



## Male age influence on courtship behaviors in three geographical strains of *Drosophila malerkotliana*

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### Abstract

In 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 it is noticed that in all the three geographical strains of *D. malerkotliana* female mated with middle aged males showed greater courtship acts than those female mated with young male and old male. The female generally showed lesser responses (decamping, extruding and ignoring) to middle aged males than to young and old males and here there was significant variation in female decamping, ignoring and extruding behavior toward males of different ages.

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

### Introduction

In sexually reproducing organisms, each of the offspring has one father and one mother, so the average reproductive success is equal for both males and females. However, if a male gains a disproportionate share of reproduction, he will take away reproductive opportunities from other males, leading to a high reproductive variance among males. On the contrary female will not take away reproductive opportunities from other females, leading to a smaller variance in reproductive success. The higher the reproductive variance, the stronger the effects of sexual selection.

However, theoretical analyses of sexual selection suggest at least three ways sexual selection might come about (1) Female choice: Female mate choice is the subject of a large area of research in behavioural ecology. Over the past three decades, many theoretical and empirical studies have investigated the patterns and consequences of female mating decisions (Andersson, 1994; Shuster and Wade, 2003; Barbosa and Magurran, 2006) [2, 19, 31].

Females use wide varieties of male traits such as larger morphological traits, bigger and brighter color patterns, more vigorous visual displays, and faster, longer, and louder calls to select their mates (Andersson, 1994; Ryan and Keddy-Hector, 1992) [2, 18]. Female preferred males with such preferred traits to obtain can provide material resources that increase her or offspring fitness (direct benefits; Heywood, 1989; Price *et al.*, 1993) [10, 16], alleles that increase offspring viability (good genes; Fisher, 1930; Grafen, 1990; Pomiankowski, 1988; Zahavi, 1975) [7, 8, 15, 21], or alleles that affect the attractiveness of male offspring (sexy sons; Fisher, 1930; Kirkpatrick, 1982) [7, 12].

### 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  $21 \pm 1^\circ\text{C}$  at a relative humidity of 70% using a 12: 12 h light: dark cycle. This procedure was continued for 2<sup>nd</sup> generations to allow them to acclimatize to the laboratory conditions. At the 3<sup>rd</sup> generation, synchronized eggs ( $\pm 30$  min) were collected separately from each of three experimental stocks using Delcours's procedure (1969) [5]. 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  $62 \pm 2$  days. In addition to this, mating activities of males were also studied from day 1<sup>st</sup> of their eclosion until 60<sup>th</sup> day. Results showed that showed least male courtship activities were found at 1<sup>st</sup> day, whereas from 2<sup>nd</sup> day and onwards (up to 46-47 days) male showed all the courtship activities and all of them mated with the female. At 47<sup>th</sup> 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 20<sup>th</sup> 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 20<sup>th</sup> day and the first set of flies reached 47<sup>th</sup> 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.

### Male age influence on courtship activities

One male (5–6 days old) and one female (young/middle-aged/old-aged) were individually aspirated into a mating chamber (Elens-Wattiaux 1964) <sup>[6]</sup> and observations were made for one hour. Female mating latency (time between introduction and initiation of copulation) and female copulation duration (time between initiation of copulation and termination of copulation) were recorded for each pair. We also quantified courtship acts such as tapping, scissoring, vibration, licking, circling, ignoring, extruding, and decamping, following the procedure of Hegde and Krishna (1997) <sup>[9]</sup>. These courtship acts are described as follows:

#### Tapping

The male initiates courtship with a foreleg motion partially extending and simultaneously elevating one or both forelegs, followed by a downward striking motion, thus bringing the ventral surface of the tarsus in contact with the partner.

#### Scissoring

The courting male opens and closes both wings with a scissor like movement during the interval between wing vibrations.

#### Vibration

The male expands one wing laterally from the resting position, and then moves one or both wings rapidly up and down.

#### Licking

The courting male positions himself closely behind the female, extends his proboscis, and licks her genitalia.

