

Male age influence on male remating and progeny production in *Drosophila malerkotliana*

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Abstract: Reproductive success of the male is not only depends on his success in mating, but the number of females he could inseminate in a given time. The age effect on male remating and progeny production has been studied in *Drosophila malerkotliana*. It was found that middle aged males had a greater percentage of remating than those of young or old males. Male mated two females in an hour had copulated longer, laid a greater number of eggs and produced the greatest number of progeny than those males which mated with one female in an hour. This result found to be similar in all the male age classes studied. It was also noted that young male copulated longest in the experiment involving male mated only one female in 1 hour. Female that mated with middle aged male laid least fecundity in the experiment involving male mated only one female in 1 hour. Whereas female that mated with middle aged male had produced greater progeny in the experiment in which male mated with one/two females in 1 hour. Among the male mated with 1st and 2nd female in an hour male invested more in 1st mated female compared to 2nd mated female.

Keywords: *Drosophila Malerkotliana*, Male Age, Male Remating, Fertility

1. Introduction

All biological processes directly related to reproduction plays an important role in determining fitness. Collectively, these processes determine the fertility component of fitness, which fertility is defined in its broadest sense (Turner and Anderson 1983). This is because reproductive capacity is particularly a good index of fitness in organisms that go through repeated cycles of rapid population growth and it has evolved as a way for species to maximize their potential for survival [44].

The sexual behaviors of various species of *Drosophila*, with particular reference to their basic courtship patterns, genetic control, role of stimuli, and contributions of the 2 sexes to variations in mating. Repeated mating has been studied extensively [6,10,29].

Remating is common among many species of *Drosophila* under both natural and laboratory conditions. Multiple mating in *Drosophila* bears a direct relation to fitness [24]. Remating time of male and female in different species of *Drosophila* has been studied [31,39, 40,43]. Remating and sperm storage are specific features that can play important roles in determining female fecundity, male mating success. Remating reduces the maternal survival [26, 28,30].

Markow Quaid and Kerr [27] have found that females are able to distinguish between mated and unmated males and those that had a previous mating experience, showing preference for virgin males.

It has been widely demonstrated that males of different species of *Drosophila* can inseminate more than a single female, and the mating ability of males is influenced by genetic and other factors [32,34,36,37]. According to Gromko [12], multiple mating is widely believed to be advantageous for males, and selection of males can produce a correlated response in females.

A great diversity of male traits is known to influence female mating decision. Some of the factors that can determine male mating success include size, diet and early adult experience [1, 7, 8, 19, 25]. Male size in insects may affect reproductive activities including success in intrasexual encounters, sperm precedence and female fecundity. For many of these activities a larger size confers a reproductive advantage. One male characteristic that has received a lot of attention as a possible cue for female mating decision is age [5, 15, 22, 42, 46]. The effect of male age on reproductive success has been of great interest to evolutionary biologists.

The importance of male age on sexual behavior is not

straightforward, and in most cases, only 1 or a few aspects of male fitness like success in inter-male competition, fecundity, fertility, and longevity have been studied [2, 33, 42,]. In fact, remating is also a strategy for improving the fitness of individuals of polygamous populations. In *Drosophila*, the influence of female remating on fitness has been studied [41]. The further influence of male age on male remating ability has been largely ignored.

Therefore present study has been undertaken in *D. malerkotliana*. It is a cosmopolitan species is a member of the *bipunctinata* complex of the *ananassae* subgroup [3-4]. It has a wide ecogeographical distribution ranging from India through South east Asia and New Guinea to Fiji and Samoa in the Pacific [4]. It is a common occurrence in the Indian subcontinent and has attracted the attention of various Indian workers who are using this species for past the few years. They have carried out extensive studies on population and behavior genetics of this species and have established the phylogenetic relationship between *D. malerkotliana* and other members of the *bipunctinata* complex based on chromosome analysis, hybridization studies and isozyme analysis [17, 18, 20, 35, 45]. These findings provide interesting and important information concerning certain aspects of evolutionary genetics of this species. However, in this species, male age effect on male mating ability and progeny production has not been studied therefore the present study has been undertaken in *D. malerkotliana*. Hence the present investigation has been undertaken in *D. malerkotliana* to understand male age influence on the male mating ability and progeny production.

