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Male age effect on male remating and fitness in *Phorticella striata*

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Abstract: Male traits known to influence on female mating decision were also known to influence on male remating ability too. In *Phorticella striata* age of male effect on male remating and progeny production has been studied. It was noticed that middle aged male had greater percentage of remating than those of young and old males. In all the three male age classes male mated with two females in 1 hour had copulated significantly for longer time, mated females laid greater number of eggs and more progeny than male mated with one female in 1 hour. Further, among male mated with two females in 1 hour, male mated with 1st female copulated longer, mated female laid greater number of eggs and progeny production than male mateed with 2nd female in 1 hour. Thus, these studies in *Phorticella striata* suggests that male age has significant influence on male remating and progeny production. Middle aged male had greater remating and progeny production than young and old males.

Keywords: Male Remating, Fitness, Male age, *Phorticella striata*

1. Introduction

Mating is the most important and fundamental process to select the best partner and to produce progeny. Darwin's theory of sexual selection can help model the basic partners of choosing a mate. According to Darwin (1859), sexual selection is the struggle for existence in the face of other organic beings or external conditions. This means the struggle of the one sex (usually the male), trying to perpetuate his genes by winning the acceptance and mating with the female. The result of failure is normally not death, but few or no offspring. The refused male's genes are not carried on and thus gene diversity in the alleles diminishes. In the opinion of Darwin (1871), sexual selection is actually a non-random differential reproductive success that results from competition for access to mates. However, the optimal strategy for males may not be to mate with as many females as possible, but rather to fertilize as many eggs as possible. The influence of sexual selection can only affect one sex directly (usually the male) and other indirectly (usually the female). It also requires that there is an uneven ratio to male to female organisms so as to keep the competition intense.

Studies on sexual behaviour in numerous species of *Drosophila* with particular reference to its genetic control, contribution of two sexes to the variation in mating activity and repeat mating are well documented [1-7]. It is widely demonstrated that males of different species of *Drosophila* can inseminate more than one female and mating ability of males is influenced by genetic and other factors [8-13].

A male's ability to secure copulations may depend on his phenotype, which can vary according to his physiological condition [14]. 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 [15]. Ex. 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 [16-19, 15].Since 1972 age based female male preference has been a great interest to evolutionary biologists. Most of these male traits related to female mate decision has concentrated in the viewpoint of female and offspring fitness. However, how these male traits influence on male reproductive success has not been studied. Therefore present study has been undertaken in

Phorticella striata to study effect of male ageon male reproductive success. *Phorticella striata* [20] is a drosophillid insect discovered from Karnataka, India belonging to group Drosophillidae. This species also has all characteristics of a good laboratory tool to analyze genetic and evolutionary problems as that of the genus *Drosophila* [21]. In this species the size related mating and reproductive success has been studied by Sharath Chandra and Hegde (2003). They found that larger flies had greater reproductive success than small flies. But for this maiden lone attempt no work has been done on behavioral genetics, cytogenetics and evolutionary genetics of this species. Also, no work so far has been done to study male age influence on remating ability and fitness. Therefore, present investigation has been undertaken in *P. striata* to test the male age effect on male remating and fitness.

2. Materials and Methodology

2.1. Establishment of Experimental Stock

Flies used in the present investigation was obtained from experimental stock of *P. striata* established from progenies of ten wild caught females (iso-female line) collected at Mysore, Karnataka state, India. These flies were cultured (25 males and 25 females) in quarter pint milk bottles (250ml) containing wheat cream agar medium and maintained them in 22±1^{o}c at a RH of 70% of 12 hr light: 12 hr dark 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

For obtaining males of different age classes before start of experiment, longevity of male *P. striata* was studied by transferring unmated males 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 longevity was recorded. A total of 50 replicates were made and mean longevity data calculated showed 49±2days. In addition to this, mating activities of males was also studied from day 1st to 45th day. Results showed that from 2nd day to 44th day male showed all the courtship activities and it started to decline after 45th day. Therefore, we assigned 2-3 days for young, 22-23 day for middle aged and 42-43 day for old males.

