

Evidence of Female Preference for Older Males in *Drosophila bipectinata*

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Krishnashetty Somashekar and Mysore Siddaiah Krishna (2011) Evidence of female preference for older males in *Drosophila bipectinata*. *Zoological Studies* 50(1): 1-15. Male age influence on mating success was studied using 3 geographic strains of *Drosophila bipectinata*. When investigating 3 age classes of males, it was noted that females preferred to mate with the older of 2 males more frequently than with the younger one. A non-significant difference was also noted in the mean wing length between the selected and rejected males by females in a female-choice experiment. Old males mated faster, copulated longer, and inseminated more females in a given unit of time than did young or intermediate-aged males. Furthermore, females mated with older males had shorter lifespan compared to females mated with young and intermediate-aged males. These studies suggest that irrespective of geographic strain, *D. bipectinata* females prefer to mate with older males, and the older of the 2 competing males had a greater mating advantage in terms of inter-male success, mated faster, and inseminating more females in a given unit of time compared to young and intermediate-aged males. Females mating with older males lived a shorter time than females mating with young and intermediate-aged males. <http://zoolstud.sinica.edu.tw/Journals/50.1/1.pdf>

Key words: *Drosophila bipectinata*, Female preference, Wing length, Male age.

A great diversity of male traits is known to influence female mating decisions. One male characteristic that has received a lot of attention as a possible cue for female mate preference is age (Zuk 1988, Hansen and Price 1995, Kokko 1998, Brooks and Kemp 2001, Beck et al. 2002). The success of males in achieving mating is often linked to the reproductive benefits which females derive (Jennions and Petrie 1997, Bussière et al. 2005). Males typically vary in their ability to provide benefits, and determining how females detect what males have to offer has revealed much about the processes that drive the evolution of mate choice. Females often use phenotypic cues that serve as indicators of mate choice benefits (Bussière et al. 2005, Andersson and Simmons 2006, Kokko et al. 2006, Jacot et al. 2007).

Different models were proposed for female preference for males of different age classes.

Both theoretical and empirical evidence of female preference for male age has demonstrated preferences for old, young, and even middle-aged males in a variety of species (Jones et al. 2000, Brooks and Kemp 2001), and much research has been devoted to understanding the benefits females derive from mating with males of a particular age. Trivers (1972) and Halliday (1978) offered the 1st verbal arguments for age as an important factor affecting female mate choice. They stated that all else being equal, females should prefer older males since they have demonstrated their capacity for survival. A few years later, Manning (1985) developed a theoretical argument in support of their idea. 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 life. Thus, older

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males are genetically superior, and females who mate with such males would be benefited by passing on those genes to their offspring.

Numerous examples were reported 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 (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). However, in 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, Vandenberghe et al. 1993), for species in which males do not provide care to offspring of females (Jarvi et al. 1982, Weatuerhead 1984), and in cases of extra-pair copulation (Moller 1992).

The 1st theoretical challenge to the idea that younger males make better mates was put forth by Hansen and Price (1995). Those authors included a life history perspective in their model to show that older males do not have an average higher genetic quality, and they are not genetically superior to younger males. They provided 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 germline 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 subsequent years, a theoretical study by Hansen and Price (1995) incorporated additional life history variables into models of 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 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). However, these models also 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). Differences in conclusions of those models 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.

Drosophila has played an integral role in the development of sexual selection theory, and a great deal is known about the patterns and fitness consequences of female mate choice (Spieth 1952, Partridge 1980, Fowler and Partridge 1989, Chapman et al. 1993, Gromko and Markow 1993, Hegde and Krishna 1997, Krishna and Hegde 1997 2003). Moreover, recent behavioral research revealed that male *Drosophila* varies greatly in its level of interest in females, providing evidence that males have also evolved to selectively mate (Gowaty et al. 2003). Furthermore, the reproductive biology of *Drosophila* is useful for investigating whether female mate choice is influenced by male quality and the cost of choosing.

Drosophila bipectinata, a wild species, commonly occurs on the Indian subcontinent and has attracted the attention of various Indian workers during the past few years, who have carried out extensive studies on population and behavioral genetics of this species (Hegde and Krishnamurthy 1979, reviewed in Benerjee and Singh 1997). Close phylogenetic relationships among *D. bipectinata* complexes were documented based on the results of chromosome analyses, hybridization studies, and isozyme analyses. Indian populations of *D. bipectinata* do not show high levels of genetic differentiation for inversion polymorphisms (Krishna and Hegde 1997 2003, Mishra and Singh 2005, reviewed in Singh 2008 and references therein). However, male-age influence on female preference has not been studied. Therefore, the present study was carried out using 3 different geographic strains of *D. bipectinata* to test the hypothesis of whether or not females of *D. bipectinata* prefer to mate with older males and whether they obtain direct benefits of doing so.

