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RESEARCH ARTICLE

AGE BASED FEMALE MATE PREFERENCE IN *PHORTICELLA STRIATA*

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ABSTRACT

Sexual selection often involves female preference for males of certain age, and a body of theoretical information predicts preference for old males in species in which mating is resource independent and both parents do not show parental care. *Phorticella striata* is one such species in which mating is resource independent and both parents do not show parental care. Outbred population of *P. striata* has been used to understand age based female mate preference. It was noticed that in *P. striata* females of all age preferred older males more frequently than young or middle aged males. Older males showed significantly greater courtship acts, mated faster and copulated longer than young or middle aged males. Females mated with old males laid significantly greater number of eggs and progeny than females mated with either young or middle aged males. Further, female mated with older males lived significantly for a shorter time than when she mated with either young or middle aged males. Thus these studies confirmed that female evolved towards older male in species in which mating is resource independent and both parents do not show parental care.

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INTRODUCTION

Beginning with Darwin, sexual selection by female mate choice has received much attention by researchers which emphasizes on the evolution and maintenance of mating preferences for sexual traits. Therefore, it forms a large area of research in behavioural ecology (Andersson, 1994). However, success of males in mating is linked to traits namely size, diet, early adult experience, colour, etc., in turn how females use such traits to derive both direct and indirect benefits (Andersson and Simmons, 2006). Male age and adult sexual experience are other two important traits used by female to select the potential mate (Radwan, 2003). Studies of age based female mate preference have suggested that male age could be used as an honest indicator of male quality. In spite of this, both theoretical and empirical evidence from different species belonging to different genera have shown occurrence of female preference for young, middle aged and old males (Avent *et al.*, 2008). Different models have been proposed to explain female preference for male age classes to derive either direct or indirect benefits. Among these models Good gene model and antagonistic pleiotropic model are important. According to Good gene model or viability indicator models of age-related mate choice predict female preference for older males as they have already proven their ability to survive upto older age and mating with such males she can receive materials benefits that affect female fitness or genetic benefits that affect offspring

fitness. It was widely suggested that in species in which female receive only sperm and seminal ejaculate, in such species female should prefer older males because viability selection leads to older males of higher genotypic quality than younger males (Trivers, 1972; Manning, 1985; Kirkpatrick, 1992; Andersson, 1994). Empirical evidences are also available to female preference for older males (Zuk, 1988; Simmons and Zuk, 1992). In addition to this Kokko and Lindstrom (1996) have showed using a simulation model that a strong female preference for older mates is likely to evolve under a variety of environmental conditions.

Although the idea that females prefer to mate with older males because of higher genetic quality of older males is intuitively appealing, such age based preferences may have evolved for other reasons. Hansen and Price (1995) argued that young to intermediate age males have the highest breeding values for fitness, and thus are of higher genetic quality than older males. Therefore, if females choose mates based on genetic quality alone, females should prefer younger rather than older males. Some empirical evidence supports the contention that younger males are of higher genotypic quality than older males. In collared flycatchers (*Ficedula albicollis*), the offspring of females that mated with younger males had higher lifetime reproductive success than offspring of females that mated with older males (Alatalo *et al.*, 1986). Similarly, in *D. melanogaster*, male age negatively affected larval survival and mating ability of sons (Price and Hansen, 1998). If female preference for older males is not due to higher genetic quality of older males, females may exhibit preferences for older males solely due to positive correlations between male age and

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values of sexually-selected traits (Hansen and Price, 1995). Consequently, empirical evidence for female preference for older males does not necessarily support the idea that female mate choice for older mates evolved due to "good genes". The conclusions of various models are probably a result of their contrary approaches towards changes in physiological state of males with age and existence of life-history trade-offs. Therefore, all these models emphasise that female can discriminate potential mate on the basis of male age classes. Thus, one important conclusion from all these studies show that longevity associated with fitness made in early arguments of age-based mate choice is not always correct (Brooks and Kemp, 2001). Studies in human and nonhuman (vertebrate and invertebrate) showed that sperm traits, fertilisation success, female fitness and offspring fitness decreased with increasing of male age (Table 1 & 2). Paradoxically females in different species belonging to different genera show preference for older males. Further, little work has been undertaken that investigates whether male fertility changes with age and whether females can discriminate among more or less fertile males in a single experimental system. Until more species and genera are studied, it will be difficult to draw firm conclusions. Hence, more studies regarding age related selection are needed.