The first set of flies emerged were allowed to age for 42-43 day (to obtain old males). When these flies reached 20th day the next set of new flies were isolated and allowed them to age for 22-23 days (to obtain middle aged males). When the second set of flies reached 20th day and the first set of flies reached 40th day, then the new set of flies were 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 were collected from the same culture and maintained them individually under uniform environmental conditions as described above. This procedure allow 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 Effect on % of Male Remating

Pair wise mating has been carried out to test the male age effect on % of male mating ability. A 5-6 day old virgin female and a male (young/middle aged/old) were aspirated into an Elens-Wattaiux mating chamber [22] and observed for 1 hour. Flies which did not mated within 1 hour was discarded. If the flies mated they were allowed to complete copulation and duration of copulation was recorded (time between initiation to termination of copulation of each pair). Soon after mating the mated female fly was transferred to a vial containing wheat cream agar medium to check for larval activity. Mated male fly was once again provided with 5-6 days old virgin female and observation was continued upto 1 hour. If the second female did not mated within time was discarded. If mating occurred duration of copulation was recorded and mated female was transferred to a new vial to observe larval activity. Fifty trials were carried out separately for each of the three male age classes (young-50, middle aged-50, old age-50). Percentage of male remating ability was recorded.

2.4. Male Age Effect on Male Remating Ability (Number of Females Inseminated in 1 Hour) and Male Fitness

To study this 5-6 day old virgin female and a male (young/middle aged and old) were aspirated into an Elens-Wattaiux mating chamber [22] and observed for 1 hour. Flies which did not mated within 1 hour was discarded. If the flies mated, they were allowed to complete copulation and duration of copulation was recorded (time between initiation to termination of copulation of each pair). Soon after mating mated female was transferred to a new vial containing wheat cream agar medium on every 24th hour. This was continued until the death of the female. Mated male was once again provided with 5-6 days old virgin female and observation was continued upto 1 hour. If mating did not occurred with second female within time was considered as male mated once in 1 hour. If mating occurred with the second female the second mated female was transferred to a vial containing wheat cream medium every 24thhour. This was continued upto the death of the second female. Total number of eggs laid and progeny produced by male (Young/Middle aged/Old) mated with one female in 1 hour and male mated with two females in 1 hour were separately used (young-50, middle aged-50, old male-50). Before applying Anova and Tukey's post-hoc test data of male mated with two females in 1 hour were added together for calculating duration of copulation, total number of eggs laid and progeny produced. Two-way Anova followed by Tukey's post-hoc test was carried out on above data of male mated with one female in 1 hour and male mated with two females in 1 hour. Similarly for male mated with 1st and 2nd females.

3. Result

Percentage of remating of young, middle aged and old males of *P. striata* is provided in figure-1. It was noticed that middle aged males had highest % of remating compared to young and old males.

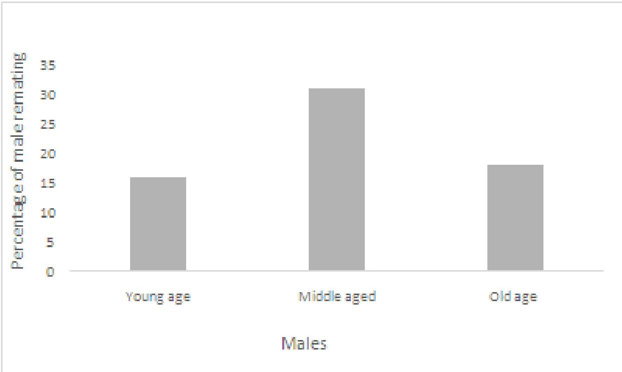
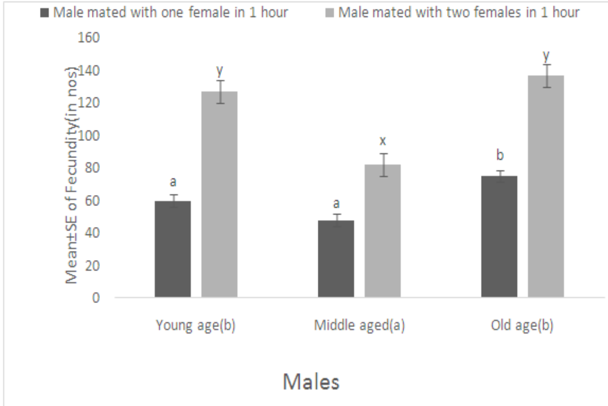


Figure1.*Percentage of male remating in Phorticella striata*

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 insignificantly different among male age classes by Tukey's test.