MATERIALS AND METHODS

Isofemale lines of *D. bipectinata* used in the present study were established from wild-caught females collected at Dharwad, Bellur, and Mysore, India in Aug. 2007. These stocks were cultured and maintained using 40 flies (20 males and 20 females) per quarter-pint milk bottles (250 ml) containing wheat cream agar medium at $21 \pm 1^\circ\text{C}$ and a relative humidity of 70% in a 12: 12-h light: dark cycle for 3 generations to acclimatize the flies to laboratory conditions. After 3 generation of laboratory culture, eggs were separately collected from each of the above isofemale stocks using Delcour's (1969) procedure. Eggs (100) were seeded in a vial containing wheat cream agar medium. When adults emerged, virgin females and males were isolated within 3 h of eclosion and were aspirated into a new vial containing wheat cream agar medium. These flies were aged as required for the experiment. Flies collected first were assigned to 32-33 d-old males, flies collected next were assigned to 17-18 d-old males. Following this, flies collected were assigned to 2-3 d-old males. These males of different age classes and virgin 5-6 d-old females were cultured and maintained using the above-described laboratory conditions and were transferred once a week until they were used in the experiment.

Female mate choice experiment

Young (2-3 d-old), intermediate-aged (17-18 d-old), and old (32-33 d-old) males and 5-6 d-old females were used to study female preference for males of different age classes in a female-choice experiment. A 5-6 d-old female and 2 males of different age classes (young and intermediate-aged, intermediate-aged and old, or young and old) were separately and individually aspirated into an Elens-Wattiaux mating chamber. Indian ink was painted onto one of the males on the thoracic region. The effect of the paint was tested before commencing the experiment by painting a young male in 1 trial and an intermediate-aged/old male in an alternate trial and allowing them to mate. This pair was observed for 1 h. This procedure was continued for 50 trials separately for each of the 3 geographic isofemale lines. In 25 of 50 trials, a young male was painted and in the remaining 25 of 50 trials, the old male was painted. In the Mysore strain, in 24 of 50 trials, painted males mated, and in the remaining 26 of 50 trials, unpainted males mated ($\chi^2 = 0.08$;

$d.f. = 1$; $p > 0.05$). Similarly, in the Dharwad strain, in 23 of 50 trials, painted males mated. In the remaining 27 of 50 trials, unpainted males mated ($\chi^2 = 0.08$; $d.f. = 1$; $p > 0.05$). In the Bellur strain, in 25 of 50 trials, painted males mated, and in the remaining 25 of 50 trials, unpainted males mated ($\chi^2 = 0.00$; $d.f. = 1$; $p > 0.05$). Thus, these results indicated that painting of one of the competing males in the female-choice experiment did not have an effect on the performance of the flies. When mating occurred, the copulating pair was aspirated from the mating chamber. The pair was observed for 1 h, and mated flies were recorded and transferred to a new vial. The male rejected by the female was also transferred to a new vial. The wing lengths of both selected and rejected males were later measured. In total, 50 trials were separately conducted for each of the 3 geographic strains. Chi-square and logistic regression analyses were applied to the data of the female mate choice experiment. Wing lengths of 50 selected and rejected males in each of the female-choice experiments were individually measured following the procedures of Hegde and Krishna (1997), and a paired *t*-test was applied to the data.

Male age, mating activities, and female longevity

A 5-6 d-old virgin female and an unmated young, intermediate-aged, or old male were used to study the influence of males of different age classes on mating, courtship activities, and female longevity. A female and a male (young, intermediate-aged, or old) were aspirated into an Elens-Wattiaux mating chamber (Elens-Wattiaux 1964) and observed for 1 h. If the pair did not mate within 1 h, it was discarded. When mating occurred, the mated pair was allowed to complete copulation. Mating latency (the time between the introduction of the male and female together into a mating chamber and the initiation of copulation) and copulation duration (the time between initiation of copulation and termination of copulation) were recorded. Soon after copulation, the mated female from each pair was transferred to a new vial containing wheat cream agar medium and was maintained in the above laboratory conditions to study the longevity of females mated with males of different age classes. Each mated female was individually transferred once every 4 d to a new vial containing wheat cream agar medium, and this procedure was continued until her death. The number of days lived by each female, calculated from its eclosion from a pupa until death, was

recorded as female longevity. In total, 50 trials were separately run for each combination of a female mated with young, intermediate-aged, and old males. These were carried out separately for each of the 3 isofemale lines of *D. bipectinata*. Two-way analysis of variance (ANOVA) followed by Duncan's multiple-range test (DMRT) was carried out on the mean mating latency, copulation duration data, and female longevity.

Male age and male mating ability

A 5-6 d-old virgin female and an unmated young, intermediate-aged, or old male were used to study the influence of males of different age classes on male mating ability (number of females inseminated by each male). A female and male (young, intermediate-aged, or old) were aspirated into an Elens-Wattiaux mating chamber (Elens-Wattiaux 1964) and observed for 1 h. If the pair did not mate within 1 h, it was discarded. When mating occurred, a mated pair was allowed to complete copulation. Soon after copulation, the mated female was individually aspirated into a new vial containing wheat cream agar medium to check that insemination has occurred (by observing larval activity). The mated male was allowed to mate with a 2nd virgin female (5-6 d old). If mating occurred with the 2nd female, they were allowed to complete copulation, and insemination of the female was checked as described above. This process was continued, and the number of females inseminated by each male in 1 h was recorded as the male mating ability. In total, 50 young, intermediate, and old mated males were separately tested. This was separately carried out for each of the 3 geographic isofemale lines. Two-way ANOVA followed by DMRT was carried out on data of male mating ability. We used SPSS 10.1 program (SPSS, USA) for all analyses.

