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RESEARCH ARTICLE

EVIDENCE OF FEMALE PREFERENCE FOR MIDDLE AGED MALE IN *DROSOPHILA MALERKOTLIANA*

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ABSTRACT

Studies of age based female mate preference suggested that female preference for male age in different taxa was found to be mixed and various hypotheses have been proposed with conflicting prediction. The present study in *D. malerkotliana* noticed that females of all ages preferred to mate with a middle aged males more frequently than young or old males. Further middle aged males showed significantly greater courtship acts to courting females. The female showed least rejection behavior and copulated longer with middle aged male than young or old males. Female mated with middle aged male had produced a significantly greater number of progeny but insignificant variation was found in eggs laid by female mated with different male age classes. Female mated with middle aged male lived shorter. Thus our study in *D. malerkotliana* shows the occurrence of age based female mate preference and female preferred middle aged male to obtain direct fitness benefits.

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INTRODUCTION

In studies of sexual selection female of a species choose among potential mates based on variation found in male mating signals. Such preferences commonly evolve because females benefit from mating with preferred male phenotypes (Knapp and Kovach 1991, Petrie 1994). In turn male success in mating linked to reproductive benefits he offers to the mating female. Females receive benefits from mating with the preferred male traits which include material benefits that affect fitness of females and material benefits that affects offspring fitness or genetic benefits that affects offspring fitness (Andersson, 1994). Thus female choice based on a signal correlated with the quality of a mating benefit is possible only if the male signal provides reliable information about the quality of the benefit (Avent *et al.*, 2008). A great diversity of male traits such as body size, courtship song, mating activities, colors are known to influence on female mate preference (Hegde and Krishna, 1997; Somashekar and Krishna, 2011). Male age is one such character known to affect mating and reproductive success of males as a result female can use this trait has an honest indicator of male quality (Avent *et al.*, 2008).

Age based female mate preference studies have suggested that the female of a species use male age as a reliable signal of male quality thereby mating with a preferred male age class can obtain fitness benefits (Prokop *et al.*, 2007). However in such studies what is really matters for the fitness of female progeny is not the quality of her partner but of his gametes because

females mating with male receives not only genes but also receives sperms and a variety of seminal ejaculate. Studies of sperm traits and fertilization success in studies of human and non-human has found a reduction in quality and or quantity of sperm, fertilization success and offspring fitness suggesting that the quality of gametes actually decreased with male age. For example. Due to the accumulation of deleterious mutation in his germ line (Hansen and Price, 1995; Radwan, 2003). However female preference with respect to male age differs among species (Table 1). Some species showing preference for older males because the reliability of male signaling increases with age because they have proven their survival ability while other studies have shown that females were attracted to and/or mated preferentially with younger male further studies have also shown that intermediate age males were favored. Father in some insect species female did not show a preference for male age classes.

Female mate preference for male age have different arguments. According to Trivers (1972) and Halliday (1978) have stated that all else being equal, females should prefer older males since they have demonstrated their capacity for survival. A few years later this idea was supported by Manning (1985). He proposed that older males on average, likely carry fewer somatic mutations than young males since selection would have removed individuals with deleterious mutations early in one life. Thus, older Males are genetically superior and females who mate with such males would be benefited by passing on those genes to their offspring. Empirical evidences are also available in which females prefer to mate with older males. By doing so, females of many species may obtain direct benefits through the superior paternal care provided by older males

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(Burly and Moran, 1979; Yasakawa, 1981; Price, 1984; Grant and Grant, 1987; Komers and Dhinsa, 1989; Buchholz, 1991; Cote and Hunte, 1993; Lee and Park, 2009). Further it was also noticed that species in which males do not provide such benefits, females may choose older males to obtain offspring of higher genetic quality. This was suggested for species with no parental care (Davison, 1981; Manning, 1987, 1989; Zuk 1987, 1988; Simmons, 1988; Stidel *et al.*, 1991; Simmons and Zuk, 1992; Vandenbergh *et al.*, 1993) for species in which males do not provide care for the offspring of females (Jharvi *et al.*, 1982, Weather head, 1984) and in cases of extra-pair copulation (Moller, 1992).