Species in which males show no parental care and do not offers direct benefits in the form of nuptial gifts to the mating female are suitable for testing the above hypothesis (Andersson and Simmons, 2006). The species of the genus *Drosophila* and genus *Phorticella* forms a very good material to test the female preference for male age classes because males and females of these species do not show parental care or provide nuptial gifts to the mating females. Males of these species transfer accessory proteins and sperms to the mating female in copulation. Recently, very few studies have been carried out in *Drosophila* to test good gene model (Avent *et al.*, 2008; Somashekar and Krishna, 2011; Prathibha and Krishna, 2011). They found females of *Drosophila* species discriminate their mates on the basis of male age classes and prefers to mate with older males more frequently than with either young or middle aged males. In contrast to this Hansen and Price (1995), have suggested that young to middle aged males have highest breeding values for fitness and thus are of higher genetic quality than older males. If females choose mates based on genetic quality alone, female should prefer younger males rather than older males. Therefore, in the present investigation, *Phorticella striata*, belonging to the genus *Phorticella* an allied genus of *Drosophila* has been selected.

Phorticella striata (Nirmala and Krishnamurthy, 1975) is a drosophilid insect discovered from Karnataka, India belonging to group Drosophilidae. This species also has all characteristics of a good laboratory tool to analyze genetic and evolutionary problems as that of the genus *Drosophila* (Sharath Chandra and Hegde, 2003). 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 reproductive performance and offspring fitness. Therefore, present investigation has been undertaken in *P. striata* to test whether females of this species are able to discriminate their mates on the basis of male age classes. If so, whether female obtained greater direct benefit from preferred males or not.

MATERIALS AND METHODS

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 at $22\pm 1^{\circ}\text{C}$ RH of 70% of 12 hr light: 12 hr 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.

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 ± 2 days. 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 and 42-43 day for old age 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 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 2-3 day (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.

Male age influence on female mate preference

Female mate preference test has been used to understand whether females of *P. striata* can discriminate males on the basis of male age classes. Female mate preference involved a 5-6 days old virgin female (young/ middle aged/ old) and two males of different male age classes (young and middle/ middle and old/ young and old) were individually aspirated into the Elens-Wattisux chamber (Elans Wattiaux, 1964) and observation was made for 1 hour. When mating occurred, pairs in copulation were aspirated out of the mating chamber and transferred into a separate vial containing wheat cream agar

media. The selected and rejected males in female mate choice experiment were used to measure wing length using the procedure of Hegde and Krishna (1997). Fifty trials were run separately for each combination of female mate choice experiment. The effect of paint was tested before commencing the experiment by painting one of the two young/middle/old males on the thoracic region and allowed them to mate. This pair was observed for 1 hour and 50 trials were run separately for each combination. The presence of paint had no effect on the probability of mating (all groups $p > 0.05$).

Male age influence on mating activities

Pair wise mating has been carried out to test male age courtship acts in mating success. A 5-6 day old virgin female and a male (young/ middle-aged/ old) were aspirated into an Elens-Wattiaux mating chamber (Elens Wattiaux, 1964) and observed for 1 hour. If the pair did not mate within 1 hour was discarded, if mating occurred, we recorded male and female courtship activities such as tapping, scissoring, circling, vibration, decamping, ignoring and extruding activities in courtship following procedure of Hegde and Krishna (1997). Mating latency (the time between the introduction of male and female together into a mating chamber until the initiation of copulation) and copulation duration (the time between initiation to termination of copulation of each pair) were recorded.

Male age influence on Female fitness

Soon after copulation as above, mated female from each pair was transferred into fresh vial containing wheat cream agar media once in every 24 hours until the death of mated female to study fecundity, fertility, sex-ratio and longevity. Total number of eggs laid (fecundity) and progeny produced (fertility) by each mated female was recorded and sex of the progeny (sex ratio) was also noted. Female longevity was measured from the time of its emergence from pupa until her death. A total of 50 successfully mated pairs were studied for each of the male age classes.

