Greater son mating success of old age male in *Drosophila bipectinata.*

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Introduction

Many *Drosophila* species exhibit genetic variation in traits affecting mate choice (Scott and Richmond, 1988; Marin, 1994; Aspi and Hoikkala, 1995). This variation forms the basis for sexual selection (exercised) by the females within a species and may also give rise to isolation between geographically separated populations (Markow, 1991). Studies on genetic variation in traits affecting mate choice and progeny production are thus essential for understanding how species-specific courtship behaviour evolved.

Mating success of the flies of different *Drosophila* species depends on a variety of factors including male courtship vigour, female receptivity, male size, and the signals emitted during the courtship (studies reviewed in Spiess 1987). Male age is another character known to influence on mating success (Zuk, 1988; Beck *et al.,* 2002).

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; Bucholz, 1991; Cote and Hunte, 1993; Lee and Park, 2009). In species in which males do not provide such benefits, however, 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, 1987; Simmons and Zuk, 1992; Vandenberghe *e .al*., 1993), or species in which males do not provide care to offspring of females (Jarvi *et al*., 1992; Weatherhead, 1984), and in cases of extra-pair copulation (Moller, 1992).

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 by Banerjee 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). Females of this species prefer to mate old aged males more frequently over young or middle aged males (Somashekar and Krishna. in press). Therefore, offspring of female mated with young, middle, and old age males were used in the present study to investigate male age influence on son mating success using multiple mating and pair wise mating (no choice method) in three different strains of *D. bipectinata* to test the hypothesis good gene model, which emphasizes that females prefer older males to obtain genetically better offspring.

Materials and Methods

Experimental stocks of *D. bipectinata* were established from isofemale lines collected at Dharwad, Bellur, and Mysore were used. These stocks (main experimental stocks) were cultured using forty flies (20 males and 20 females) per quarter pint milk bottles (250 ml) containing wheat cream agar medium at 21º ± 1ºC at a relative humidity of 70%. These stocks were maintained at a 12:12 L:D cycle. After three generations of culture, eggs were collected separately from each of the above stocks using Delcour's (1969) procedure. Eggs (100) were seeded in a vial 3×1 containing wheat cream agar medium. When adults emerged from these vials, virgin females and unmated males were isolated within 3 hrs of their eclosion and were aspirated in to a new vial containing wheat cream agar medium. These flies were aged as required for the experiment [young age males (2-3 days); middle age males (24-25 days); old age males (46-47 days)]. These males of different age classes were maintained individually in a vial containing wheat cream agar medium using the same laboratory conditions and were transferred once in a week until they were used in the experiment. Female flies were also aged for 5-6 days and were maintained using above laboratory condition. Offspring were obtained separately by crossing each of the three male age classes with 5-6 day old virgin females. When adults emerge from these crosses, unmated sons were isolated as above. At the same time 5-6 day old virgin females were also obtained from the main experimental stock to study male age influence on son mating success.

In the multiple mating experiment, five 5-6 days old virgin females (obtained from main experimental stock) and five each of 5-6 days old unmated sons of young and old / young and middle / middle and old age male were aspirated individually into an Elens-Wattiaux mating chamber (1964). Indian ink was painted to sons of young age males in one trial and sons of old age male in another trial on the thorax region. [The effect on mating was tested before commencing the experiment by painting 5 out of 10 sons of young age male/ sons of old age male/ sons of middle age males and allowing them to mate with 5-6 days virgin females. Observation was made for 1 hour for each trial. When mating occurs, pairs in copulation were aspirated out of the mating chamber without disturbing other flies in the mating chamber. A total of 10 trials were made separately for each of the sons of young, middle, and old age classes. In sons of young age males in 24 out of 50 mated pairs, son painted were mated while in remaining 26 mated pairs sons did not paint were mated ($\chi^2 = 0.08$; df = 1; $P > 0.005$). Similarly in sons of old age males, 23 out of 50 mated pairs sons painted were mated while in the remaining 27 mated pairs sons did not paint were mated ($\chi^2 = 0.32$; df = 1; $P > 0.005$). In sons of middle age males 22 out of 50 mated pairs sons painted were mated while in the remaining 28 mated pairs sons did not paint were mated ($\chi^2 = 0.72$; df = 1; $P > 0.005$). Thus, these results suggest that painting did not have influence on mating success and performance of mating flies]. Observation was made for 1 hr. When mating occurred, pairs in copulation werr spirated out of the mating chamber without disturbing other flies in the mating chamber. A total of 10 trials were made for each combination of multiple mating experiments, and number of mated pairs was recorded. Mean wing length of selected and rejected sons in multiple mating experiment were also measured using the procedure of Hegde and Krishna (1997). The mating success data was subjected to Chi-square analysis, and mean wing length data of selected and rejected sons were subjected to Paired 't' test using SPSS 10.0 programme.