In contrast to this younger males make better mates were put forth by Hansen and Price (1995). They included a life history perspective in their model to explain that older males do not have an average higher genetic quality, and they are not genetically superior to younger males. To explain this they put forth 4 arguments: 1) there are negative genetic correlations between early and late fitness components; 2) males usually suffer a decrease in fertility with age; 3) younger males are better adapted to the current environment; and 4) older males have accumulated more germ line mutations. They concluded by stating that females choosing older males are probably not doing so for genetic benefits, although they did not offer any alternative hypothesis.

In 1995 Hansen and price incorporated additional life history variables to explain age-based mate choice. When factors such as the allocation of resources to sexual traits, variations in male condition and age-specific survival probability were included the models showed that the preferences for older males could evolve (Kokko and Lindstrom, 1996; Kokko, 1998; Hansen and Price, 1999; Beck and Powell 2000, Beck *et al.*, 2002, Beck and Promislow, 2007). They predicted that preferences for younger males are equally possible depending upon the conditions specified. One important conclusion from those studies was that the assumption of equating longevity with fitness that was made in early verbal arguments of age-based mate choice is not always correct (Brooks and Kemp, 2001). Thus differences in explanations are probably a result of their contrary approaches towards changes in the physiological state of males with age and the existence of life-history trade-offs. Until more species and genera are studied, it will be difficult to draw firm conclusions. Hence more studies are needed in this regard. Studies also suggested that species which do not show parental care are more suitable for testing these models. These experimental results of age based mate preference suggest more studies are needed to evaluate age based female mate preference. The species of the *Drosophila* in which males do not provide parental care nor gives any nuptial gifts to mating female therefore it forms an ideal model to test above models. Recent studies of age based female preference in species of *Drosophila* showed mixed response i .e. Species of *D. bipunctata*, *D. annannasae*, *D. pseudopscura* showed that females of these species preferred old male while females of *D. melanogaster* prefers to mate a young male (Avent *et al.*, 2008; Moulin *et al.*, 2001). Therefore more studies are required to understand age based female preference in species of *Drosophila*. Therefore present study has been undertaken in *D. malerkotiana*. It is a cosmopolitan species is a member of the

bipunctata complex of the *ananassae* subgroup (Bock, 1971; Bock and Wheeler, 1972). It has a wide ecogeographical distribution ranging from India through South east Asia and New Guinea to Fiji and Samoa in the Pacific (Bock and Wheeler, 1972). 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 few years. They have carried out extensive studies on population and behavior genetics of this species and have established the phylogenetic relationship between *D. malerkotiana* and other members of the *bipunctata* complex based on chromosome analysis, hybridization studies and isozyme analysis (Yang *et al.*, 1972; Jha and Rehman , 1972; Hegde and Krishnamurthy, 1979; Singh *et al.*, 1981; Hegde and Krishna, 1997). These findings provide interesting and important information concerning certain aspects of evolutionary genetics of this species. However in this species is not known whether females of this species discriminate males on the basis of their age classes, if so what its effect on female direct fitness benefits. Therefore present study has been undertaken in *D. malerkotiana* to understand females of this species can discriminate males on the basis of age . If so, whether female mated with preferred male age class obtains direct fitness benefits or not.

MATERIALS AND METHODS

Progenies of 150 naturally inseminated isofemale lines of *D. malerkotiana* collected in Mysore, Karnataka, India was used to establish experimental stock. In each generation progeny obtained were mixed together and redistributed to 20 different culture bottles containing wheat cream agar medium each with 20 males and 20 females. These culture bottles were maintained them at $21 \pm 1^\circ\text{C}$ at a relative humidity of 70% using a 12: 12 h light: dark cycle. This procedure was continued for 4 generations to allow them to acclimatize to the laboratory conditions. At the 5th generation, synchronized eggs (± 30 min) were collected using Delcour's procedure (1969). When adults emerged, virgin females and unmated males were isolated within 3 h of eclosion and were aged as required for the experiment.

Assigning of age classes to males

For obtaining males of different age classes before the start of experiment, longevity of male *D. malerkotiana* 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 was made and mean longevity was found to be 62 ± 2 days. In addition to this, mating activities of males were also studied from day 1st of their inclusion until 60th day. Results showed that showed least male courtship activities at 1st day whereas from 2nd day and onwards (up to 46-47 days) male showed all the courtship activities and all of them mated with the female . At 47th day male showed least courtship activities and rarely mated with the female. Hence age classes assigned to males were 2-3 days for young, 24-25 days for middle and 46- 47 days for older males. The first set of flies emerged were allowed to age for 46-47 day (to obtain old males). When these flies reached 20th 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 20th day and the first set of flies reached 47th day, then the new set of flies was isolated and was aged for 2-3day (to obtain young males). This procedure helps us to culture all young, middle and old male in the same environment and also conduct the experiment at the same time.