RESULTS

Male age influence on mating success

In the present study when female was given a choice to select males (young, middle aged and old) in combination of two male age classes, it was noticed that females of all the three age classes preferred older of the two competing males more

frequently in the experiment involving a female and two males. Mating success of old males in crosses involving old and young males was 68% with young female, 70% with middle aged female and 74% with old aged female (Table 1). Mating success of old males in crosses with old and middle aged males was 80% with young female, 82% with middle aged female and 78% with old female. Similarly, mating success of middle aged males in crosses with middle aged and young males was 56% with young female, 58% with middle aged female and 62% with old female. Generalised linear model with a binomial link function has been applied (in which the mating success of the youngest male is the dependent variable and his age class and the age class of the alternative mate are the fixed factor and the difference in the wing length of the selected and rejected competing males is the covariant) carried out on female mate preference for male age classes showed significant variation. But difference in mean wing length of selected and rejected males in female mate choice experiment showed insignificant variation (Table 1).

Male age influence on mating activities

Figure 1(a) shows mean mating latency and copulation duration of young, middle aged and old males of *P. striata*. Time taken to initiate copulation was lowest in old males and was found to be least in middle aged males, while duration of copulation increased with increasing age of males.

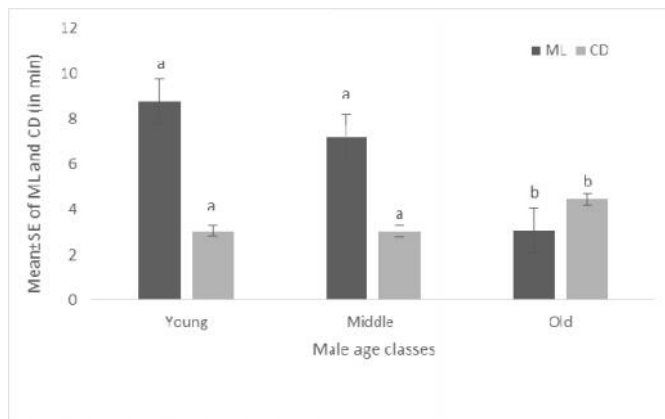


Fig. 1(a). Male age influence on Mating Latency (ML) and Copulation Duration (CD) of *Phorticella striata* (ML- F value-8.559, df-2, $p < 0.001$; CD- F value-10.355, df-2, $p < 0.001$.)

Different small letters in superscript on the bar graph indicate significance by Tukey's post-hoc test.

Table 1. Female mate preference for male age classes in *Phorticella striata*. (N=50; df-1)

Female age	Males		Wald chi-square (mating success)	Sig. level	Diff. in wing length (S-R) Mean±SE	Wald chi-square (wing length of S-R)	Sig. level
	Young (2-3 d)	Old (42-43 d)					
Young (2-3 d)	16 (32%)	34 (68%)	164.728	.000**	.0678±2.956	.669	.414 ^{NS}
Middle (17-18 d)	15 (30%)	35 (70%)	171.587	.000**	.0932±1.498	.018	.893 ^{NS}
Old (32-33 d)	13 (26%)	37 (74%)	147.911	.000**	.1022±1.494	.078	.780 ^{NS}
	Middle (22-23d)	Young (2-3 d)					
Young (2-3 d)	22 (44%)	28 (56%)	170.544	.000**	.0542±4.27	.426	.514 ^{NS}
Middle (17-18 d)	21 (42%)	29 (58%)	169.590	.000**	.0814±2.518	.488	.485 ^{NS}
Old (32-33 d)	19 (38%)	31 (62%)	186.626	.000**	.1100±1.299	.009	.926 ^{NS}
	Old (42-43 d)	Middle (22-23 d)					
Young (2-3 d)	10 (20%)	40 (80%)	163.513	.000**	.0672±3.488	.150	.699 ^{NS}
Middle (17-18 d)	09 (18%)	41 (82%)	227.712	.000**	.0700±2.765	.125	.723 ^{NS}
Old (32-33 d)	11 (22%)	39 (78%)	306.812	.000**	.0722±1.263	1.186	.276 ^{NS}

S – selected male; R – rejected male.

