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Age based female mate preference in *Drosophila malerkotliana*

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Abstract
Female prefers to mate with middle age aged males more frequently over young or old males. Further it was found that in these female prefers middle aged male to obtain direct benefits greater fecundity and fertility. It was found that in all the three geographical strains middle aged male had copulated longer time with female compared to young and old males. Among three geographical strains studied flies of Chamarajanagar strain had copulated longest while flies of Bellur strain copulated for shortest time.

Keywords: *Drosophila malerkotliana*, female age, mate preference, wing length

Introduction

In sexual selection female use greater diversity of male traits such as body size, mating calls, courtship song etc. Male age is another trait which vary the average quantity with age therefore males vary typically in their ability to provide benefits with increasing age of male and how female determining to detect and preferred to mate with them revealed much about the process that drive the evolution of age based female mate preference. Different theories have been put forward to explain variation in quality of male with increasing age and how female obtain either direct benefits from mating males of different male age classes. However many of the theories predicting age based preference are predicted on assumptions about the life history of organism, which will be strongly influenced by ecology (Kokko,1998) [9] Example Manning (1985) argued that older males are proven survivors, but exactly how good a predictor of survival ability age is will depend strongly male age may be a weak predictor of quality if most male mortality occurs through largely stochastic events for which males carry little heritable variation in ability to survive.

Mating behavioral studies in *Drosophila* have assumed that females control mating success, i.e., whether mating takes place and with whom (Speith 1952). Female mate choice not only involves the female preference for males but also involves male-male competition (Hegde and Krishna 1997, Avent *et al*. 2008, Somashekar and Krishna 2010) [8, 2, 17]. Those studies suggested that male-male competition can reinforce female mate preference rather than operating in an antagonistic fashion (Moore and Moore 1999). Even with male mate choice, it is difficult to separate among male choice, female-female competition, and differences in female motivations to mate (Byrne and Rice 2006) [5]. Therefore, we can hypothesize that in *D. ananassae*, middle-aged females are more eager to mate than younger or older females. This agrees with earlier studies of sexual selection in different species of *Drosophila* (Speith 1952, Manning 1961, Hegde and Krishna 1997, Byrne and Rice 2006) [8, 5].

Studies on mating behavior in *Drosophila* suggest that male mating success not only depends on male preferences, but successful copulation also requires female receptivity (Spieth 1952) [18]. In *Drosophila*, sexually mature females show a range of behaviors to thwart unwanted advances of courting males, such as decamping, wing flicking, kicking, and ovipositor extrusion (Spieth 1952) [18], while immature females do not show such rejection behaviors. This is because newly emerged (immature) females do not perform any of these rejection behaviors (Manning 1961).

The female mate preference for older male may not tightly linked to different species. Further in most of the experiment only one population/ strain was used therefore on cannot rule out whether or not it is applicable other population of the some species. Recently to the best of our knowledge in two species *D. bipectinata* polyandrous species and *D. sudobscura*, monodromous species tested female preference for male age in different population of these species. They found that different population of these species did indeed differ in the key mating traits of copulation duration and latency but they show a constant and strong preference for older males. However it is not know that whether or not in species preference for young or middle age male are tightly linked to ecology. Therefore present experiment has been undertaken in *D. malerkotliana* in this species female prefers to mate with middle age aged males more frequently over young or old males. Further it was found that in these female prefers middle aged male to obtain direct benefits greater fecundity and fertility. Hence in the present study three different geographical strains /populations of this species having different ecological background were used to understand factors driving middle aged males to be preferred in this species are tightly linked to ecology or not.

Materials and Methods

Experimental stocks

Progenies of 150 naturally inseminated iso female lines of *D. malerkotliana* collected separately at three geographical strains of Karnataka i.e. Mysore, Chamarajanagar and Bellur were used to establish experimental stocks. 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 ± 1°C at a relative humidity of 70% using a 12: 12 h light: dark cycle. This procedure was continued for 2nd generations to allow

them to acclimatize to the laboratory conditions. At the 3rd generation, synchronized eggs (± 30 min) were collected separately from each of three experimental stocks 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. malerkotliana* 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 procedure was continued until their death and longevity was recorded. A total of 50 replicates was made and mean longevity was found to be 62±2days. In addition to this, mating activities of males were also studied from day 1st of their eclosion until 60th day. Results showed that showed least male courtship activities were found 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 aged 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 were 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 were aged for 2-3day (to obtain young males). This procedure helped us to culture all three male age classes young, middle aged and old and to conduct the experiment at the same time in same environment.