Mate-choice test (Male age effects on female mate preference)

The female mate preference test has been used to understand females of *D. malerkotliana* can discriminate their mates on the basis of male age classes. Female mate preference involved a 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-Wattiaux chamber (1964) and observed them 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 to measure the wing length of selected male and rejected males in female mate choice experiment using the procedure of Hegde and Krishna (1997). Fifty trails 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 aged /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 effect on courtship and mating activities

Pairwise mating has been carried out to test male age effect on of *D. malerkotliana*. A female and a male (young, middle-aged or old) were aspirated into a lens-Wattiaux mating chamber (1964) and observed in 1hour the pair did not mate within 1hour was discarded. When 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 in a mating chamber until the initiation of copulation) and copulation duration (the time between initiations of termination of copulation of each pair) were recorded.

Male age effect on female fitness (fecundity, fertility, and female longevity)

Soon after copulation (as obtained above), mated female from each pair was transferred to a new vial containing wheat cream agar medium once in 24 hours until her death. Total number of eggs laid (fecundity) and progeny emerged (fertility) from these eggs and their sex was also recorded. From about the time of its emergence until death of the mated female was considered as female longevity. Fifty trails were run separately for mating activities, fecundity, fertility and female longevity of female mated with young, middle aged and old male (young=50, middle =50 and old = 50).

RESULTS

Mating success

When female of *D. malerkotliana* was given a choice to select males of young, middle aged and old in combination of two male age classes. Females of all the three age classes preferred middle aged males more frequently than young or old males in an experiment involving a female and two males. Mating success of middle aged males in crosses involving middle aged and young male was found to be 72% with young female, 70% with middle aged females and 74% with old females (Table.2). Mating success of old male in crosses with young and old males was found to be 76% with young, 78% with middle aged female and 74% with old female. Similarly, mating success of middle aged male in crosses involving old and middle aged males was found to be 76% with young female, 74% with middle aged female and 68% with old females. Generalized linear model with a binomial link function has been applied (In which the mating success of 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 body size of two competing males is the covariant) on data of female mate choice experiment showed significant variation in mating success between different male age classes. Difference in selected and rejected male wing length in female mate choice experiment showed insignificant variation. (Table 2).

In Table 2. Middle aged males showed highest tapping, scissoring and licking behavior to courting female while young males showed lowest courtship activities to courting female. In turn female showed least rejection responses (decamping, extruding and ignoring) to middle aged males than to young or old males. One way ANOVA followed by post hoc test carried out on above data showed significant variation in male's courtship activities between male age classes and also between female rejection responses of female to males of different age classes. Courtship activities of middle aged males were found to be significantly greater to courting female compared to young or old male courtship acts to courting female as shown by Tukey's post hoc test. Principal component analysis and scree plot applied on above data showed that among male courtship acts to courting female tapping and scissoring acts of male had significant influence on mating success of middle aged males compared to licking, wing vibration and circling behaviors of males to court females. This was also evident in the scree plot of Principal component analysis (fig 1a). The Principal component analysis carried out on female rejection responses to courting male of different age classes showed that female rejection responses such as ignorance, decamping and extruding acts to courting male of different male age classes had a greater influence on mating success of middle aged males and it was also evident in the scree plot (fig 1b).

Male age effect on Mating latency and Copulation duration

Mating latency and Copulation duration data of young, middle aged and old males of *D. malerkotliana* is provided in Fig 2. It was noticed that the time taken for the initiation of copulation (Mating latency) was found to be least in female