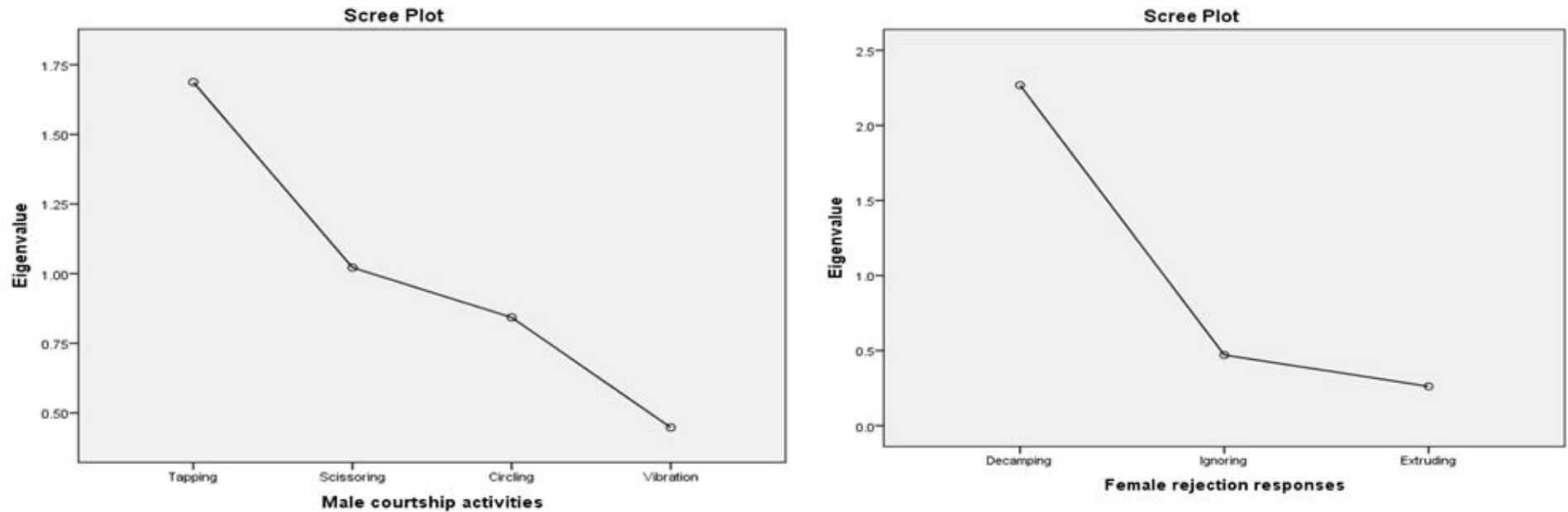
Table 2 Principle component analysis of male age effect on courtship activities in *Phorticella striata*.(N=50; df-2)

Courtship activities	Male age classes	Mean±SE			F-value	Total	Initial Eigenvalues		Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
		Young (2-3 d)	Middle (22-23 d)	Old (42-43 d)			% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
Male courtship activities	Tapping	2.200±.069 ^b	1.480±.076 ^a	1.300±.076 ^a	40.678**	1.345	33.613	33.613	1.345	33.613	33.613	1.321	33.030	33.030
	Scissoring	2.520±.082 ^b	2.140±.134 ^a	1.840±.087 ^a	10.775**	1.093	27.319	60.932	1.093	27.319	60.932	1.116	27.903	60.932
	Circling	1.540±.086 ^b	0.960±.085 ^a	0.560±.070 ^b	36.674**	.958	23.945	84.877						
	Vibration	2.360±.0742 ^a	2.540±.091 ^a	2.320±.066 ^a	2.252 ^{NS}	.605	15.123	100.000						
Female courtship activities	Decamping	0.860±.121 ^a	0.160±.072 ^b	1.080±.127 ^a	19.185**	1.553	51.781	51.781	1.553	51.781	51.781	1.549	51.619	51.619
	Ignoring	0.720±.117 ^b	0.160±.072 ^a	1.220±.128 ^c	23.653**	1.004	33.471	85.252	1.004	33.471	85.252	1.009	33.633	85.252
	Extruding	0.340±.093 ^a	0.080±.055 ^a	0.680±.125 ^b	9.817**	.442	14.748	100.000						

Extraction Method: Principle Component Analysis. **p<0.001; NS-Non significant

S-Selected; R-Rejected

Different small letters in superscript indicate significance by Tukey's post-hoc test

**Fig 1(b).** Scree plot of Principle Component Analysis for courtship activities in *Phorticella striata*

One-way Anova followed by Tukey’s post-hoc test applied on mating latency and copulation duration data showed significant variation between male age classes [Fig 1a]. Tukey’s post hoc test showed that old males had taken significantly greater time to initiate mating than middle aged males, but it was insignificantly greater compared to young males. Duration of copulation was found to be significantly longer in female mated with old male than female mated with young male but it was found to be insignificantly greater than female mated with middle aged male by Tukey’s post hoc test.