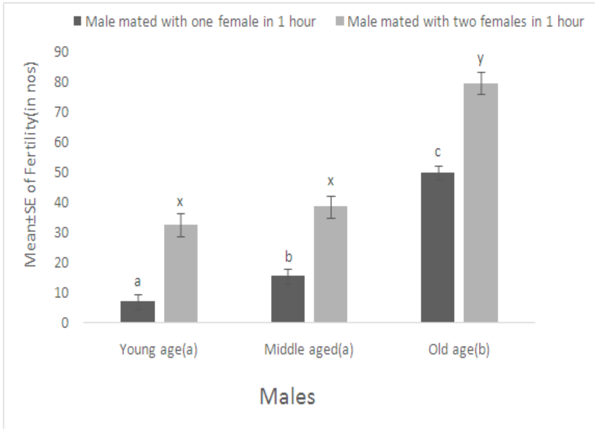
Different letters on the bar graph indicate significant at 0.05 level by Tukey's post-hoc test.

Figure2a.*Male age influence on Copulation duration(in min) of male mated with one female in 1 hour and two females in 1 hour in Phorticella striata.*



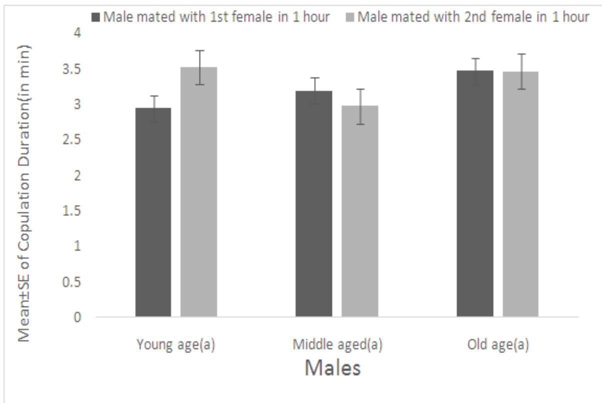
Different letters on the bar graph indicate significant at 0.05 level by Tukey's post-hoc test.

Figure2b.*Male age influence on Fecundity (in nos) of male mated with one female in 1 hour and two females in 1 hour in Phorticella striata.*



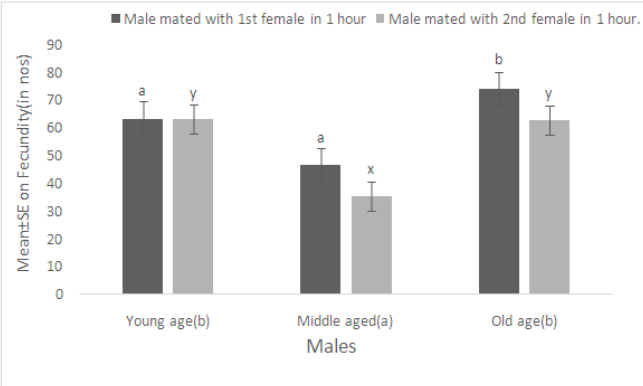
Different letters on the bar graph indicate significant at 0.05 level by Tukey's post-hoc test.

Figure2c.*Male age influence on Fertility (in nos) of male mated with one female in 1 hour and two females in 1 hour in Phorticella striata.*



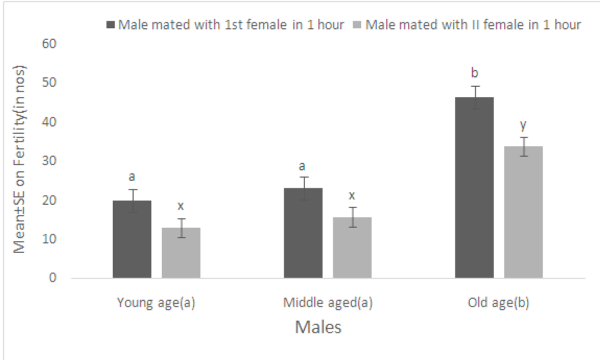
Different letters on the bar graph indicate significant at 0.05 level by Tukey's post-hoc test.

Figure3a.*Male age influence on Copulation duration(in min) of male mated with 1st female in 1 hour and 2nd female in 1 hour in Phorticella striata.*



Different letters on the bar graph indicate significant at 0.05 level by Tukey's post-hoc test.

Figure3b.*Male age influence on Fecundity (in nos) of male mated with 1st female in 1 hour and 2nd female in 1 hour in Phorticella striata.*



Different letters on the bar graph indicate significant at 0.05 level by Tukey's post-hoc test.