RESULTS

Females generally chose to mate with the older of the 2 competing males (Table 1). Old males were successful in crosses involving young and old males ($n = 50$) in 62% of cases with Mysore females, 68% of Dharwad females, and 70% of Bellur females; old males were successful in crosses involving intermediate-aged and old males ($n = 50$) in 64% of cases with Mysore females, 60% of Dharwad females, and 56% of Bellur females; intermediate-aged males were successful in crosses involving young and intermediate-aged males ($n = 50$) in 60% of cases with Mysore females, 72% of Dharwad females, and 68% of Bellur females.

A logistics regression analysis was also carried out on the mating-success data, and results are given in table 2. For all strains, it was noted that male age had a significant influence on mating success with females.

Mean male wing lengths of selected and rejected males in the female-choice experiment are given in table 3. In crosses of a female with young and old males, it was noted that mean wing length of selected and rejected males did not significantly vary in the Dharwad and Mysore strains, but they showed a significant variation in the Bellur strain. Similar results were also found in crosses of a female with young and intermediate-aged males. In contrast to this, in crosses of a female with intermediate-aged and old males, a non-significant variation in mean wing lengths of selected and rejected males was seen in the Bellur strain of *D. bipectinata*. It was also further noted that in the Dharwad and Mysore strains, rejected males had significantly longer wing lengths than selected males.

Figure 1 shows the mean mating latency of males (time taken for copulation initiation) of

Table 1. Mating success of *Drosophila bipectinata* males from 3 age classes and 3 different geographical strains

Female (5-6 d old)	Males				Males				Males			
	Young	Old	χ^2 value	p value	Old	Inter mediate	χ^2 value	p value	Inter mediate	Young	χ^2 value	p value
Mysore	19	31 (62%)	2.66	< 0.10	32 (64%)	18	3.92	< 0.05	33 (66%)	19	5.12	< 0.05
Dharwad	16	34 (68%)	6.48	< 0.01	30 (60%)	20	2.00	< 0.10	36 (72%)	14	9.68	< 0.001
Bellur	15	35 (70%)	8.00	< 0.001	28 (56%)	22	0.75	< 0.90	34 (78%)	16	6.48	< 0.01

Young (2-3 d old), Intermediate (17-18 d old), and Old (32-33 d old).

different age classes in the 3 different geographic strains of *D. bipectinata*. The highest mean mating latency was found in the Dharwad strain, while the shortest mean mating latency was in the Bellur strain. Similarly, in all strains, young males showed the greatest mean mating latency, while old males had the shortest mean mating latency. As speed is the reverse of time, the Bellur strain was a fast mater, while the Dharwad strain was a slow mater. Similarly, old males were fast maters, while young males were slow maters. It was also noted that mating latency decreased with an increase in male age. Two-way ANOVA followed by DMRT applied to the mean mating latency data (Table 4) showed significant differences in mean mating latencies among the 3 geographic strains, among males of different age classes, and also for interactions among males of different age classes and strains. DMRT showed that among the 3 strains, the Mysore strain had the highest mean mating latency compared to the other 2 strains. The mean mating latency of old males was the shortest compared to young and intermediate-aged males. The mean mating latency of intermediate-aged males was significantly lower than that of young males.

The mean copulation durations of males of different age classes in the 3 geographic strains of *D. bipectinata* are provided in figure 2. The Dharwad strain copulated longer than the Mysore and Bellur strains. In all strains, old males copulated longer than did young and intermediate-aged males. Among the 3 strains, the shortest copulation duration was found in young males.

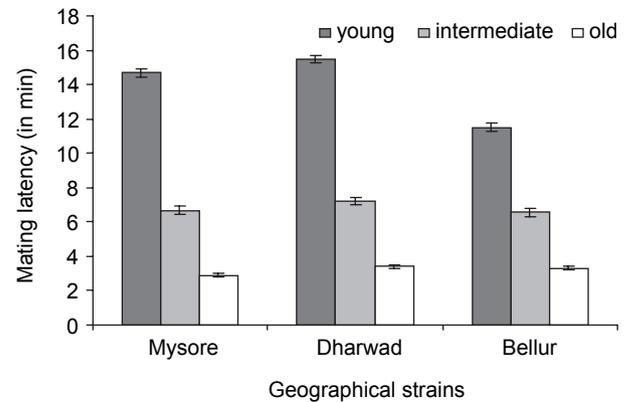


Fig. 1. Male age influence on mating latency in 3 geographic strains of *Drosophila bipectinata*.

Table 2. Logistic regression analysis of mating success of *Drosophila bipectinata* males from 3 age classes and 3 different geographical strains

Effect	-2 log likelihood reduced model	Chi-squared	d.f.	Significance
Male age (Y vs. O)	18.590	0.774	2	0.679
Male age (Y vs. I)	18.935	1.149	2	0.563
Male age (I vs. O)	18.638	0.668	2	0.716
Strain	13.528	0.774	2	0.679

Y, young (2-3 d old); I, Intermediate-aged (17-18 d old); O, Old (32-33 d old).