Mean male courtship acts to female in mating is provided in Table 2. It was observed that old males showed highest courtship acts than young and middle aged males. In turn, female showed least rejection responses to old males compared to young and middle aged males. One-way Anova followed by Tukey’s post-hoc test showed that except vibration all other male courtship acts differed significantly between male age classes. Courtship activities of old male to mating female was found to be significantly greater than young and middle aged males. Females showed significantly lesser rejection responses to old males compared to young and middle aged males by Tukey’s post hoc test. Principle Component Analysis followed by Scree plot applied on male courtship activities of different male age classes showed that among the courtship acts tapping and scissoring behaviour had significantly greater influence on mating success of older males [Table 2; Fig. 1b]. Among the female rejection responses decamping and ignoring have shown greater influence than extruding in mating success of older males by Principle component analysis and scree plot [Table 2; Fig. 1b].

Male age influence on Fecundity and Fertility

Figure 1c shows male age influence on fecundity and fertility of *P. striata*. Total number of eggs (fecundity) laid by females are shown to be highest in females mated to old aged males and it was found to be least in females mated to middle aged males.

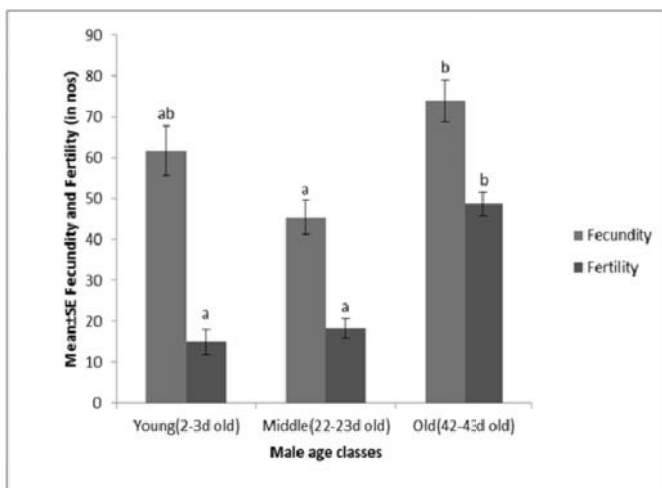


Fig 1(c). Male age influence on Fecundity(fec) and Fertility(fer) of *Phorticella striata*. (Fec-F value-7.675, df-2, p<0.001; Fer- F value-44.091, df-2, p<0.001)

Different small letters in superscript on the bar graph indicate significance by Tukey’s post-hoc test.

Progeny (fertility) of females mated with young, middle aged and old males of *P. striata* has shown to be increased with increasing of male age. Highest progeny was recorded from females mated with old males, while it was least in females mated with young males. One-way Anova and Tukey’s test carried out on the above data showed significant difference in both fecundity and fertility of females mated with different male age classes. Fecundity and fertility of females mated with older males was shown to be significantly highest when compared to females mated to young and middle aged males. Between females mated to young and middle aged males both fecundity and fertility was found to have insignificant difference by Tukey’s post hoc test.

Male age influence on female longevity

Survival curve analysis on longevity of females mated with males of different male age classes is provided in [Tables 4 a, 4b; Fig. 1d]. The survival functions is defined as the probability of surviving at least till time t. The hazard function h(t) is the conditional probability of dying at time having survived till that time. The graph of S(t) against t is called the survival curve.

Table 3. Male age influence on courtship activities, mating activities and female fitness in *Phorticella striata*

MATING, COURTSHIP ACTIVITIES/FEMALE FITNESS	MALE AGE
MALE AGE	1.000
MATING LATENCY	-.002 ^{NS}
COPULATION DURATION	.251**
TAPPING	.446**
SCISSORING	.225**
CIRCLING	.330**
VIBRATION	-.116 ^{NS}
DECAMPING	.091 ^{NS}
IGNORING	.225**
EXTRUDING	.194*
FECUNDITY	.125 ^{NS}
FERTILITY	.602**