Figure3c.*Male age influence on Fertility (in nos) of male mated with 1st female in 1 hour and 2nd female in 1 hour in Phorticella striata.*

Table 1.*Two-way Anova of male age influence between male mated once and twice in 1 hour in P. striata.*

Parameter	Source	Type III Sum of Squares	df	Mean Square	F
Copulation duration	Mating status	522.192	1	522.192	133.568**
	Age	129.749	2	64.875	16.594**
	Mating status*Age	36.609	2	18.305	4.682*
	Error	1149.408	294	3.910	
	Total	9930.418	300		
Fecundity	Mating status	219673.080	1	219673.080	145.829**
	Age	87317.787	2	43658.893	28.983**
	Mating status*Age	15352.880	2	7676.440	5.096*
	Error	442874.240	294	1506.375	
	Total	3088066.000	300		
Fertility	Mating status	51587.853	1	51587.853	102.206**
	Age	117478.427	2	58739.213	116.374**
	Mating status*Age	596.027	2	298.013	.590^{NS}
	Error	148394.880	294	504.744	
	Total	731574.000	300		

**p<0.001; *p<0.05; NS-Non significant.

Table 2.*Two-way Anova of male age influence between 1st female and 2nd female mated to a male in 1 hour in P. striata.*

Parameter	Source	Type III Sum of Squares	df	Mean Square	F
Copulation duration	Mating status	1.051	1	1.051	.445^{NS}
	Age	7.470	2	3.735	1.582^{NS}
	Mating status*Age	8.616	2	4.308	1.825^{NS}
	Error	693.955	294	2.360	
	Total	3892.594	300		
Fecundity	Mating status	4316.813	1	4316.813	2.636^{NS}
	Age	42181.227	2	21090.613	12.880**
	Mating status*Age	2090.667	2	1045.333	.638^{NS}
	Error	481410.080	294	1637.449	
	Total	1522794.000	300		
Fertility	Mating status	6021.120	1	6021.120	16.126**
	Age	33116.720	2	16558.360	44.348**
	Mating status*Age	509.840	2	254.920	.683^{NS}
	Error	109772.000	294	373.374	
	Total	338724.000	300		

**p<0.001; NS-Non significant.

Figure 3ashows that the duration of copulation of male mated with 1st female increased with increase in age of male while duration of copulation of male mated with the 2nd female was longer at young age than it decreased at middle aged then once again duration of copulation increased at old age. Two-way Anova followed by Tukey's post-hoc test carried out on above data showed insignificant variation between male age classes, between male mated with 1st mated and 2nd mated female [Table 2].

Total number of eggs laid by male mated with one female in 1 hour and two females in 1 hour is provided in the figure 2b. Male mated with two females in 1 hour had laid greater number of eggs than males mated with one female in 1 hour. This result was found to be similar in all the three male age classes studied. Among the male age classes, females mated with old males had laid greatest number of eggs and female mated with middle-aged male had laid least number of eggs. Two-way Anova followed by Tukey's post-hoc test carried out on the above data showed significant variation in total number of eggs laid between male age classes, between male mated with one female in 1 hour and male mated with two females in 1 hour. Also, interaction between male mating status and male age classes. Tukey's post-hoc test showed that old male mated with one female in 1 hour had laid significantly greater number of eggs compared to females mated with young or middle-aged males. Young males mated with two females in 1 hour had laid significantly greater number of eggs than middle-aged males mated with two females in 1 hour but significantly different in old males mated with two females in 1 hour.

Figure 3b shows total number of eggs laid between the 1st female and 2nd female mated with the same male in 1 hour. The number of eggs laid by female mated with old male was highest than the number of eggs laid by females mated with middle-aged male but it was found to be least when compared with females mated to young male in both the 1st and 2nd mated females in all the three male age classes. Further, among mated females it was noticed that the 1st female mated with old male had laid greater number of eggs than 1stfemale mated with young male but 1stfemale mated with middle-aged males had laid least number of eggs. Further, 1st mated female had laid greater number of eggs than the 2nd mated female mated with old and middle-aged males while it was almost the same in females of both mated to young males. Two-way Anova followed by Tukey's post-hoc test applied on fecundity data showed significant variation between male age classes but found insignificant variation between 1st and 2nd mated female. Tukey's test showed that females mated to middle-aged males had laid least number of eggs than females mated to old and young males.

Figure 2c shows results of progeny obtained by male mated with one female in 1 hour and two females in 1 hour. It was noticed that number of progeny obtained from male mated with one female in 1 hour was lesser than those of progeny obtained from male mated with two females in 1 hour. The number of progeny obtained from male mated with one/two females in 1 hour increased with increasing of male age. Two-way Anova followed by Tukey's post-hoc test showed significant variation between mating status (male mated with one female and two females in 1 hour) and also between male age classes. Tukey's test showed that young male mated with 1st/2nd female had produced significantly less number of progeny compared to middle aged/old male mated with 1st/2nd female.