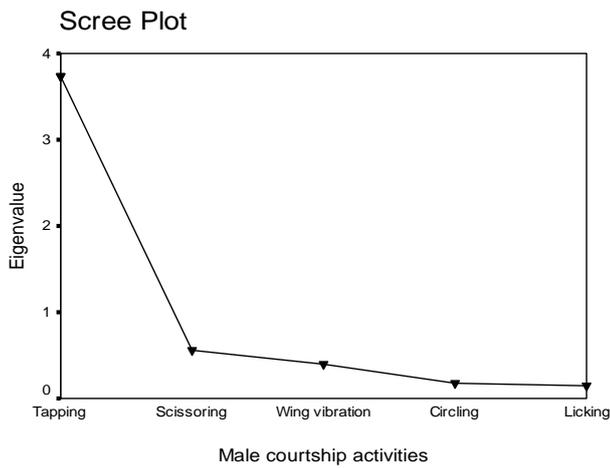
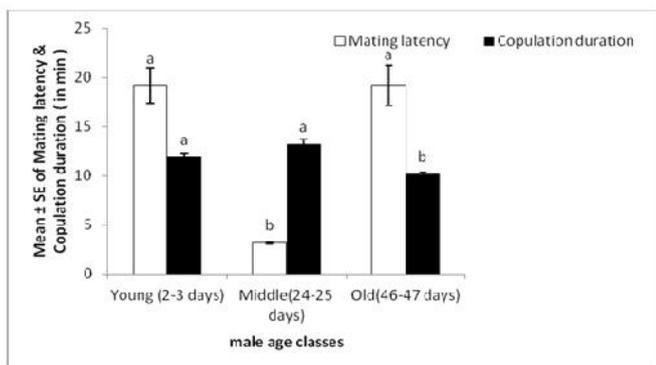


Fig. 1a. Principle component analysis of male age effect on male courtship activities in *D. malerkotliana*.



Fig. 1 b. Principle component analysis of male age effect on female rejection responses in *D. malerkotliana*.

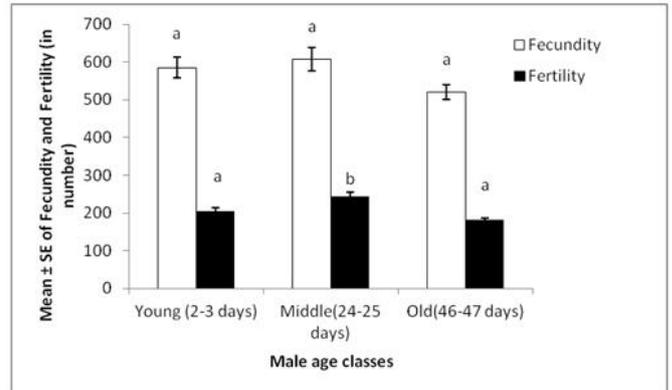


(ML- F value-34.416, df -2, 147; $p < 0.0001$, CD- F value- 14.212, df -2, 147; $p < 0.001$)

Fig 2. Male age effect on the Mating latency and Copulation duration of *D. malerkotliana*.

mating with a middle aged male. Whereas female mated with young or old males had taken more or less same to initiation of copulation. In contrast to this, duration of copulation was found to be highest in female mated with middle aged males and it was found to be lowest in female mated with older males. One way ANOVA followed by Tukey's post hoc test applied on above data showed significant variation in mating latency and duration of copulation between male age classes. Tukey's post

hoc test showed that the time taken for the initiation of the copulation of female mated with a middle aged male was found to significantly shorter than those female mated with young or old males. It was also noticed that the duration of copulation of female mated with old male was significantly shorter than those females mated with either young or middle aged males by Tukey's test.



(Fec - F value- 3.836, df -2,147; $p < 0.05$, Fert- F value- 12.361, df -2,147; $p < 0.001$)

Fig 3. Male age effect on the Fecundity and Fertility of *D.malerkotliana*



(F value-189.85, df -2,147; $p < 0.0001$)

Fig 4 a. Male age effect on the Female longevity of *D. malerkotliana*

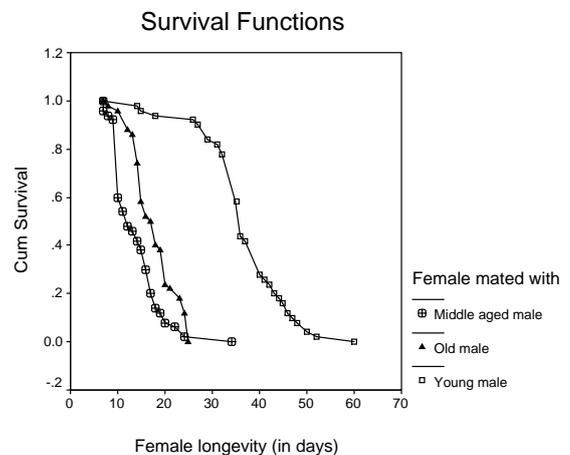


Fig 4b . Male age effect on the Female Survival ability of *D. malerkotliana*

Table 1. Female mate choice for young, intermediate and old age males in different insect species