** p-value< 0.001 level; * p-value <.05 level; NS-Non significant.

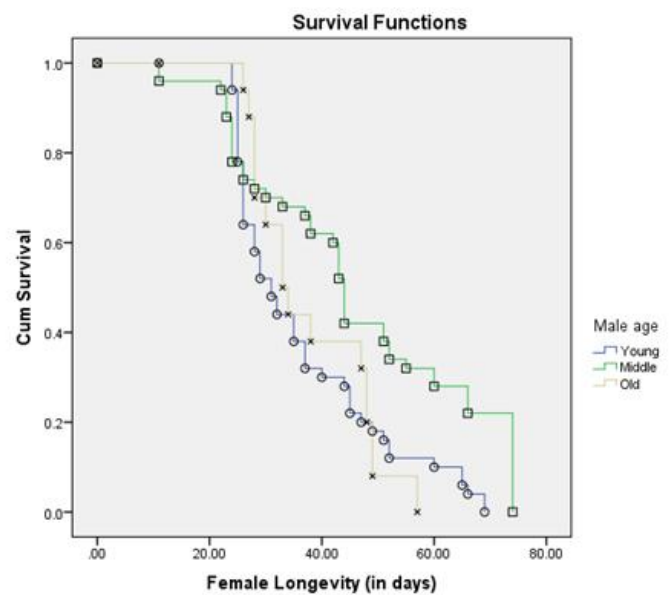


Fig. 1(d). Male age effect on the Female Survival ability of *Phorticella striata*

Table 4a. Kaplan Meir analysis for Female longevity in *Phorticella striata* for female mated with male of different age classes

Male age classes	Mean ^a				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
Young	36.620	1.937	32.823	40.417	31.000	2.019	27.043	34.957
Middle	46.320	2.805	40.822	51.818	44.000	.776	42.480	45.520
Old	37.980	1.446	35.146	40.814	33.000	1.414	30.228	35.772

Table 4b. Overall tests of the equality of female Survival times of female mated with male of different age classes of *Phorticella striata*

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	15.417	2	.000
Breslow (Generalized Wilcoxon)	6.706	2	.035
Tarone-Ware	9.898	2	.007

The Kaplan Meier method was used to estimate this curve from the observed survival time without the assumption of an underlying probability distribution. Comparison of two survival curves was done by using a statistical hypothesis test called the log rank test. It is used to test the null hypothesis that says there is no difference between female's survival curves (i.e., the probability of an event occurring at any point of time is the same for each male age class). It was noticed that in *P. striata* females mated with old males survival was found to be least than females mated with middle aged or young males. Table 3 shows Spearman's rho-correlation applied between male age, courtship activities and fitness. It was noticed that male age was insignificant negatively correlated with mating latency and wings vibration of behavior to courting female while it was significant positively correlated with duration of copulation, courtship activities and fertility. Where as insignificant positive correlation was found between male age classes with decamping behaviour of female to courting male and fecundity.

DISCUSSION

Previous studies of age based mate preference suggests that in species of insects in which mating is resource independent and male or female do not show parental care when female was given a choice to select male on the basis of age she should prefer older males to obtain good gene because old males had already proved their survival ability to become upto old. As a result they might have carried genes responsible to live longer. If these genes were transferred to their offspring thereby offspring may also live longer (Trivers, 1972; Andersson, 1994; Radwan, 2003). *P. striata* is one such insect in which mating is resource independent and both male and female do not provide parental care. When females of *P. striata* was given a choice to select males on the basis of age it was found that females of all age classes preferred older of the two competing males confirming the prediction of age based male preference of resource independent system, thereby confirming the prediction that in species in which males contribute only sperm female preference on male age is more likely to evolve in a population if preferences are directed toward older males. Our results also supports the work of Avent *et al.*, (2008) in *D. pseudobscura* and *D. persimilis*, Prathibha and Krishna (2011) in *D. ananassae*, Somashekar and Krishna (2011) in *D. bipectinata*. They also found female preference for old males in these species.

This is also found to be true with other insects and birds (Zuk, 1998; Conner, 1989; Hasselquist *et al.*, 1996) and also with theoretical models (Kokko, 1997 and 1998). Thus, these studies suggest that female preference for older male is found to be an evolutionary stable strategy. However, in *D. melanogaster* Moulin *et al.* (2001) who while studying courtship song components of males aged 2-6 days have mentioned that courtship song increased with increasing male age (from 2 days to 6 days) and they have not shown female preference for male age classes. Except in studies of *D. ananassae* and *D. bipectinata*, in remaining studies male age did not span with male longevity of these respective species. Female age classes of *P. striata* used in the present study were selected based on the female rejection behavior toward courting males. Rejection behavior of females toward courting male was analysed from 2nd day to 32nd day. Females rarely mate at 33rd day, therefore in the present experiment we used female age classes as follows: young females 2-3d; middle aged females, 17-18d; old females 32-33d.