Table 3. Mean wing length (mm) of selected and rejected males of *Drosophila bipectinata* from 3 different strains in the female-choice experiment

Crosses	Female (5-6 d old)	Male age (d)	n	d.f.	Male wing length (mm)		t-value	p value
					Selected	Rejected		
Bellur strain		Young, old	50	49	1.62 ± 0.61	1.66 ± 0.08	6.1800	< 0.001
		Young, intermediate	50	49	1.58 ± 0.42	1.63 ± 0.21	2.612	< 0.05
		Intermediate, old	50	49	1.57 ± 0.71	1.56 ± 0.35	0.098	non-significant
Dharwad strain		Young, old	50	49	1.60 ± 0.31	1.59 ± 0.42	1.922	< 0.10
		Young, intermediate	50	49	1.59 ± 0.02	1.63 ± 0.33	1.922	< 0.10
		Intermediate, old	50	49	1.63 ± 0.15	1.59 ± 0.61	3.190	< 0.001
Mysore strain		Young, old	50	49	1.52 ± 0.26	1.57 ± 0.71	0.096	non-significant
		Young, intermediate	50	49	1.54 ± 0.32	1.55 ± 0.08	0.438	non-significant
		Intermediate, old	50	49	1.59 ± 0.41	1.64 ± 0.31	3.020	< 0.001

Young (2-3 d old), Intermediate (17-18 d old), Old (32-33 d old).

The mean copulation duration increased with an increase in male age except in the Mysore strain. Two-way ANOVA applied to the mean copulation duration data showed significant differences among male age classes and for interactions among the different strains and male age classes (Table 5). However, insignificant differences were noted in mean copulation durations among the geographic strains. DMRT carried out on mean copulation durations of males of different age classes showed that the mean copulation duration of old males was significantly greater than those of young and intermediate-aged males. Similarly, the mean copulation duration of intermediate-aged males was significantly greater than that of young males.

Figure 3 shows the mean longevity of females mated with males of different age classes in the 3 geographic strains of *D. bipectinata*. Females of the Bellur strain had the greatest longevity, while females of the Mysore strain had shortest longevity. In all 3 strains, females that had mated with old males lived a shorter time than females that had mated with intermediate-aged and young males. The longevity of females that had mated with males of the different age classes decreased with an increasing male age. Two-way ANOVA

followed by DMRT showed significant differences in the mean longevity of females that had mated with males of different age classes, among the 3 geographic strains, and for interactions among males of different age classes and geographic strains (Table 6). DMRT showed that in all the 3 strains, females that had mated with old males had significantly lower female longevity than those that had mated with intermediate-aged and young

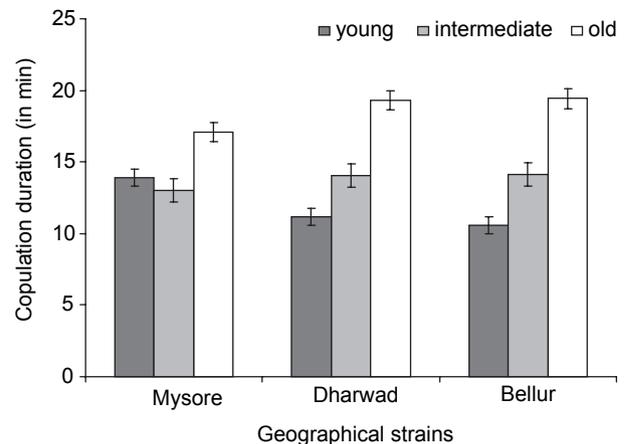


Fig. 2. Male age influence on copulation duration in 3 geographic strains of *Drosophila bipectinata*.

Table 4. Two-way ANOVA of male age influence on mating latency in 3 different geographic strains of *Drosophila bipectinata*

Source	Sum of squares	d.f.	Mean square	F-value	p value
Population	188.893	2	94.447	19.885	< 0.0001
Age	8864.653	2	4432.327	933.170	< 0.0001
Population x age	275.493	4	68.873	14.500	< 0.0001
Error	2094.640	441	4.750		
Total	40,032.000	450			
Corrected total	11,423.680	449			

Table 5. Two-way ANOVA of male age influence on copulation duration in 3 different geographic strains of *Drosophila bipectinata*

Source	Sum of squares	d.f.	Mean square	F-value	p value
Population	2.698	2	1.349	0.296	non-significant
Age	3616.298	2	1808.149	397.188	< 0.0001
Population x age	524.462	4	131.116	28.802	< 0.0001
Error	2007.600	441	4.552		
Total	103,892.000	450			
Corrected total	6151.058	449			

males. Similarly, the mean longevity of females that had mated with intermediate-aged males was significantly greater than that of females that had mated with young males. DMRT also showed that among the 3 strains, the Mysore strain had significantly lower longevity than the Dharwad and Bellur strains.