#### Circling

After posturing at the side or rear of a non-receptive female, the male faces the female as he moves about. The male may move to face her and then retraces his path to the rear, or may at other times move completely around her in a circle.

#### Ignoring

The non-receptive female simply continues with whatever activity in which she was previously engaged, apparently ignoring actions by the male.

#### Extruding

The non-receptive female presses the vaginal plates together, contracting certain abdominal muscles and apparently relaxing others.

#### Decamping

The non-receptive female attempts to escape by running, jumping, or flying away from the courting male.

Two different observers recorded the behavior of the male and female simultaneously for one hour. The number of pairs mated was also recorded.

### Results

#### Influence of male age on male courtship activities in three geographical strains of *D. malerkotliana*

In all the three geographical strains of *D. malerkotliana*

female mated with middle aged males showed greater courtship acts than those female mated with young male (Figure 1a-e). Two-way ANOVA followed by Tukey's post hoc test showed males of Bellur strain showed least courtship acts and difference were found to be insignificant in the interaction between geographical strains and male age (Table 1).

#### Influence of male age on female rejection responses in three geographical strains of *D. malerkotliana*

The female generally showed lesser responses (decamping, extruding and ignoring) to middle aged males than to young and old males (Figure 2a-c). Similar results were measured for all the three geographical strains. In addition, there was significant variation in female decamping, ignoring and extruding behavior toward males of different ages. Females showed significantly less rejection responses to middle aged males (Table 2). Among three geographical strains females of Mysore strain showed least rejection responses to males while highest female rejection was found in males of Chamarajanagar strain.