2. Materials and Methods

2.1. Establishment of Experimental Stock

Flies used in the present investigation were obtained from the experimental stock of *D. malerkotliana* established from progenies of fifty wild caught females (ISO-female line) collected at Chamundi Hills, Mysore, Karnataka state, India. In each generation fly were mixed together and redistributed to different culture bottles each with 20 males and 20 females. These flies were cultured using wheat cream agar media and maintained them at $22\pm 1^\circ\text{C}$ at RH of 70% of 12 hour light: 12 hour day cycle for three generations to allow them to acclimatize to laboratory conditions. At 4th generation, virgin females and unmated males were isolated within three hours of their eclosion and were aged as required for the experiment.

2.2. Assigning Male Age Classes

Before the start of the experiment male age classes were assigned by studying longevity of male *D. malerkotliana*. Unmated males were individually transferred into a vial containing wheat cream agar medium once a week and maintained them in above lab condition. This process was continued until their death and the longevity was recorded

from the time of its eclosion until death in the days. A total of 50 replicates was made and mean longevity data calculated was found to be 60 ± 2 days. In addition to this, mating activities of males were also studied from day 1st to 55th day. Results showed that from 2nd day to 54th day male showed all the courtship activities and later it started to decline. Therefore, male age classes where we assigned 2-3 days for young, 24-25 day for middle aged and 46-47 days for older males.

The first set of flies emerged from cultured bottles were allowed to age for 46-47 days (to obtain old males). When these flies reached 25th day the next set of new flies were isolated and allowed to age for 24-25 days (to obtain middle aged males). When the second set of flies reached 25th day and the first set of flies reached 45th day, then the new set of flies was isolated and was aged for 2-3day (to obtain young males). These young, middle and old aged males along with 5-6 day old virgin females obtained from the same culture and maintained them individually under uniform environmental conditions as described above. This procedure allowed us to grow the flies of all the three age classes in the same environmental condition and conduct the experiment at the same time.

2.3. Male Age Influence on Percentage of Male Mating Ability

To study male age effect on male remating. A 5-6 day old virgin female and a male (young/middle-aged/old) were aspirated into an Elens-Wattaiux mating chamber (ElensWattaiux, 1964) and observed for an hour. Flies which did not mate within an hour were discarded. If the flies mate, they were allowed to complete copulation and the duration of copulation was recorded (time between initiation to termination of copulation of each pair). Soon after mating the mated female was transferred to a vial containing wheat cream agar medium to check for larval activity. Mated male was once again provided with 5-6 days old virgin female and observation was continued up to an hour. If the second female that did not mate within the time was equally discarded. If mating occurred, the duration of copulation was recorded and mated female was transferred to a new vial to observe larval activity. A total of 50 trials was carried out separately for each of the three male age classes (young-50, middle aged-50, old male-50). The percentage of male remating ability was recorded.

2.4. Male Age Influence on Male Mating Ability and Male Fitness

Five to six days old virgin females and a male (young/middle aged and older) were aspirated into an Elens-Wattaiux mating chamber [9] and observed for an hour. Flies which did not mate within an hour were discarded. If the flies mated, they were allowed to complete copulation and duration of copulation was recorded (time between initiations to termination of copulation of each pair). Soon after mating mated female was transferred into

a vial containing wheat cream agar medium every 24 hours. This was continued until the death of the female. Mated male was once again provided with 5-6 day virgin female and observation was continued up to an hour. If mating did not occur with second female within the time, then it was considered as male mated once in an hour. If mating occurred with the mated second female was allowed to complete copulation and the duration of copulation was recorded. Mated female was transferred to a vial containing wheat cream, medium once in 24 hours and was continued up to the death of the second female. The total number of eggs laid and the progeny produced by male mated with the first and second female was recorded. Fifty male mated with one female in an hour and mated with two females in an hour were separately used for each male age class (young-50, middle aged-50, old male-50). Data of male mated with second female were added together to calculate duration of copulation, fecundity and fertility. Duration of copulation, eggs laid and the progeny produced data by male mated with one female within 1 hour, male mated with two females within 1 hour. Male mated with first female and second female was subjected to two way ANOVA followed by Tukey's post hoc test.