The mean male mating abilities of young and old males in the 3 geographic strains of *D. bipectinata* are given in figure 4. The Bellur strain inseminated more females, while the Mysore strain inseminated the fewest females in a given unit of time. In all 3 geographic strains, old males inseminated more females compared to young and intermediate-aged males. The mean male mating ability data subjected to two-way ANOVA followed by DMRT showed significant differences in the mean male mating ability among the 3 geographic strains, among males of the 3 age classes, and also for interactions among the geographic strains and males of different age classes (Table 7). DMRT showed that males of the Mysore strain

inseminated significantly fewer females compared to males of the Dharwad and Bellur strains.

DISCUSSION

In studies of insects species with no parental care, it was proposed that females may choose to mate with older males to obtain good genes because older males have survived for a longer time and thus proven their genetic ability (Trivers 1972, Markow and Akney 1984, Andersson 1994, Radwan 2003, Prokop et al. 2007). *Drosophila* is 1 such insect; in *Drosophila*, mating is resource independent in that males do not contribute resources to their mate or protect their mate or offspring. In the present study, 3 geographic strains of *D. bipectinata* established from isofemale strains were used to study female preference for males of different age classes. The present study provides evidence that in all 3 geographic strains, female *D. bipectinata* discriminated between males

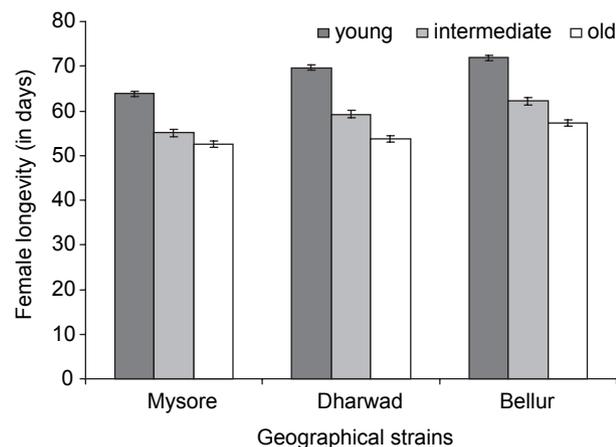


Fig. 3. Male age influence on longevities of females in 3 geographical strains of *Drosophila bipectinata*.

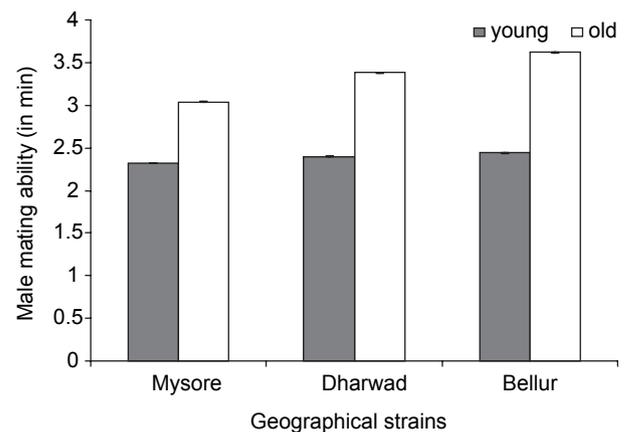


Fig. 4. Influence of male age on male mating ability in 3 geographical strains of *Drosophila bipectinata*.

Table 6. Two-way ANOVA of male age influence on longevity of female in 3 different geographical strains of *Drosophila bipectinata*

Source	Sum of squares	d.f.	Mean square	F-value	p value
Population	3262	2	1631.200	52.263	< 0.0001
Age	15,398.893	2	7699.447	246.684	< 0.0001
Population x age	308.267	4	77.067	2.469	< 0.05
Error	13,764.400	441	31.212		
Total	1,685,296.000	450			
Corrected total	32,734.000	449			

on the basis of age and preferred to mate with older males more frequently than with young and intermediate-aged males (Tables 1, 2). Herein, females mated more frequently with old males than young males in the presence of male-male competition and accepted old males more rapidly than young and intermediate-aged males in the female-choice experiment. Although the 2-male experiment (female-choice experiment) design allowed male-male competition to occur, a similar result was also noted in a no-choice experiment (single-male tests; unpubl. data), which suggests that male-male competition was not an important factor influencing female choice. Results of both the female-choice and no-choice experiments provide strong support of a female preference for older males. Our results also agree with the work of Avent et al. (2008) who when working on *D. pseudoobscura*, also found a female preference for older males in both female-choice and no-choice experiments. These studies thus suggest that male-male competition can reinforce female mate preferences rather than operating in an antagonistic fashion (Moore and Moore 1999).

Our results of a female preference for old males are consistent with some previous observations in *D. melanogaster* and *D. simulans* (Moulin et al. 2001), in *D. pseudoobscura* (Avent et al. 2008), in other insects and birds (Zuk 1988, Conner 1989, Hasselquist et al. 1996), and with theoretical models (Kokko 1997 1998). Those studies suggested that female preference for old mates may be an evolutionarily stable strategy. All male flies used in our experiment were virgins, and so preference for old males was not influenced by differences in male mating history (Jones et al. 2007). The males used were 32-33, 17-18, and 2-3 d old. In *D. bipectinata*, males are fully sexually mature at 2 d old (Krishna and Hegde 1997). It was also noted that 50% of *D. bipectinata*

males survived to 32-33 d, although in the laboratories at $21 \pm 1^\circ\text{C}$, the mean longevity of *D. bipectinata* is 60 ± 2 d. In nature, these flies can live up to 58 ± 2 d. Thus, choosing between males of these ages is likely to occur fairly frequently in both wild and laboratory populations (although it is unlikely that males would remain unmated for 32-33 d in either the laboratory or the wild).