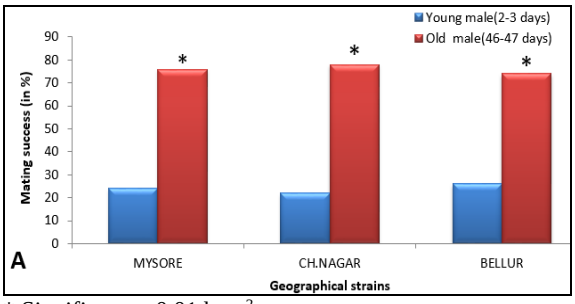
Female mate preference for male age

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. 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) [7] 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 and rejected males in female mate preference test using the procedure of Hegde and Krishna (1997) [8]. Fifty trails were run separately for each combination. The effect of paint was tested before commencing the experiment by painting one of the two young /middle aged /old males in 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).

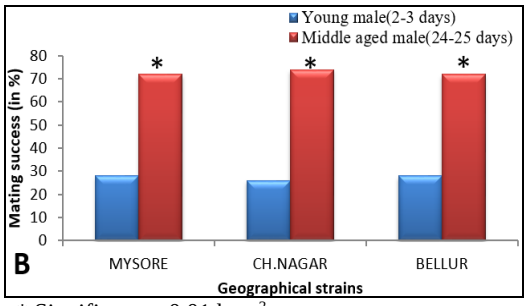
Results

The females in all the three geographical strains of *D. malerkotliana* discriminate between males on the basis of age and females prefers to mate with the middle aged males more frequently over young and old male (Figure 1a-c). Crosses involving a female and two males (young and middle aged male, young and old male, middle aged and old male). Further, the difference in mean wing length of selected and rejected males in female mate choice experiment showed insignificant variation (Table 1).

It was found that in all the three geographical strains middle aged male had copulated longer time with female compared to young and old males. Among three geographical strains studied flies of Chamarajanagar strain had copulated longest while flies of Bellur strain copulated for shortest time. It was noticed that fecundity (total number of eggs laid) was higher in females mated with a middle aged male when compared to female mated with either young or old males. Flies of Mysore strain had produced a greater number of progeny than the flies of Bellur and Chamarajanagar strains and also in all the three geographical strains females mated with middle aged males had produced more number progeny than females mated to young and old ages males respectively. Flies of Mysore and Chamarajanagar strains had produced a greater number of progeny than the flies of Bellur strain.



* Significant at 0.01 by x^2 test



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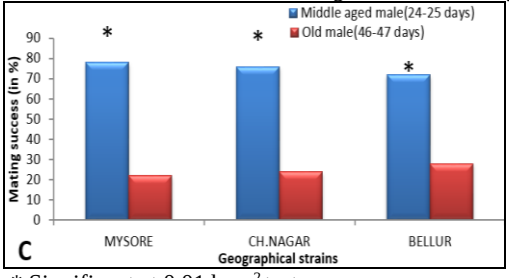

* Significant at 0.01 by x^2 test

Fig 1: a-c. Female mate preference for male age classes of *D. malerkotliana* (N=50; df-1).

Table 1: Wing length (mm) of selected and rejected males (in female choice experiment) of *D. malerkotliana*

Geographical strains	Crosses		Selected (Wing length) (mm)	Rejected (Wing length) (mm)	Paired t value
	Female	Male			
Mysore	5-6 days	Young and Old	1.644±0.0102	1.646±0.010	0.289 NS
		Middle and Young	1.646±0.0102	1.653±0.010	1.285 NS
		Old and Middle	1.653±0.0101	1.635±0.010	1.127 NS
Chamarajanagar	5-6 days	Young and Old	1.661±0.0098	1.668±0.010	0.602 NS
		Middle and Young	1.668±0.0101	1.635±0.009	0.026 NS
		Old and Middle	1.635±0.0091	1.635±0.010	1.032 NS
Bellur	5-6 days	Young and Old	1.653±0.0101	1.671±0.009	2.292 NS
		Middle and Young	1.671±0.0095	1.634±0.009	0.204 NS
		Old and Middle	1.634±0.0091	1.635±0.010	1.127 NS

NS-Non significant.

