7
Viability	73.20±9.6	74.30±6.3	135.80±7.0	29	20.92	.000	143±21.3	207.4±82.9
Fertility	48.50±6.4	63.30±5.17	120.50±6.0	29	41.58	.000	69±6.1	143±23.0
Sex ratio (Male: Female)	1:1.13	1:1.10	1:0.89	29			1:1.15	0.98:1

Table 4. showing fitness of *D.gangotrii* after first and second mount (Paired t Test, value significance at 0.05 levels)

Fitness Parameter	1st Mount	2nd Mount	t	df	Sig.(2-tailed)
Fecundity	55.40±9.13	90.90±10.27	-3.233	9	.010
Hatchability	36.10±6.68	48.20±7.15	-1.564	9	.152
Viability	23.30±3.80	39.10±5.77	-2.931	9	.017
Fertility	18.60±3.69	29.80±4.47	-2.826	9	.020
Sex Ratio (Male:Female)	1:1.17	1:0.63	-2.234	9	.052

Table 4 shows that *D.gangotrii* exhibits two mounts. Fecundity of the first mount was 55.4±9.1 while after second mount it was 90.9±10 and the difference in the fecundity between first mount and second mount was significant (t = -3.233; df=9; P<0.05). However, the hatchability of first mount (73.150±7.8) and second mount (48.2±6.6) did not show any significant variation (t = -1.56; P>0.05). Viability and fertility was also significantly (P<0.05) different between the first and second mount in this species. Sex ratio of the progeny of first mount was (1:1.17) and that of second mount was (1:0.63) and the mean difference between them was significant with t = -2.234 (P<0.05). Except hatchability all other fitness traits showed significant variation between first and second mount.

DISCUSSION

In our earlier study, we noticed that in some species of montium group of *Drosophila* the male remounts the same female for second and subsequent time (Sowmya and Hegde, 2009). In most *Drosophila* species, there are instances of second mount only if the first mount is unsuccessful and the pairs separate immediately after second mount. There are also instances of female remating with the same or different male after a considerable gap of time. In the present study during the courtship we have noticed that male often remount the same female after the separation of the pair from the first mount. For example *D.gangotrii* male when mounted first remained in cupola for 201±120.6 sec and got separated and again immediately mounted. The pair remained together again for about 23±8.0 sec. *D.nagarholensis* remounted thrice (Table 1). The remounting of the same male to the same female might have taken place because of the incomplete transfer of sperms from male to female in the first mount. The second and third remounting by the males of *D.gangotrii* and *D.nagarholensis* might have occurred for the same reason. It is also interesting to note that the copulation duration gradually declines in the second and subsequent matings. This further confirms that the second and subsequent mounts have occurred due to inadequate transfer of sperms to the female. The same male mounting the same female after copulation or the first mount is the rare phenomenon in *Drosophila*. *D.nagarholensis* had the longest copulation duration (607±60.1sec). *D.nagarholensis* was fast mating with short courtship and mating latencies compared to other species. *D.nagarholensis* and *D.gangotrii* exhibit remating also like any other species of *Drosophila* and even during remating by another male the phenomenon of remounting was observed (Table 2). There was no male remounting of *D.bipectinata* and *D. kikkawaii* and also the copulation duration of them were 420±80.3 and 540±30.6 respectively. This confirms that the phenomenon of remounting enhances the number of sperms during courtship. In general longer copulation leads to higher reproductive success for males. Accordingly *D.nagarholensis* has high mating success than others. Hegde and Naseerulla (1992) have demonstrated that the mating speed could also affect the transfer sperms from male to female during the courtship of Drosophila which further supports the present finding that the remounting enhances the number of sperms transferred from male to female. Another important observation of the present study is the female remating within few minutes of the first mating. Table 2 shows that quite a good number of females of species of montuim subgroup of *Drosophila* remated within two hours of the first mating. Female remating is not uncommon in *Drosophila.* For example, Snook (1998) observed female remating in *D.pseudoobscura*, *D.persimilis, and D.affinis* using the periodic designs. He found >80% remating in *D.pseudooscura*,>80% in *D.persimilis* and >90% in *D.affinis*. Snook (1998) also compared in each species and found that invariably all species show shorter duration of copulation in the second mating. Singh and Singh (2001) reported female mating in ten strains of *D.annanasae*, and compared the duration of copulation between first and second mating. They found that invariably all strains shows significantly shorter duration of copulation in the second mating. Recently, Bundgaard and Barker (2000) also found shorter duration of copulation in the second mating compared to the first mating in *D.bizzatii* females. On the contrary, in the present studies, in species with remounting, both the copulation duration and the mating speed (courtship latency and mating latency) decreased when the females re-mate. Since courtship and mating latencies indicate the vigor of males and receptivity of females, the present study also indicates that the vigor of male and receptivity of females are decreased after the first mating of species which exhibit this remounting phenomenon. This finding contrasts the work of Singh and Singh (2001) and Bundgaard and Barker (2000).

Fitness consists of many components such as fecundity, hatchability, fertility, viability, sex ratio etc. The analysis of these parameters in *D.nagarholensis* and *D.gangotrii* after each mounts shows that the fitness gets enhanced after each mount. Perhaps that is the purpose of this repeated mounting of female by the same male. In most species of Drosophila, the flies enhance their fitness by repeated mating. However, re-mating can enhance the fitness of only female and not the male because in this act any male could be involved. In contrast to this repeated mounting (remounting) enhances the fitness of both the partners involved in the process of mating. The most important factor of mating is the influx of male seminal fluid protein. Male seminal fluid protein can increase egg production and decrease female receptivity after mating has occurred (Wolfner, 1997; Chapman, 2001; Wolfner, 2002; Kubli, 2003; Liu and Kubli, 2003; Lawniczak and Begun, 2004; Mc Graw *et al*., 2004). This is evident in the present study because the mating speed and re-mating duration has been decreased in *D.nagarholensis* and *D.gangotrii*. A more recent finding suggest that a particular “Sex peptide” can increase female egg production (Chapman *et al*., 2003; Liu and Kubli, 2003), and causes releases of Juvenile hormone (Moshitzky *et al*., 1996) it was previously known that juvenile hormone may positively affect *Drosophila* oogenesis and egg production process (Soller *et al*., 1999; Dubrovskaya *et al*., 2002). In the present study, as male shows re-mounting, availability of the sperm stored is more during second mate and third mate in *D.nagarholensis*, and *D.gangotri* hence there is an increases in offspring production and fitness.

Remating is an insurance against sterility and sub-fertility. Remating is necessary when a single insemination does not provide the female with sufficient sperm to ensure high fertility during the female’s reproductive period (Halliday and Arnold, 1987; Gibson and Jewell, 1982; Walker, 1980). The reason may be limited sperm storage capacity. This maybe economic optimization by which female reduces the energy cost of keeping sperm alive for a long time. In the present study the two species of the montium subgroup, *D.gangotri* and *D.nagarholensis*, also showed female re-mating. Even during female re-mating, the repeated male mounting occurred. Thus the remounting the same female by the male is not just a mechanism of introduction of more sperms to the female reproductive organs but it could be for enhancing the fitness by improving the quality of semen.

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