One potential problem one may encounter in this type of study of rearing males of different age classes in groups before they are exposed to females is that experience of male-male interactions increase with age and the increase in male experience can affect male courtship as noted in *D. melanogaster* (Svetic and Ferveur 2005). In the present study, it was noted that males maintained in groups of 4 and other kept individually in separate vials did not show a significant influence on mating latency or courtship time, suggesting that keeping virgin males in groups of 4 did not affect the performance of mating activities. Therefore, the increased success of old males in our female-choice experiment was unlikely due to being kept with other males for longer periods, but instead was an effect of age itself. When studying *D. pseudoobscura*, Noor (1997) and Avent et al. (2008) also found that virgin males kept at high densities for 8 d had increased mating latency and reduced courtship intensity compared to males raised in isolation, making experienced males less successful in courtship. They found that males kept in isolation were less successful in courtship. They also suggested that increased success of old males in their experiment was not because males had been maintained in high densities, but instead was an effect of age itself.

Thus, those studies suggest that there are many potential cues that females can use when making a choice between old and young males.

Table 7. Two-way ANOVA of male age influence on male mating ability in 3 different geographic strains of *Drosophila bipectinata*

Source	Sum of squares	d.f.	Mean square	F-value	p value
Population	6.207	2	3.103	10.774	< 0.0001
Age	69.120	1	69.120	239.977	< 0.0001
Population × age	2.660	2	1.330	4.618	< 0.05
Error	84.680	294	0.288		
Total	2628.000	300			
Corrected total	162.667	299			

Potential proximate mechanisms include an increased intensity of courtship (Noor 1997) or other behavioral changes, differences in cuticular hydrocarbons (Kim et al. 2004), and differences in courtship song with age (Moulin et al. 2001). However, it is not known at present which of the above cues used by females correlates with male age in *D. bipectinata*.

In contrast to this when studying *D. melanogaster*, Hansen and Price (1995) and Price and Hansen (1998) also argued that females preferring older males might not necessarily be true for a number of reasons, including the accumulation of mutations in the germline, negative genetic correlations between early and late fitness components, and ongoing adaptations in the population. Thus, those studies suggested that the role of female choice in promoting sexual selection is largely tied to the observation that females invest more heavily in reproduction, thus leading to females being more limiting. A female that chooses a male endowed with lots of good genes will also pass on those good genes to her progeny. Traits that aid survival of the male will undoubtedly be valuable resources to pass on to her progeny; thus, her choice is purely adaptive in that it leads to strong material benefits for her own offspring. In the present study we noted that when a female was given a choice to mate with young and intermediate-aged males, females mated with intermediate-aged male more frequently than young males. Similarly, when a female was given a choice to mate with intermediate-aged and old males (Tables 1, 2) females preferred to mate with old males more frequently than intermediate-aged males. Thus, these studies also suggest that females of *D. bipectinata* prefer to mate with the older of the 2 males.

Body size in male insects may affect reproductive activities including success in intrasexual encounters, sperm precedence, and female fecundity. For many of these activities, a larger size confers a reproductive advantage even in *Drosophila*. Other insects studies showed that although olfactory, visual, and auditory stimuli are some of the obvious influences on courtship behavior, physical characters such as body size and shape seem to be the determining factors for successful courtship and mating (Ewing 1961, Fulker 1966, Monclus and Prevosti 1971, Partridge et al. 1987, Santos et al. 1988, Krebs 1991, Ruiz et al. 1991, Naseerulla and Hegde 1992, Aspi and Hoikkala 1995, Hegde and Krishna 1997, Krishna and Hegde 2003, Guru Prasad et al. 2008). Those

studies also demonstrated greater mating success of larger flies.

In the present study, we also measured the wing length of selected and rejected males in the female-choice experiment to study relationships among female mate choices, male size, and male age (Table 3). It can be noted from table 2 that in all 3 geographic strains except in 1 cross of the Bellur strain (a female was given a choice of young and old males), the mean wing length of the selected male did not significantly differ from that of the rejected male, while in some crosses the rejected males also had significantly greater wing length than the selected males. This suggests a female preference for male age instead of male size. Our results also suggest that in cases when the sizes of selected and rejected males were equal or did not significantly differ, female preference was based on male age. It should be stressed that flies were reared under low-density conditions (100 eggs/vial) to reduce the influence of environmental components on the variance of body size and mating activity. Furthermore, the absence of size variations noted in our experimental population might have been due to flies being maintained under uniform laboratory conditions and also being established from isofemale lines. Therefore, size variations among individuals of a strain were found to be insignificant. Furthermore in *Drosophila*, the size of the adult fly is fixed at the time of emergence from the pupa, and therefore body size will not increase with increasing age of the flies (Santos et al. 1992).