3. Results

The percentage of males remating at young, middle and old age of *D. malerkotliana* is provided in figure-1. It was noticed that middle aged males had the highest percentage of remating potential compared to the young and old males.

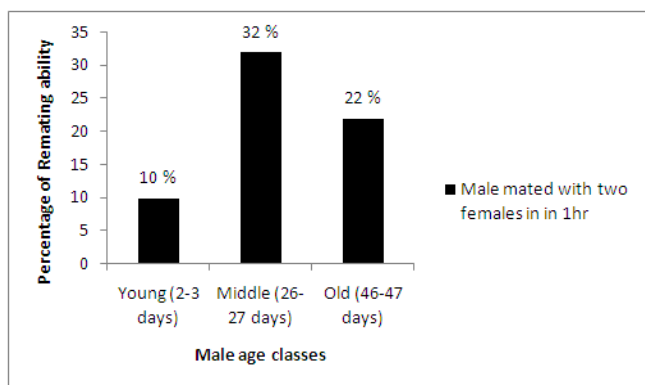


Figure 1. Percentage of mating success of male mated twice with female within one hour in *D. malerkotliana*

3.1. Male age Influence on Copulation Duration

Figure 2a revealed that duration of copulation was found to be longer in male mated with two females in 1 hour than that those male mated with one female in 1 hour. This was found to be similar in all the male age classes. Among male age classes old male copulated longer than middle aged and

young males. Two-way ANOVA followed by Tukey's post-hoc test applied on above data showed significant variation in copulation duration between male age classes, between male mated with one female and two females in 1 hour [Table-1]. Tukey's post-hoc test showed that old male copulated significantly longer than middle aged and young male mated with one female in 1 hour. In contrast to this copulation duration of male mated with two females in 1 hour was found to be insignificant varied different between male age classes.

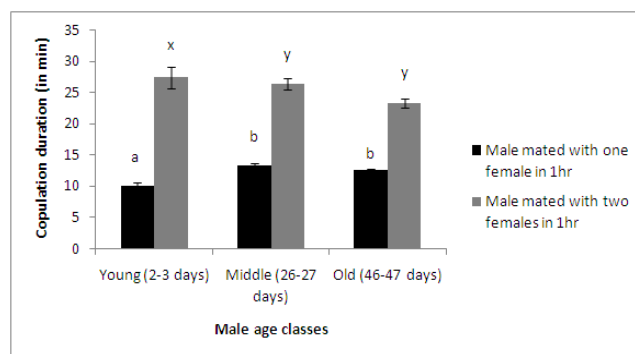


Figure 2a. Male age influence on Copulation duration (in min) of male mated with one female in 1 hour and two females in 1 hour in *D. malerkotliana*. (Values are Mean \pm SE)

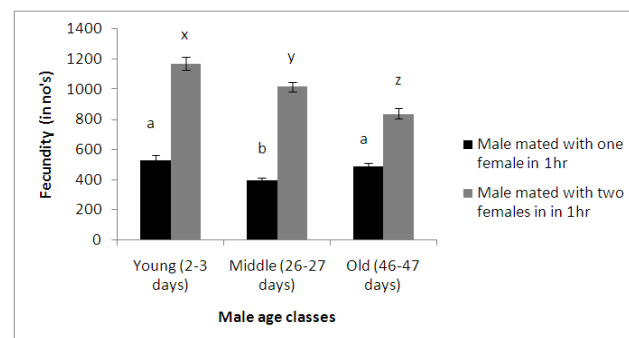


Figure 2b. Male age influence on Fecundity (in nos) of male mated with one female in 1 hour and two females in 1 hour in *D. malerkotliana*. (Values are Mean \pm SE)