Species	Female mate preference for			Reference
	Young male	Intermediate male	Older male	
<i>Ephippiger ephippiger</i> (Orthoptera: Tettigonioidea)	+	-	-	(Ritchie et al. 1995)
<i>Rhizoglyphus robini</i> (Acari: Astigmata)	+	-	-	(Radwan et al. 2005)
<i>Choristoneura rosaceana</i> (Lepidoptera: Tortricidae)	-	+	-	(Delisle 1995)
<i>Lutzomyia longipalpis</i> (Diptera: psychodidae)	-	+	-	(Jones et al. 2000)
<i>Dermestes maculatus</i> (Coleoptera: Dermestidae)	-	+	-	(Jones and Elgar 2004)
<i>Bolitotherus cornutus</i> (Coleoptera: Tenebrionidae)	-	-	+	(Conner 1989)
<i>Platthemis Lydia</i> (Odonata: Libellulidae)	-	-	+	(Koenig 1991)
<i>Phthorimaea operculella</i> (Lepidoptera: Gelechiidae)	-	-	+	(Cameron et al. 2005)
<i>Drosophila pseudoobscura</i> (Diptera: Drosophilidae)	-	-	+	(Avent et al. 2008)
<i>Bicyclus anynana</i> (Lepidoptera: Nymphalidae)	-	-	+	(Fischer et al. 2008)
<i>Sepsis cynipsea</i> (Diptera: Sepsidae)	-	-	-	(Martin et al. 2003)
<i>Callosobruchus maculatus</i> (Coleoptera: Bruchidae)	-	-	-	(Savalli and Fox 1999; Fricke and Maklakov 2007)

Table 2. Female mate preference for male age classes in Outbred population of *D.malerkotliana* (N=50; df. 1).

Female age	Males		Wald chi-square (mating success)	Sig. level	Difference in wing length(S-R) mean ± SE	Wald Chi-square (wing length of S-R)	Sig. level
	Young	Old					
Young	12 (24%)	38 (76%)	6.128	0.013	0.01 ± 0.002	1.384	0.239
Middle	11 (22%)	39 (78%)	7.918	0.005	0.01 ± 0.001	1.167	0.280
Old	13 (26%)	37 (74%)	4.682	0.030	0.01 ± 0.002	1.255	0.263
	Middle	Young					
Young	36 (72%)	14 (28%)	4.762	0.290	0.01 ± 0.002	0.994	0.319
Middle	35 (70%)	15 (30%)	6.371	0.012	0.01 ± 0.001	1.041	0.307
Old	37 (74%)	13 (26%)	4.725	0.030	0.01 ± 0.002	1.024	0.312
	Old	Middle					
Young	12 (24%)	38 (76%)	6.128	0.013	0.01 ± 0.004	0.123	0.726
Middle	13 (26%)	37 (74%)	4.682	0.030	0.01 ± 0.002	0.673	0.412
Old	16 (32%)	34 (68%)	3.089	0.079	0.02 ± 0.04	0.198	0.656

S=selected male; R= rejected male

Table 3. Principle component analysis of male age effect on courtship activities in Outbred population of *D. malerkotliana*. (N=50; df. 1).

Population	Male courtship activities	Male age classes Mean ± SE			F value	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
		Young	Middle	Old		Total	% Variance	Cumulative %	Total	% Variance	Cumulative %	Total	% Variance	Cumulative %
Male courtship activities	Tapping	8.52 ± .6775 ^a	12.60 ± 1.54 ^b	14.46 ± 1.30 ^b	6.34 *	2.877	57.531	57.531	2.877	57.531	57.531	1.032	20.649	20.649
	Scissoring	5.92 ± .5742 ^a	13.94 ± 1.45 ^b	11.56 ± 1.22 ^b	12.89 **	.933	18.659	76.189	.933	18.659	76.189	1.024	20.481	41.130
	Circling	7.66 ± 0.69 ^a	12.78 ± 1.10 ^b	12.52 ± 1.60 ^b	5.82 *	.502	10.043	86.233	.502	10.043	86.233	1.021	20.423	61.553
	Licking	4.82 ± 0.79 ^a	10.52 ± 1.10 ^b	12.58 ± 1.62 ^b	10.84 **	.414	8.285	94.518	.414	8.285	94.518	.990	19.796	81.349
Female rejection responses	Decamping	2.60 ± 0.34 ^a	2.86 ± 0.34 ^a	6.88 ± 0.79 ^b	20.08 **	1.909	63.636	63.636	1.909	63.636	3.636	1.008	33.614	33.614
	Extruding	2.02 ± 0.25 ^a	1.70 ± 0.22 ^a	4.96 ± 0.73 ^b	14.74 **	.645	21.485	85.121	-	-	21.485	1.002	33.388	67.002
	Ignoring	3.02 ± 0.48 ^a	1.28 ± 0.18 ^b	3.54 ± 0.45 ^a	8.77 **	.446	14.879	100.000	-	-	14.879	.990	32.998	100.000