In *Drosophila*, successful mating depends on male activity and female receptivity, because the female is usually the discriminating partner in the mating act, i.e., she actively accepts or rejects a courting male (Bastock 1956, Bateman 1948). In *Drosophila*, mating is resource independent, in that males do not contribute resources to their mate or protect their mate or offspring. Therefore, the display given by males before mating is the only grounds on which females can differentiate between potential mates (Hoikkala and Liimatainen 1992). Earlier studies also showed that the greater the activity of courting pairs, the greater the mating success (Krishna and Hegde 1997), indicating that activity during courtship is important for greater mating success.

In addition to mate choice, age in male insects can also affect reproductive activities such as mating latency and copulation duration. Mating latency or courtship time, i.e., the time

from the beginning of courtship to copulation (Spieth and Ringo 1983), is a good estimate of sexual receptivity of females and sexual activity in males. During this period, acts of courtship such as tapping, scissoring, vibrating, and circling are performed by the male, to increase the receptivity of female and to make her sexually excited (Manning 1961, Spieth 1968a b). Thus, these studies suggest that mating latency might also be used to indicate both the activity level of males and the receptivity of females. The higher the activity of males and the receptivity of females, the shorter is the mating latency. Our study (Fig. 1, Table 4) revealed that mating latency significantly varied among the different geographic strains, which suggests the existence of interpopulational variations in mating latency and confirms earlier studies in different species of *Drosophila* (Long et al. 1980). In all 3 geographic strains, old males spent significantly less time in mating latency than young males. Flies which spend less time are fast maters, while those flies which take more time are slow maters. This suggests an influence of male age on mating latency. This supports the work of Long et al. (1980) who studied *D. melanogaster* and found an influence of male age on courtship. During mating latency, older males more quickly performed courtship acts such as tapping, scissoring, vibrating, licking, circling, etc., thereby more rapidly increasing the receptivity of females than young males, suggesting that older males had greater activity than younger males. Consequently, old males had a shorter mating latency.

Courtship activity of a male or female in courtship culminates in copulation (Spiess 1970). Copulation duration was frequently observed and measured in many insects. In the genus *Drosophila*, copulation duration is highly variable, ranging from 30 s in *D. muller* to more than 1 h in some species (Spieth 1952). Copulation duration is the time elapsed between initiation of copulation by both the male and female until separation of the male and female of a copulating pair. During copulation, sperm from the male are transferred to the female reproductive tract, and therefore the duration of copulation has great significance in an animal's life.

Copulation duration is known to be influenced by male size, female size, male age, strain, etc., as seen in our study in all 3 strains (Fig. 2, Table 5). Females mating with old males copulated longer than females mating with young and intermediate-aged males. There are 3 main hypotheses to

explain this (Avent et al. 2008). First, old males may be unable to rapidly transfer sperm and hence require longer copulations. Second, old males may transfer larger quantities of sperm requiring more time. Third, old males might transfer more accessory fluid in their ejaculates during extended copulations. The 1st explanation suggests that old males are worse mates than young males. The 2nd and 3rd explanations suggest that old males invest more resources per mating, which could be the underlying cause of a female preference for old 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 copulations of old males result in the transfer of more sperm, because in many insects, longer copulation durations are associated with the transfer of more sperm (Thornhill and Alcock 1983, Wedell et al. 2002). However, in *D. melanogaster*, sperm transfer is completed in the first 20% of the overall copulation duration (Gilchrist and Partridge 2000). Sperm transfer commences after 90 s of copulation in *D. pseudoobscura* (Snook 2000). It is also possible that the extended copulation duration increases transfer of seminal fluids which are released at a constant rate during copulation and can significantly affect a female's egg-laying rate and delay re-mating (Gilchrist and Partridge 2000). However, whether this is true in *D. bipectinata* is not presently known.

In the present study, 32-33 d-old males were unmated for 32-33 d. These males had not encountered females for up to 32-33 d. These old males copulated longer than young and intermediate-aged males. However, there is no reason why old males should have copulated longer. There are strong theoretical reasons to expect that old males which have never encountered females for so many days would invest more resources in the 1st female they encounter (Parker 1970, Wedell et al. 2002). Old males have lower residual reproduction value and may increase their ejaculate investment per mating with increasing age (Roff 1992). Also, old males may have accumulated a larger quantity of ejaculates (sperm and seminal fluids) that take a longer time to transfer to females (Jones et al. 2007). Avent et al. (2008), who studied *D. pseudoobscura*, also found high ejaculate quantities because males had been kept virgin for 14 d. However, in most natural populations, the operational sex ratio is male biased (Turner

1986). Males will have relatively few opportunities to mate, and so 32-33 d-old males in the present study might be likely to carry almost their full sperm complement. Therefore, the possibility that longer copulations could function as physical mate guarding by males (Parker 1970, McLain 1989) can be discounted, because in many species of *Drosophila*, females do not re-mate in less than 24 h of a previous mating encounter (Snook and So 2000). It was noted that the copulation duration did not significantly vary among the 3 geographic strains, suggesting an absence of intrapopulation variation in copulation duration. This supports a lack of intrapopulation variations in copulation duration in earlier studies of *Drosophila* (Krebs 1991, Guru Prasad et al. 2008). Furthermore, in the present study, in all 3 geographic strains, old males copulated longer than young and intermediate-aged males (Fig. 2, Table 5).