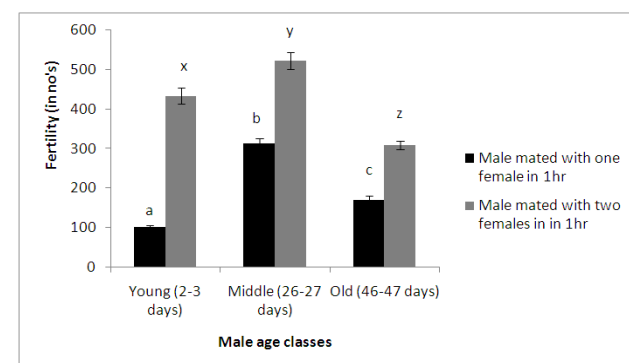
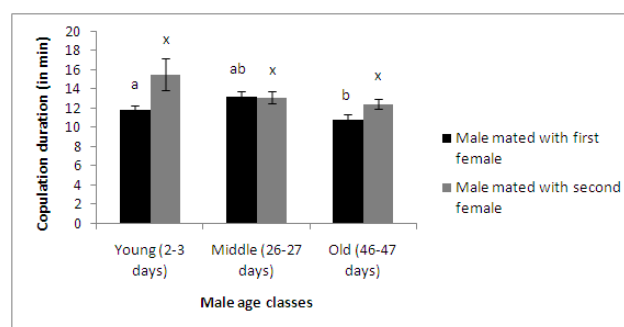


Figure 2c. Male age influence on Fertility (in nos) of male mated with one female in 1 hour and two females in 1 hour in *D. malerkotliana*. (Values are Mean \pm SE)

Table 1. Two-way ANOVA of male age effect on fitness traits between male mated with one female and two females in *D. malerkotliana*

Parameter	Source	Type III Sum of Squares	df	Mean Square	F- value	Sig
Copulation duration	Age	464.007	2	232.003	5.458	.005
	Mating status	14145.333	1	14145.333	332.765	.000
	Age * Mating status	127.847	2	63.923	1.504	.224 ^{NS}
	Error	12497.480	294	42.508		
	Total	134020.000	300			
Fecundity	Age	2108926.407	2	1054463.203	20.478	.000
	Mating status	14093301.763	1	14093301.763	273.700	.000
	Age * Mating status	787221.927	2	393610.963	7.644	.001
	Error	15138602.340	294	51491.845		
	Total	219852919.000	300			
Fertility	Age	932240.580	2	466120.290	46.697	.000
	Mating status	3323479.253	1	3323479.253	332.952	.000
	Age * Mating status	319439.687	2	159719.843	16.001	.000
	Error	2934668.760	294	9981.867		
	Total	37500766.000	300			

Figure 3 a. Shows the duration of copulation of male mated with 1st female and 2nd female in 1 hour with young, middle-aged and older males. Duration of copulation with 1st female was highest in middle aged males compared to that of young and old males while duration of copulation with 2nd female was highest in young male and it was found to be more or less the same in middle aged and old males. Two-way ANOVA followed by Tukey's post-hoc test carried out on above data showed significant variation between male age classes and between 1st female and 2nd female. However insignificant variation was noticed in the interaction between male age and mating status (Table 2). Tukey's test showed that among male age classes, middle aged female mated with first female copulated longer compared to young or old male mated with first female (Fig-3a).

**Figure 3a.** Male age influence on Copulation duration (in min) of male mated with the 1st female in 1 hour and 2nd female in 1 hour in *D. malerkotliana* (Values are Mean ± SE)**Table 2.** Two-way Anova of male age effect on fitness traits between 1st and 2nd mated female in *D. malerkotliana*

Parameter	Source	Type III Sum of Squares	df	Mean Square	F- value	Sig
Copulation duration	Age	224.747	2	112.373	3.257	.040
	Mating status	225.333	1	225.333	6.531	.011
	Age * Mating status	173.307	2	86.653	2.512	.083 ^{NS}
	Error	10143.280	294	34.501		
	Total	60432.000	300			
Fecundity	Age	1367187.547	2	683593.773	17.478	.000
	Mating status	1487129.613	1	1487129.613	38.022	.000
	Age * Mating status	369029.547	2	184514.773	4.718	.010
	Error	11499055.880	294	39112.435		
	Total	90894950.000	300			
Fertility	Age	582202.087	2	291101.043	32.781	.000
	Mating status	13.230	1	13.230	.001	.969 ^{NS}
	Age * Mating status	143953.380	2	71976.690	8.105	.000
	Error	2610786.220	294	8880.225		
	Total	16657409.000	300			