** Significant at p < 0.001. NS- Insignificant (p > 0.05).

A different letter in the superscript indicates significance at 0.05 levels by Tukey's post hoc test

Table 4a. Kaplan Meir analysis for Female longevity of female mated with male of different age classes of *D. malerkotliana*

Males classes	age	Estimate	Std Error	Mean		Estimate	Std Error	Median	
				95% Confidence Interval				95% Confidence Interval	
				Lower Bound	Upper Bound			Lower Bound	Upper Bound
Young		37.16	1.23	34.76	39.56	36.00	.50	35.02	36.98
Middle		13.94	.74	12.50	15.38	12.00	1.57	8.92	15.08
Old		17.72	.64	16.47	18.97	17.00	1.18	14.69	19.31

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

	Chi- square	Df	Significance
Log Rank (Mantel- Cox)	134.42	2	.0000
Breslow (Generalized Wilcoxon)	105.37	2	.0000
Tarone- Ware	119.52	2	.0000

Male age effect on female Fecundity and fertility

Fecundity (total number of eggs laid) and fertility (total progeny produced) data of female mated with young, middle, old age males of *D. malerkotliana* is provided in Figure.3. It was found that females mated with middle aged males had laid a greater number of eggs and progeny compared to female mated with old or young males. One way ANOVA followed by Tukey's post-hoc test applied to mean above fecundity and fertility data showed insignificant variation in between male age classes. Female mated with old male produced significantly lesser progeny than those female mated either by middle aged or young males. Further female mated with middle aged males had a significantly greater number of progeny when compared to female mated with young or old males.

Male age effect on Female longevity

Survival curve analysis of longevity of female mated with males of different age classes (Figure 4b and Table 4a and 4b). Two functions that are dependent on time are of particular interest the survival functions are defined as the probability of surviving at least till time t . The hazard function $h(t)$ is the conditional probability of dying at the time having survived till that time. The graph of $S(t)$ against t is called the survival curve. The Kaplan Meir 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 *D. malerkotliana* female mated with older males survived last than the middle aged and young males.

DISCUSSION

Previous models for the evolution of female preference based on male age have suggested that in species in which mating is resource independent and either male or female do not show parental care in such species female evolved preference towards intermediate or older males and bias against younger males if the mutation rate is constants with increasing age of males (Beck and Powell, 2000; Beck *et al.*, 2002; kokko, 1998). *D. malerkotliana* is one such species of the genus *Drosophila* here mating is resource independent and both male and female of this species receives not only genes but also accessory gland proteins and sperms from mating males. In the genus *Drosophila* very few species have been employed to study age based female mate preference even in these studies female prefers to mate with older of the two competing males. They used 5-6 days old virgin females for their experiment (Avent *et al.*, 2008; Somashekar and Krishna, 2011; Prathibha and Krishna, 2011).

In contrast to this in our study in *D. malerkotliana* females of all the age prefers to mate with middle aged males more frequently over young and old males suggesting that females of this species evolving towards middle aged males. Female age classes of *D. malerkotliana* used in the present study was selected based on the female rejection behavior toward courting males. The rejection behavior of females toward courting male was analyzed from 2nd day to 32th 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, older females 32-33d. Four hypotheses have been proposed to explain the female preference for middle aged males. The production and disposal of middle aged male pheromones may be important in attracting the female. Second the middle aged may be more vigorous in courtship and convince the female faster than young or old males therefore it would be expected to be more likely to achieve mating with females. Thirdly female shows least rejection responses to preferred male age classes (middle aged males). Fourthly female may obtain direct fitness benefits by mating with preferred male age (middle aged males). Our results in *D. malerkotliana* support the similar results reported in *C. browningi* (Liu *et al.*, 2010) and *Dermestes maculatus* (Jones and Elger, 2004).