### Conclusion

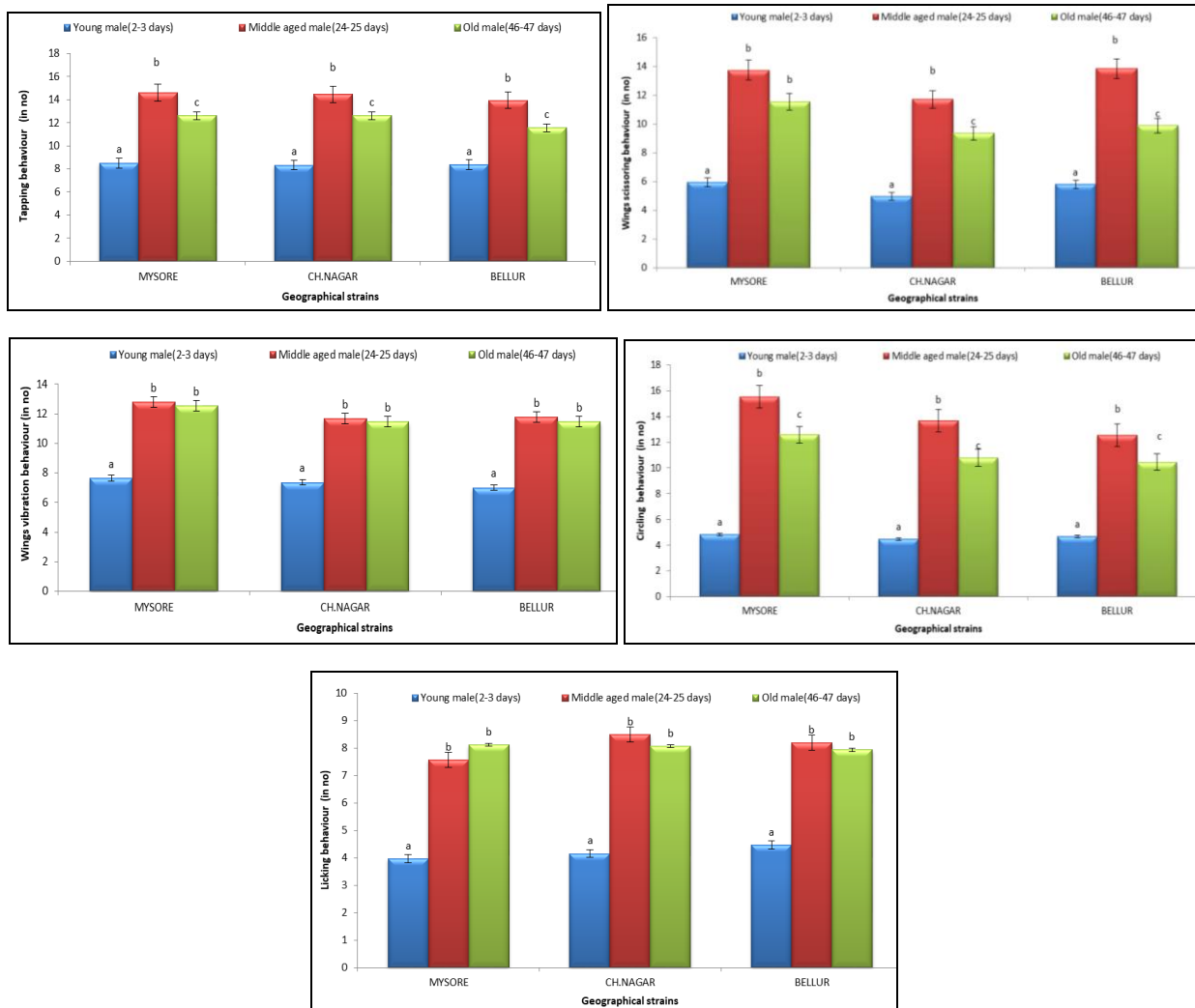
In species *Drosophila* mating is resource independent, mating success of courting pair depends on the activity levels of male and female during mating. Therefore the display given by males before mating is the only grounds on which females can differentiate between potential mates because courtship 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 Limatainon, 1992; Hedge and Krishna, 1997) <sup>[11, 9]</sup>. Greater the activities of males greater 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 (Figure 1a-e). This supports the 2<sup>nd</sup> hypothesis proposed above that middle aged males were more vigorous during mating thereby female preferred to mate with middle aged males. 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. While in *D. melanogaster* (Abolhasan *et al.*, 2015) <sup>[1]</sup> young males had shown significantly greater courtship activities than those middle or old aged males. Thus, these studies suggest that in species of *Drosophila* courtship activities of males are important in achieving successful mating by male. Males which perform greater courtship acts convince the females faster for mating (Figures 1a-e) and Table 1) 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 (Figure 2 and Table 2).

Female preference with respect to male age classes also differs among species, some species showing preferences for older males because the reliability of male signalling

increases with age and because they have proven survival ability (Kokko and Lindstrom, 1996; Proulx *et al.*, 2002) [13, 17], including fungus beetle, *Bolitotherus cornutus* (Coleoptera: Tenebrionidae) (Conner, 1989) [4], three species of crickets (*Orthoptera: Gryllidae*) (Zuk, 1988; Simmons and Zuk, 1992) [22, 20]. These experimental results of age based mate preference suggest that insect species have developed different mate preference strategies to enhance their reproductive success.