3.2. Male Age Influence on Fecundity

The total number of eggs laid by male mated with one female (once mated) in 1 hour and two females (twice mated) in 1 hour is provided in the figure -2b. In both the cases male mated with two females in 1 hour had laid the greatest number of eggs than males mated with one female in 1 hour. Among male age classes middle aged male, which mated with one female in 1 hour had lowest fecundity compared to the young or old, male, while

fecundity of the 2nd female decreased with increasing age of the males. Two-way ANOVA followed by Tukey's post-hoc test carried out on the above data showed significant variation in the total number of eggs laid between male age classes, between male mated with one female (once mated) in 1 hour and male mated with two females (twice mated) in 1 hour. Also, interaction between male mating status and male age classes. Tukey's post-hoc test showed that young male mated with one female in 1 hour had laid a significantly greater number of eggs compared to females

that mated with middle-aged or old males. Similarly, middle aged male that mated with one female in 1 hour had laid a significantly greater number of eggs compared to the females that mated with old males by Tukey's test.

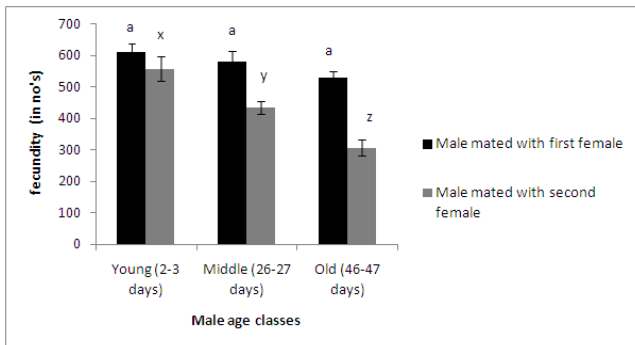


Figure 3b. Male age influence on Fecundity (in nos) of male mated with the 1st female in 1 hour and 2nd female in 1 hour in *D. malerkotliana* (Values are Mean \pm SE)

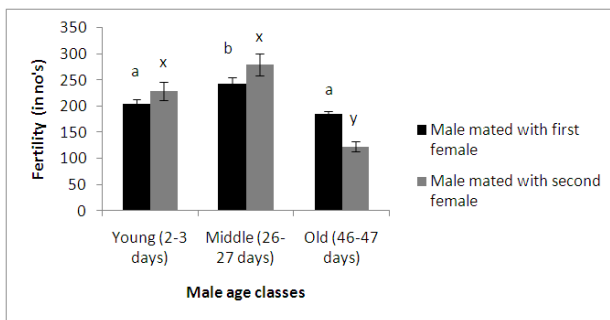


Figure 3c. Male age influence on Fertility (in nos) of male mated with the 1st female in 1 hour and 2nd female in 1 hour in *D. malerkotliana* (Values are Mean \pm SE)

Figure- 3b. Shows the total number of eggs laid by the male mated with 1st and 2nd female. The number of eggs laid by first female mated was greater within that of than second mated female in all the three male age classes studied. Two-way ANOVA followed by Tukey's post-hoc test applied on above fecundity showed significant variation between male age classes, between 1st mated and 2nd mated females and also the interaction between male age and mating status. Tukey's post hoc test showed that among male age classes young male mated with first female had significantly greater number of eggs compared to the middle aged or old aged males mated with first female.

3.3. Male Age Influence on Fertility (Progeny Production)

Figure-2c. Shows the total number of progeny obtained from male mated with one female in 1 hour and male mated with two female in 1 hour. It was noticed that females mated with middle aged male had a greater number of progeny than females mated with old or young males. Furthermore, male mated with two females in 1 hour had greater progeny than male mated with one female in 1 hour. Two-way ANOVA followed by Tukey's post-hoc test

applied on fertility data showed significant variation among male age classes, between male mated with one female in one hour and two females in one hour.