Note: male age-young male (2-3 days); middle aged male (24-25days); old male (46-47days)

Discussion

Most compelling studies on sexual selection suggest that mate choice can be the most important decision in an individual's life, at least in sexually reproducing animals. Studies of age based female mate preference 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) [3, 2, 9]. *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 our study in *D. malerkotliana* females prefer to mate with middle aged males more frequently over young and old males (Figure 1) suggesting that females of this species discriminate their mates on the basis of their age. This result confirms earlier results of age based female mate preference in other species of *Drosophila* (Somashekar and Krishna, 2011; Prathibha and Krishna, 2010; Aholhasan and Krishna, 2014) [17, 14, 1]. However, in contrast to the results in *D. malerkotliana* studies in *D. melanogaster* female prefers to mated more frequently with younger of the two competing males (Aholhasan and Krishna, 2014) [1]. These studies in species of *Drosophila* show the existence of species specific difference in the age based female mate preference. In *D. malerkotliana* female mate preference for middle aged male suggesting that this species evolving towards middle aged males.

In the present study unmated males of *D. malerkotliana* which were reared in groups of 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 of different male age classes or rearing conditions before exposure to mating. Further, in the present study rejected males had slightly longer wings than selecting 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 (Table.1).

Male size is another potential factor known to influence on male mating success in *Drosophila* and other insects (Partridge *et al.,* 1987) [12]. Both laboratory and field studies showed that greater mating success of larger males over smaller males (Partridge *et al.,* 1987; Santos *et al.,* 1988, 1992; Hegde and Krishna, 1997)[12, 16, 8]. Therefore, we measured wing length of selected and rejected males in female choice experiment to rule out the possibility of male size effect on the observed greater mating success of middle aged males in *D. malerkotliana*. Table 1 revealed insignificant variation in mean wing length between selected and rejected males, suggesting that the observed greater mating success of young males in female mate choice experiment was not the result of larger size instead it could be the effect of male age itself. Further, in the present study, flies were reared in low density (100 egg/quarter pint milk bottle) to reduce the effect of larval density on body size variation and mating activities. Furthermore, in *Drosophila*, the size of the fly was fixed at the time of eclosion as a result size may not increase with increase in male age (Santos *et al.,* 1992) [12]. This agrees with the work of Somashekar and Krishna (2011) [17] in *D. bipectinata*, Prathibha *et al.,* (2011) [15] in *D. ananassae* that female preference for older males in their study was not influenced by male size. Thus, these studies confirm that females of *Drosophila* species discriminate their mates on the basis of age.

The females used in our experiments were five to six days old virgins obtained from same culture bottle in which males of different age were also isolated and aged them. Therefore, observed greater mating success of middle aged males in female mate choice experiment was not due to the effect of female age instead it was due to male age related variation. Delayed male maturation is another potential behavioral trait required to attract females (Stearns, 1992) [19]. In insects, sexual maturity is correlated with physiological traits such as testis size, sperm length and accessory gland size (Pitnick, 1991) [13]. In our study young, middle aged and old, male age used in mating experiments showed all the courtship activities and females mated with these males laid eggs. Therefore, all the three ages used in the experiments were behaviorally mature and capable of mating and producing offspring. Further, all the three male age used were able to transfer sperm to the female. Furthermore, overall sizes of the accessory gland of young, middle, and old males were insignificantly different. Therefore, observed female preference for male age was not due to difference in the maturation of male age in *D. malerkotliana.*

Four hypotheses have been proposed to explain the female preference for middle aged males in *D. malerkotliana.* 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. Third female shows least rejection responses to preferred male age classes (middle aged males). Fourth female may obtain direct fitness benefits by mating with preferred male age (middle aged males).

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