In the present study we used female mate choice situation. In such experiment it was difficult to separate female has a choice to mate with preferred male in male-male interaction on one hand and competition of competing males to obtain a female for mating on the other hand. In spite of this, one of the advantage of female choice experiment is that female has an equal opportunity to approach and reject the advances of males regardless of their age. In turn males also has an opportunity to approach and reject the female. This gives an equal opportunity for both males and females sampling themselves. Further, on the activity levels of competing males, female can discriminate potential males (Hegde and Krishna, 1997). Using similar protocol on female choice experiment Avent *et al.*, (2008) in *D. pseudobscura*, Prathibha and Krishna (2011) in *D. ananassae* and Somashekar and Krishna (2011) in *D. bipectinata* have also found female preference for old age males in both female choice situation and no-choice situation.

In contrast to our study in *P. striata* in above studies they used only 5-6 days old female. However, they did not study whether females of all ages can discriminate male on the basis of age or preferred to mate with old males or not. Thus, these studies suggest that the male-male interaction observed in female-choice experiment can reinforce female mate preferences rather than operating in an antagonistic fashion (Moore and Moore, 1999). Thus these studies of age based female mate preference suggests that female preference for older males may be an evolutionary stable strategy in male resource independent system. Male traits such as male mating history, rearing of males prior to the exposure to females, male size, male age, intensity of courtships, differences in cuticular hydrocarbon, courtship song, are the cues used by female to discriminate potential mates (Somashekar and Krishna, 2011). Males of different age classes used in the present investigation

of *P. striata* were unmated and were reared in group of two. Therefore, female preference for old mates observed in the present study was not influenced by the differences in male mating history and rearing condition before exposure to mating instead it was the effect of difference in the male age. Most compelling evidence suggests that in other insects and species of *Drosophila*, male size has significant influence on the male mating success (Partridge *et al.*, 1987; Santos *et al.*, 1988,1992; Hegde and Krishna, 1997). These studies have found that large males had greater mating success than small males. Therefore, in the present study wing length of selected and rejected males in female choice experiment was measured to rule out the influence of male size on mating success [Table 1]. It was found that mean wing lengths of rejected males were slightly longer compared to mean wing length of selected males in female choice experiment and difference was found to be insignificant. This clearly suggests that the observed greater mating success of older males in female mate choice situation was not due to influence of male size instead was the male age effect.

Further, flies used in the present experiment were reared under low density condition (100 eggs/vial) to reduce the influence of environmental component on the variation of body size and mating activities. Therefore, the absence of size variation in the present experiment might be due to flies being maintained under uniform condition and reared in low larval density. Further, like *Drosophila* in *P. striata* also the size of the adult fly is fixed at the time of emergence from pupa (Santos *et al.*, 1988). As a result the body size may not vary with male age. What are the reasons for greater mating success of old males or female preference for old males. The possible reasons could be, (1) older males are more active males, therefore he succeeded in convincing the female for mating (2) female may obtain direct fitness benefit by mating with older males as they carry good genes in them or she can produce greater number of eggs and progeny by mating with old males. Mating experiments of *P. striata* [Table 2; Figure 1b] shows that males of *P. striata* initiate the courtship acts and in turn female shows rejection response. These courtship elements consists of various behavioural display followed by an interchange of different sensory stimuli such as visual, acoustic, olfactory and tactile.

Therefore through these courtship elements females could identify the potential male. It was noticed that in our study in *P. striata* old male showed significantly greater courtship activities (tapping, scissoring, circling, vibration) and mated faster [Figure 1a] than middle and young age males. On the other hand, female of *P. striata* also showed greater rejection towards young males compared to middle aged and old males. This suggests that in *P. striata* older males were more active during mating, thereby convinced the female faster for mating than those of middle aged and young males. This agrees with the work of Somashekar and Krishna (2011) in *D. bipunctinata* and Prathibha and Krishna (2011) in *D. ananassae*. They also found greater male courtship activities of old males but they found less female rejection to old males which were responsible for greater mating success of old males in species of *Drosophila*. Thus, these studies suggest that activity levels of courting pair are important for successful mating and it increases with increasing age of males. The