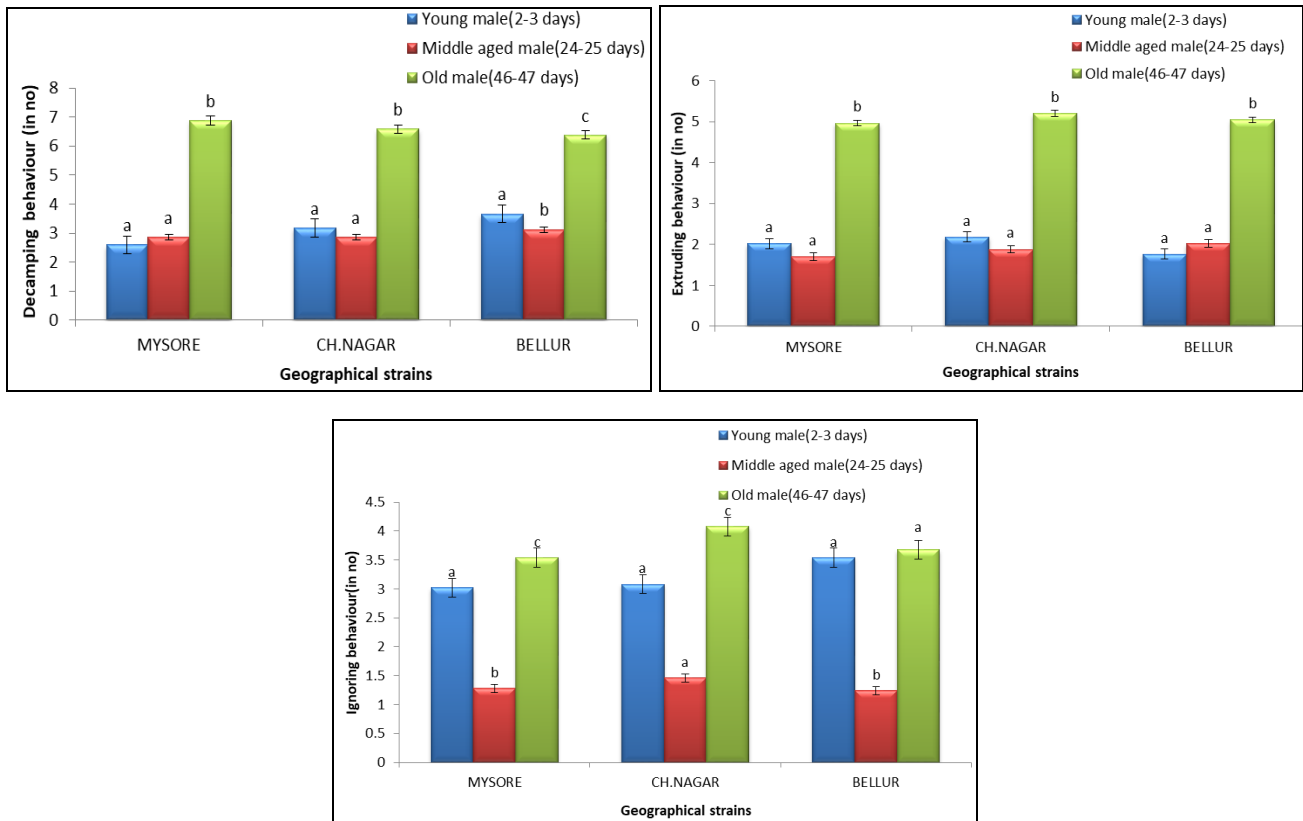
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Different letters on the bar graphs indicate significant difference by Tukey's post hoc test at 0.05 level

Fig 1: a-e. Male age influence on male courtship behaviors of *D. malerkotliana*



Different letters on the bar graphs indicate significant difference by Tukey’s post hoc test at 0.05 level

Fig 2: a-c. Male age influence on female rejection responses of *D. malerkotliana*

Table 1: Two-way ANOVA of male age influence on male courtship activities of *D. malerkotliana*.

Parameters	Dependent variable	Type III Sum of Squares	df	Mean Square	F value
Tapping	Age	2705.920	2	1352.960	20.943***
Scissoring		4336.538	2	2168.269	42.335***
Vibrating		2126.951	2	1063.476	19.917***
Circling		6656.591	2	3328.296	84.011***
Licking		1492.698	2	746.349	25.372***
Tapping	Strains	32.213	2	16.107	0.249 <sup>NS</sup>
Scissoring		235.871	2	117.936	2.303 <sup>NS</sup>
Vibrating		75.098	2	37.549	0.703 <sup>NS</sup>
Circling		124.004	2	62.002	1.565 <sup>NS</sup>
Licking		11.231	2	5.616	0.191 <sup>NS</sup>
Tapping	Age×Strains	16.827	4	4.207	0.065 <sup>NS</sup>
Scissoring		70.289	4	17.572	0.343 <sup>NS</sup>
Vibrating		11.062	4	2.766	0.052 <sup>NS</sup>
Circling		94.369	4	23.592	0.596 <sup>NS</sup>
Licking		18.089	4	4.522	0.154 <sup>NS</sup>
Tapping	Error	28489.040	441	64.601	
Scissoring		22586.700	441	51.217	
Vibrating		23547.280	441	53.395	
Circling		17471.160	441	39.617	
Licking		12972.640	441	29.416	
Tapping	Total	92494.000	450		
Scissoring		69067.000	450		
Vibrating		74474.000	450		
Circling		64712.000	450		
Licking		35194.000	450		