Studies of *Drosophila* showed that males modify female behavior and physiology in various way, which were interpreted as possible examples of sexually antagonistic coevolution. Copulation was demonstrated to 1) reduce female receptivity to courting males, 2) increase the egg production rate, and 3) decrease the female lifespan. Owing to the fact that both prior and potential future mates of females attempt to manipulate them, these interactions and their consequences for female fitness are complex; and prior mates, via passing semen to courting females (Chen 1984, Chen et al. 1988, Aigaki et al. 1991, Kalb et al. 1993) increase their egg production rate (Kalb et al. 1993). At the same time, future mates coerce females into mating more frequently than is optimal from the females' perspective (Fowler and Partridge 1989). The net consequence of all these interaction is decreases in the female lifespan (Partridge et al. 1987a b, Champman et al. 1993 1995, Rice 1996, Civetta and Clark 2000a b) and lifetime progeny production (Champman et al. 1993, Chapman and Partridge 1996). It was convincingly demonstrated that the reduction in lifespan resulting from exposure to males is a consequence of harmful effects of both male courtship and seminal fluid (Partridge et al. 1987a b, Champman et al. 1995, Vehed 1998, Lung et al. 2002).

In the present study, it was noted that females of all 3 geographic strains which mated with old males had significantly lower mean longevity than females mating with intermediate-aged and young males (Fig. 3, Table 6). This suggests that the age of a female's mate has a significant influence on her longevity. This supports earlier

studies in general and on the genus *Drosophila* in particular (Alcock 1984). A reduction in longevity of mated females was also found. This shows the negative effects of mating per se on female fitness parameters, i.e., female longevity. A potential complicating factor with respect to the sexually antagonistic paradigm relates to the effects of ejaculates and any other substances that are transferred to females during mating (Champman et al. 1995). In the present study, the reduction in longevity of females which had mated with old males may have been due to older males carrying more-harmful substance because they copulated longer than intermediate-aged males. It is possible that the longer copulations of old males may result in the transfer of more sperm and other substances, because in many insects, longer copulation durations are associated with the transfer of more sperm and other ejaculate substances (Thornhill and Alcock 1983, Wedell 2002). It is not known whether harmful effects of ejaculates increase with age in males, and this may be the cause for the reduction in longevity of females which had mated with old males. It is not known whether storing sperm and other ejaculates for 32-33 d increases the harmful effects on female fitness, i.e., longevity. However, these causes have not yet been tested.

The primary function of mating is the transfer of sperm to females, since each mating offers an opportunity to father offspring. Males can generally increase their fitness by mating with many mates, and high mating rates are thus typically associated with high male reproductive success. Male *Drosophila*, like most male insects, provides no parental care to offspring following female insemination (Thornhill and Alcock 1983). Male fitness is therefore related to the number of females inseminated and prevention of females from re-mating (Parker 1970, Jones and Elgar 2004, Jones et al. 2007). In the present study, the influence of male age on male mating ability was studied using 3 different strain of *D. bipectinata* (Fig. 4, Table 7). It was noted that the Bellur strain had the highest male mating ability, while the Mysore strain had the least. This shows the existence of intraspecific variation in male mating ability. This confirms findings of an earlier study on interpopulational variations in male mating ability in *D. bipectinata* (Guru Prasad et al. 2008). Since the male's mating ability is a fitness character, the observed variations in male mating ability among the different geographic populations suggest interactions of these different strains

with heterogeneous environments. Among the 3 geographic strains studied, the Bellur strain had greater male fitness than the Mysore strain. Male flies inseminating a number of females in a given unit of time is considered a male activity, because a male fly which performs greater activity during courtship and convinces females faster can inseminate more females than ones that do not perform greater activity during courtship and fail to increase female receptivity. Therefore, it was noted that among the 3 geographic strains, the Bellur strain had greater activity while the Mysore strain had the lowest activity. Since the Bellur strain is a fast mater, males re-mated faster and inseminated more females in a given unit of time, while the Mysore strain is a slow mater and thus inseminated fewer females in a given unit of time.

In the present study, it was also noted that in all 3 geographic strains, old males inseminated more females in a given unit of time than did young males. This suggests that male age has a significant influence on male mating ability, and old males have greater fitness than young males. This supports earlier studies on the influence of age on mating activities in *Drosophila* (Long et al. 1980, Long and Pischedda, 2005). Old males had greater activities during mating because they took less time to convince a female to mate and inseminated more females in a given unit of time than did young males. Thus, in *D. bipectinata* irrespective of the geographic strains studied, old males had greater activities during mating and greater male fitness than young males.

Our studies of *D. bipectinata* suggest that females prefer to mate with old males more frequently than young and intermediate-aged males. Old males mated faster, copulated longer, and inseminated more females than did young males. Male age did not influence body size. Furthermore, females which mated with old males lived shorter than ones which mated with young males.

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