Number of progeny obtained between male mated with 1st and 2nd female in an hour is provided in figure 3c. Results shows that the number of progeny obtained from 1st mated female was higher than the number of progeny obtained from male mated with 2nd female in 1 hour. Among male age classes number of progeny obtained from both the 1st mated and 2nd mated females increased with increase of male age. Two-way Anova followed by Tukey's post-hoc test on the data of progeny obtained from 1st and 2nd mated female shows significant variation between male age classes and also between the 1st and 2nd mated female mated with the same male in 1 hour. Among all male age classes the progeny obtained from male mated with 1st female was significantly higher than the progeny obtained from male mated with 2nd mated female.

4. Discussion

The importance of male age on sexual behaviour is not straight forward and in most cases only one or a few aspects of male fitness like success in inter male competition, fecundity, fertility and longevity has been studied. Infact male remating is also a strategy for improving the fitness of males. *P. striata* is one such polygamous species in which influence of male age on male remating has been largely ignored. Males of all the three age classes undergoes remating within 1 hour suggesting that remating is one of the inherent property of male to increase male fitness (Figure 1a). Our results support earlier studies of male remating in different species of Drosophila and other insects [23]. Male remating has also been noticed among different geographical populations in few species of *Drosophila* [23, 24]. They attributed this variation to genetic heterogeneity carried among the populations.

In the present study in *P. striata* middle aged males had greater % of remating than young and old males suggesting that male age has significant influence on % of male remating ability (Fig 1). The greater % of remating ability of middle aged male could be attributed to occurrence of age specific variation in male fitness in order to understand whether male remating really benefit the fitness of males. If male remating benefits the male then why very few males inseminate two females in 1 hour whereas some males mate with only one female in 1 hour. This study also suggests that male traits such as male size, age and other morphological features used by female in mating decisions are also known to influence on male remating ability too. Krishna and Hegde (1997) who while working in *D. bipectinata* and *D. malerkotliana* have shown influence of male size on male remating. They found that larger males could inseminate more number of females in 1 hour than small males. Therefore in our study copulation duration, fecundity and fertility of males mated with one female and two females in 1 hour has been studied in *P. striata*. Thus it was noted from these studies that male remating ability is not only influenced by genetic and environmental factor but the age of male also has its effect on male remating ability. According to Gromko (1992), multiple mating is widely believed to be advantageous to males and selection by males can produce a correlated response in females.

Figure 2a and Table 1 showed that males mated with two females in 1 hour had copulated longer, laid significantly greater number of eggs and produced greater number of progeny than males mated with one female in 1 hour in *P. striata*. This result was found to be similar in young, middle aged and old males respectively. This suggests that males of all the age classes tend to undergo remating to increase their fitness. Our results confirm earlier studies of male remating which increases male fitness in different species of *Drosophila* (Singh and Singh, 2000). Our result also suggests that old male mated with two females in 1 hour had significant greater number of eggs and produced greater number of progenies than two females mated with either middle or young male. This is because older males did not mated up to old age. As a result greater accumulation of accessory gland protein and sperms with age resulted in transferring greater accessory gland proteins and sperms more or less equally to two females mated with old males in 1 hour than females mated with either young or middle aged males.

Another important question that raised was whether males invest accessory gland proteins and sperms equally to two females mated with the same male age class within an hour. It was noticed that middle-aged males copulated shorter with 2nd female compared to they mated with the 1st female in an hour. Whereas old males had copulated more or less equally with both the 1st and 2nd female in an hour. In contrast to this young males had copulated with the 2nd female for slightly longer time than with 1st female (Fig 2a). The difference in the duration of copulation could be attributed to male age. Singh and Singh (2000) in *D. ananassae* and Guruprasadet.al.,(2008) in *D. bipectinata* have also found that males copulate shorter with 2nd female. This may be due to depletion of sperms and accessory gland secretions (Singh and Singh, 2000). Since old males had not mated for a considerable period of time, therefore there was an increased load of sperms and accessory gland proteins in old male. Thereby both 1st and 2nd females mated with them more or less for same duration compared to young or middle aged males. Although in species of *Drosophila* and other insects duration of copulation is a male determined trait [2], our study suggests that age of male also play an important role in determining duration of copulation.

Thus these studies in *Phorticella striata* suggest that male age has significant influence on male remating ability resulting increased male fitness.

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