courtship activities of male and female ends with copulation. Through this act of copulation males transfer not only genes but also seminal ejaculates which consists of accessory gland protein and sperms to mating females which brings about post mating behavioural changes such as it prevents female remating, increases egg production, etc. Therefore duration of copulation is an important fitness component. It is considered as male determined trait in many *Drosophila* species and is expression of rate of sperm transfer (Spiess, 1970). Further, copulation duration appears to influence fecundity and egg viability. In many insects, males mated for prolonged duration produces large ejaculates containing nutrients that are used by females for reproduction and somatic maintenance (Eberhard, 1996; Vahed, 1998). Thus, females obtaining larger ejaculates utilize these nutrients to lay more eggs and this operates to direct natural selection for mates that produce large ejaculates. Therefore, for fecundity function may be linked with male age (Savalli and Fox, 1998,1999). In *P. striata* copulation duration ranges from 01 min to 04 min. Further, female mated to old males had copulated significantly longer when compared to female mated to either young or middle aged males [Figure 1a]. This suggests that in *P. striata* male has significant influence on copulation duration.

Our study also agree with the work of Avent *et al.* (2008) in *D. pseudobscura*, Somashekar and Krishna (2011) in *D. bipunctinata* and Prathibha and Krishna (2011) in *D. ananassae*. They also found greater copulation duration of females mated to old males. According to Avent *et al.* (2008) differences in the copulation duration of male age classes can be explained by three hypotheses. According to 1st hypotheses longer copulation duration might be due to old males which were unable to transfer sperm rapidly. 2nd possibility for longer copulation duration among old males could be due to large quantities of sperm transfer which require more time. Thereby females mated with old age males produces greater progeny. 3rd possibility could be that old males might transfer more accessory fluid in their ejaculates during extended copulations thereby female mating with old males produced greater number of eggs. The 1st above possibility suggests that old males are worst maters than young males. While, 2nd and 3rd possibility suggests that old males invest more resources per mating. Because of this female prefers to mate with old males.

It was noticed in *P. striata* that female mated with older male had produced greater number of eggs and progeny than females mated with young or middle aged males [Figure 1c]. This suggests that longer duration of copulation of female mated with old males might be responsible for greater production of eggs and progeny of female mated with older males. Although we did not quantify the accessory gland and sperm transferred to the mated female but greater production of eggs and progeny by female mated with older male suggested indirectly that old males had transferred greater quantity of accessory gland and sperm to mated female by its longer duration of copulation thereby confirming the proposed 2nd and 3rd possible hypothesis that older males invest more resources to mated females compared to young and middle aged males. Our results in *P. striata* also confirms the studies of female preference for male age in *D. bipunctinata* (Somashekar and Krishna, 2011) and in *D. ananassae* (Prathibha and Krishna, 2011). They also found that females

mating with old males obtained greater number of eggs and progeny. However other theoretical reasons to expect that old males had greater copulation duration because they never encountered females for many days. As a result, they invest more resources in the first female they encounter (Wedell *et al.*, 2002). On the other hand, old males have lower residual reproduction. As a result they increase their ejaculate investment per mating with increasing age (Roff, 1992). Further, it is also possible that old males may have accumulated large quantity of ejaculates. Therefore, they took longer duration to transfer them to the female (Jones *et al.*, 2007). Mating was not always yielded positive effect on fitness, it also has negative effects on female fitness for eg., mating reduces female longevity. This shows occurrence of sexually antagonistic behaviour which is resulted due to the transfer of accessory gland protein through ejaculate by male to the mated female (Champman *et al.*, 1995; Wolfner 2002). Our study in *P. striata* also shows that females mated with old males lived significantly shorter compared to female mated with young or middle aged males.

This may be attributed to longer copulation duration of female mated to old male had transferred greater quantity of accessory gland protein to the mated female. In many insects longer copulation is associated with the transfer of more sperm and other ejaculate substances (Thornhill and Alcock, 1983; Wedell, 2002). Our results in *P. striata* also confirms the study of Somashekar and Krishna (2011) in *D. bipunctata* and Prathibha and Krishna in *D. ananassae* (2011). They also found reduction in longevity of female mated with old males. Careful observation of Table-4a, 4b and Figure-1d have shown that in *P. striata* mating had both positive and negative effect on female fitness suggesting occurrence of sexually antagonistic behavior which is very much essential for the evolution of male and females. Thus our study in *P. striata* suggests that females of this species preferred old males because they were more active during mating, could convince the female faster, copulated longer and obtain fitness benefits by mating with old males thereby confirming the prediction that in resource independent mating system with no parental care females prefers to mate with old males rather than young or middle aged males.

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