In pair-wise mating, a virgin 5-6 day old female (obtained from main experimental stock) and a 5-6 days old son of different male age classes (young, middle, old age) were aspirated individually into an Elens-Wattiaux mating chamber (1964). Observation was made for 1 hr, and a total of fifty trials were made for each of the three different male age classes. Total number of pairs mated in 1 hr was recorded.

Results and Discussion

It was widely suggested that in species in which males do not provide parental care or nuptial gift to mated females, females may choose to mate with older males to obtain good genes to their offspring, because these older males have survived for longer time and, thus, prove their genetic ability (Trivers, 1972; Markow and Akney, 1984; Anderson, 1994; Radwan, 2003; Prokop *et al.,* 2007). *D. bipectinata* used in the present study is one such species belonging to the genus *Drosophila*. Males of this species neither provide parental care nor provide nuptial gift to mated females except sperm and accessory gland secretion to mated females. In this species we found that females preferred to mate with old aged males more frequently over young or middle aged males (Somashekar and Krishna, in press). In the present study, therefore, offspring (sons) obtained from female mated with males of three different age classes were used to test the good gene model (Manning, 1985; Kokko and Lindstrom, 1996; Kokko, 1998), which emphasizes that females preferred old males to obtain indirect genetic benefits.

Table 1 shows son mating success of young, middle, and old age male in three different strains of *D. bipectinata*. It was noticed that in multiple mating experiments, sons of old age males were successful in crosses involving sons of young and old age males (n = 50) in 80% of cases with Mysore females, 66% of Dharwad females, and 66% of Bellur females; similarly, sons of old age males were successful in crosses involving sons of middle and old age males (n = 50) in 60% of cases with Mysore females, 66% of Dharwad females, and 74% of Bellur females. It was also noticed that sons of middle age males were successful in crosses involving sons of young and middle aged males (n = 50) in 76% cases with Mysore females, 66% of Dharwad females, and 76% of Bellur females.

Table 1. Mating success of sons of different male age classes in three different strains of *D. bipectinata* using multiple choice experiment.

Strain	Female	Son		χ^2 value	Son		χ^2 value	Son		χ^2 value
		Young age male	Old age male		Old age male	Middle age male		Middle age male	Young age male	
Mysore	5-6 days	10 (20%)	40 (80%)	18.00**	30 (60%)	20 (40%)	2.00^{ns}	38 (76%)	12 (24%)	13.52**
Dharwad	5-6 days	17 (34%)	33 (66%)	5.12**	33 (66%)	17 (34%)	5.12**	33 (66%)	17 (34%)	5.12 **
Bellur	5-6 days	17 (34%)	33 (66%)	5.12**	37 (74%)	13 (26%)	11.52**	38 (76%)	12 (24%)	13.52**

** P < 0.001; ns = insignificant

It was noticed that in all the three strains, females of *D. bipectinata* was able to discriminate between sons of young, middle, and old age males, and she generally prefers to mate with sons of old aged males more frequently than sons of middle or young age male. In the presence of male - male competition in the multiple mating experiment suggesting that in *D. bipectinata* male age has a significant influence on son mating success. Even in pair-wise mating (Table 2) sons of old aged males had the highest mating success (n = 50) in 90% of cases with Mysore strain, in 94% cases with Dharwad strain, and in 96% of cases with Bellur strain; followed by sons of middle aged males (n = 50) in 68% of cases with Mysore strain, in 70% cases with Dharwad strain, and in 76% of cases with Bellur strain; and least with sons of young age males (n = 50) in 54% of cases with Mysore strain, in 42% cases with Dharwad strain, and in 60% of cases with Bellur strain. Thus, these results support

Table 2. Mating success of sons of different male age classes in *D. bipectinata* using no choice experiment.