In the present study unmated, reared in groups of two males per vial were used therefore observed greater mating success of middle aged males was not resulted due to differences in the male mating history or rearing conditions before exposure to mating. Further we also found rejected males had slightly longer wings than selected males in female mate choice experiment suggesting observed greater mating success was not resulted due to the male size effect on mating success instead it was the effect of male age effect. In species *Drosophila* mating is resource independent, mating success of courting pair depends on the courtship activities of male and female during mating. Therefore the display given by males before mating is the only grounds on which females can differentiate between potential mates because courtship acts of male and female involves complex interaction of mechanical, chemical stimuli between courting pairs. Through this act male increases the receptivity of females for mating too (Hoikkala and Limataininon, 1992; Hedge and Krishna, 1997). Greater the activities of males greater are the success of mating. Our results in *D. malerkotliana* had shown that middle aged males performed a greater number of courtship acts such as tapping, scissoring, vibration, licking etc. Compared to female courting with young or old males. Thus middle aged males of *D. malerkotliana* through his greater courtship acts had convinced the female faster for mating compared to young or old males. This supports the hypothesis proposed above that middle aged males were more vigorous during mating thereby female preferred to mate with middle aged males. Our results in *D. malerkotliana* also confirms the activities of courting pair is

important for successful mating in species of *Drosophila* (Hegde and Krishna, 1997). In contrast to this in species of *D. bipectinata* and *D. ananassae* old males showed greater courtship acts during mating compared to middle aged and young males therefore older males were more vigorous in mating than young or middle aged males thus females of these species preferred older males in mating than young or middle aged males. Further in our study females of *D. malerkotliana* showed least rejection responses to middle aged males compared to young or old males suggesting that females of this species evolving towards middle aged males. Courtship activities of male and female culminates in copulation duration. Through this act of copulating male transfers not only genes but also seminal ejaculate which consists of accessory gland proteins and sperms to the mated female (Chapman and Wolfner, 2002). This insemination brings about modifications in female behavior and physiology in various ways 1) it reduces female receptivity thereby preventing female remating. 2) Increases egg production. 3) Decreases female longevity (Wolfner, 2002). Thus post copulatory mechanism because it affects fitness of female. Longer the duration of copulation greater will be the transfer of accessory gland proteins and sperm to the mated female (Wolfner, 2002). Our results in *D. malerkotliana* showed that female mated with middle aged male copulated significantly longer than female mated with younger or older males. Therefore three possible hypotheses have been proposed to explain greater duration of copulation by female mated with middle aged males. First middle aged males may be unable to rapidly transfer sperm and hence require longer copulation.

Second hypothesis would be middle aged males may transfer large quantities of sperm requiring more time there by female mated with middle aged males produces greater progeny size. Third hypothesis would be middle aged males might transfer more accessory gland protein in their ejaculates during extended copulations therefore female mated with middle aged males produce a greater number of eggs than females mated with young or old males. In the present study it was noticed that female mated with middle aged males had produced a significantly greater number of eggs than females mated with either young or old males. Similarly female mated with middle aged males had produced a significantly greater number of progeny than females mated with young or old males. Thus our results in *D. malerkotliana* suggest that accept the 2nd and 3rd hypothesis because female mating with middle aged males invests more resource per mating which could be the underlying cause of a female preference for middle aged males. These explanations are not mutually exclusive and cannot be directly evaluated from our study because we did not quantify the amount of sperm and accessory fluid transferred. It is possible that the longer copulation of middle aged males results in the transfer of more sperm because in many insects longer copulation durations are associated with the transfer of more sperm (Thorn hill and Alcock, 1983; Wedell *et al.*, 2002). Therefore we found a greater number of eggs and progeny produced by female mated with middle aged males in *D. malerkotliana*. However there is a clear-cut reason that why middle aged males had copulated longer. In our study it was found that reduction of female longevity if female mated with middle aged males when compared to female mated with either

young or old males. The reduction in longevity of females which had mated with middle aged males carrying a greater quantity of accessory gland this is because it is possible that the longer copulation of middle aged males may result in the transfer of more accessory gland proteins and sperms and other substances to mated female. This is because it has been shown in many insects longer duration are associated with the transfer of more sperm and other ejaculate substance (Thornhill and Alcock, 1983; Wedell, 2002). Thus these studies in *D. malerkotliana* suggest that middle aged males were more active during courtship and copulated longer than females mated with young or old males. Further female mated with middle aged males obtained greater fitness benefits.

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