\*\*\*Significant at 0.001; NS, Non-significant.

Table 2: Two-way ANOVA of male age influence on female rejection responses of *D. malerkotliana*.

Parameters	Dependent Variable	Type III Sum of Squares	df	Mean Square	F value
Decamping	Age	1275.111	2	637.556	56.107 **
Extruding		987.040	2	493.520	47.812 **
Ignoring		490.964	2	245.482	37.104 **

Decamping	Strains	5.791	2	2.896	0.255 <sup>NS</sup>
Extruding		3.053	2	1.527	0.148 <sup>NS</sup>
Ignoring		5.658	2	2.829	0.428 <sup>NS</sup>
Decamping	Age × Strains	30.969	4	7.742	0.681 <sup>NS</sup>
Extruding		5.507	4	1.377	0.133 <sup>NS</sup>
Ignoring		11.662	4	2.916	0.441 <sup>NS</sup>
Decamping	Error	5011.160	441	11.363	
Extruding		4552.080	441	10.322	
Ignoring		2917.680	441	6.616	
Decamping	Total	14396.000	450		
Extruding		9526.000	450		
Ignoring		6876.000	450		

\*\*Significant at 0.01; NS, Non-significant

**Table 3:** Two-way ANOVA of male age influence on Courtship behaviours in *D. malerkotliana*.

STRAIN	MYSORE			CH.NAGAR			BELLUR		
	Male age classes Mean± SE			Male age classes Mean± SE			Male age classes Mean± SE		
<i>Male Courtship acts</i>	YOUNG	MIDDLE	OLD	YOUNG	MIDDLE	OLD	YOUNG	MIDDLE	OLD
Tapping	8.52±0.677	14.6±1.303	12.6±1.544	8.34±0.530	14.46±1.270	12.6±1.544	8.38±0.506	13.94±1.210	11.56±1.039
Scissoring	5.94±0.573	13.74±1.426	11.5±1.2216	4.96±0.316	11.7±1.094	9.36±0.809	5±0.559.8	13.84±1.438	9.88±1.014
Wings vibration	7.64±0.696	12.78±1.104	12.52±1.606	7.36±0.459	11.66±0.859	11.46±1.296	7±0.506	11.76±0.872	11.46±1.296
Circling	4.82±0.792	15.52±1.525	12.58±1.622	4.46±0.479	13.68±1.119	10.8±1.273	4.68±0.516	12.52±0.899	10.46±1.119
Licking	3.98±0.331	7.58±0.901	8.12±1.035	4.16±0.348	8.5±0.919	8.08±0.972	4.48±0.343	8.2±0.8	7.94±0.793
<i>Female rejection responses</i>	YOUNG	MIDDLE	OLD	YOUNG	MIDDLE	OLD	YOUNG	MIDDLE	OLD
Decamping	2.6±0.342	2.86±0.344	6.88±0.790	3.18±0.279	2.86±0.242	6.58±0.681	3.66±0.34	3.12±0.229	6.38±0.644
Extruding	2.02±0.257	1.7±0.223	4.96±0.735	2.18±0.264	1.88±0.218	5.2±0.678	1.76±0.192	2.02±0.224	5.04±0.731
Ignoring	3.02±0.484	1.28±0.185	3.54±0.458	3.08±0.392	1.46±0.076	4.08±0.357	3.54±0.529	1.24±0.061	3.68±0.374

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