Strain	Female	Sons		
		Young age male	Middle age male	Old age male
Mysore	5-6 days	27 (54%)	34 (68%)	45 (90%)
Dharwad	5-6 days	21 (42%)	35 (70%)	47 (94%)
Bellur	5-6 days	30 (60%)	38 (76%)	48 (96%)

the idea that the selective advantage of female choice in *D. bipectinata* is based on indirect fitness benefits. This is in contrast to the study of Avent *et al.* (2008) who while working in *D. pseudobscura* have found that virgin females of *D. pseudobscura* obtained from main culture bottle did not discriminate sons of young and old age males. Our results also do not support the argument that the sons of old age males were less successful, because the older age males pass on the sperm with increased number of germline mutations (Price and Hansen, 1998). Price and Hansen (1998) while working in *D. melanogaster* have also found that sons of older fathers have reduced mating ability. This shows occurrence of species specific difference in obtaining indirect benefits in *Drosophila*.

Sons of young, middle, and old age males used in our experiment were unmated 5-6 days old and were reared in the same environment therefore observed greater son mating success of sons of old age males could not be attributed to difference in the life history or rearing of sons using before the experiment (Svetec and Ferveur, 2005; Jones and Elgar, 2004).

During the process of courtship, sexual selection occurs in *Drosophila*, as well as in many other animals (see Arnold, 1983). For example, in both field and laboratory studies with *D. melanogaster*, the body size of males was positively correlated with mating success (Patridge *et al.,* 1987; Santos *et al.,* 1992; Hegde and Krishna, 1997; Krishna and Hegde, 2003). In our study, therefore, we also measured wing length of selected and rejected sons by females in multiple mating experiments, and mean wing length of selected and rejected sons are provided in Table 3. It was noticed that insignificant variation was found between mean wing length of selected and rejected sons by females by Paired 't' test. Further mean wing lengths of rejected sons were longer than selected sons. This suggests that size has no influence on the observed greater mating success of old age males. Thus, these studies in *D. bipectinata* suggest that sons of old age males had greater mating success than sons of young and middle aged males supporting the hypothesis good gene model.

Table 3. Mean wing length of selected and rejected sons in multiple mating experiment of *D. bipectinata*.

Strains	Crosses		Mean wing length (mm)		
	Female	Males	Selected sons	Rejected sons	't' value
Mysore	5-6 days old	Young, Old	1.597 ± 0.015	1.596 ± 0.013	0.103^{ns}
		Young, Middle	1.610 ± 0.008	1.615 ± 0.008	0.623^{ns}
		Middle, Old	1.597 ± 0.006	1.618 ± 0.006	2.942^{ns}
Bellur	5-6 days old	Young, Old	1.571 ±0.016	1.572±0.0144	0.129^{ns}
		Young, Middle	1.607 ± 0.009	1.609 ± 0.008	0.270^{ns}
		Middle, Old	1.593 ± 0.006	1.616 ± 0.007	2.875^{ns}
Dharwad	5-6 days old	Young, Old	1.548 ± 0.0169	1.553 ± 0.014	0.648^{ns}
		Young, Middle	1.611 ± 0.0101	1.612 ± 0.008	0.330^{ns}
		Middle, Old	1.600 ± 0.007	1.622 ± 0.0772	2.621^{ns}

ns = Non significant

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A study of anti-stress property of *Convolvulus pluricaulis* (Shankhapushpi) on stress induced *Drosophila melanogaster.*

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Abstract: Anti-stress property of *Convolvulus pluricaulis* has been evaluated using *D. melanogaster*. Four groups of flies were reared simultaneously under similar conditions. The first