Results in Figure-3c. Shows that, the number of progeny obtained from male mated with 2nd female was higher than the number of progeny obtained from the male mated with the 1st female in all the three age classes studied. Two-way ANOVA followed by Tukey's post-hoc test on the above data showed significant variation between male age classes and also between the male mated with the 1st and 2nd mated female. Whereas in contrast to this, the interaction between mating status and male age classes showed insignificant variation.

4. Discussion

Age in male insects may affect the reproductive activities including success in intra sexual counters, sperm precedence and female fecundity etc. [42]. However, male reproductive success depends on the number of females inseminated by a male in a given unit of time. Therefore, male remating ability forms one of the strategies to improve male fitness [23]. Males of all the three age classes of *D. malerkotliana* remate within an hour suggesting that ability to inseminate more than one female becomes the inherent ability of male to increase his fitness. Singh and Singh [38] studied *D. ananassae* and noticed differences in male remating ability among different geographical populations. Guruprasad [14] also studied *D. bipectinata* are found differences in male remating in four geographical populations.

In the present study in *D. malerkotliana* middle aged males have a greater percentage of remating than young and old males. This suggests that the age has significant influence on the percentage of male remating ability (Fig 1). The greater percentage of remating ability of middle aged male could be attributed to the occurrence of age specific variation in male fitness. In our study it was also noticed that in all the three male age classes all the males have not remated and this varies with different male age classes. This raises the question that if male remating indeed increases male fitness, then all the males have to undergo remating. In the present study copulation duration and progeny production of male mated with one female in 1 hour and male mated with two females in 1 hour were assessed to rule out the implication of male remating in *D. malerkotliana*.

Figure It was observed in this study that male mated with two females in an hour had copulated longer and laid a significantly greater number of eggs and progeny compared to male mated with one female in an hour (Figure.2a and table 1). This suggests that male remating is really advantageous and this enhances fitness. The result of our study also conforms to the result obtained from [12] where it was observed that multiple mating in *D. melanogaster* was advantageous to males and selection of males can produce a correlated response in females. In our study it

was equally observed that young, middle aged and old males *D. malerkotliana* remated males had greater productivity. Our study conforms to the study of [38] in *Drosophila ananassae*. Furthermore, the present study is in consonance with [23] on *D. malerkotliana* and *D. bipectinata*. Thus, it was noted that in species of *Drosophila* male remating ability is not only influenced by genetic and environmental factors.

Figure.3 and Table 2 reveals that male investment to first and second mated females in 1 hour varies. Copulation duration of male with 1st female was found to be more or less the same. While male mated with 2nd female, the middle aged male copulated longer compared to old or young male that copulated with 2nd female. In contrast to this the number of eggs laid by male mated with first female was significantly greater compared to the male mated with 2nd female. This suggest male invest more in the 1st female he encountered in 1 hour.

From the results it can be seen that all age classes of males had inseminated and invested more towards fecundity in the 1st female than in the 2nd female that had mated. This result was found to be similar in males of all age groups, suggesting males to have invested more in 1st female than 2nd female. Our results confirm the work of Singh and Singh [28] in *D. ananassae* and Guruprasad [14] in *D. bipectinata*. From our results and other studies it can be demonstrated that older males increase their fitness by remating effectively with a number of individuals. At the same time make effective investment in the number of sperms and other accessory gland proteins necessary for effective fecundity and fertility evidently seen.

Figure 2c, 3c and table 1, 2 showed that female of *D. malerkotliana* mated with middle aged male had produced the greatest number of progeny (fertility) compare to old age and young males. Further second mated females with middle and young male had higher fertility rate, whereas first female that mated with old male produced highest progeny number than male mated with 2nd female. These studies also suggest differentiates investment of male mated with 1st and 2nd females. Thus, these studies suggest that in *D. malerkotliana* male remating increases male fitness.

5. Conclusion

All the studies in *D. malerkotliana* suggests that male remating increases male fitness and males had inseminated and invested more in first female than with second female. Furthermore, middle aged male had a greater percentage of remaining and produces a greater number of progeny.

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