

## Offspring of Older Males are Superior in *Drosophila bipectinata*

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**Mysore S. Krishna, Hassan T. Santhosh, and Shridhar N. Hegde (2012)** Offspring of older males are superior in *Drosophila bipectinata*. *Zoological Studies* 51(1): 72-84. *Drosophila bipectinata* offspring of old- and young-aged males reared in low (LLD) and high larval densities (HLD) were employed to test a good gene model of female preference for male age classes. It was noted that with both LLD and HLD, offspring of old-aged male had significantly greater pre-adult fitness (egg-larval hatchability and larval-adult viability) and adult fitness (son's mating success, mating latency, mating ability, progeny production, and longevity, and daughter's mating success, fecundity, and longevity) over sons of young-aged males, which suggested that larval density did not have an effect on the inheritance of characters from parent to offspring. Among larval densities, LLD offspring performed significantly better in all traits studied than did HLD offspring. Thus, *D. bipectinata* females prefer old-aged males to obtain greater fitness benefits for their offspring, thereby confirming the good-gene model. <http://zoolstud.sinica.edu.tw/Journals/51.1/72.pdf>

**Key words:** Female preference, Male age, Pre-adult fitness, Adult fitness.

It was widely proposed that in species with no parental care, females may choose to mate with older males to obtain good genes for their offspring (Price and Hansen 1998). In species of the genus *Drosophila*, males do not generally provide parental care or any resources to mating females except sperm and accessory gland proteins. Therefore species of *Drosophila* can be used as model organisms to test the 'good-gene' hypothesis. Food shortages due to larval densities in *Drosophila* are known to influence almost all fitness traits (Ribo et al. 1989); however, it is unknown whether or not food shortages have any influence on offspring fitness of female preferences for male traits. Male traits in *Drosophila*, such as size, age, color, pigmentation, courtship song, and parental care, are known to be used by females in mating to derive both direct and indirect benefits (Hegde and Krishna 1997). The expression of these traits not only depends on the genotype but also on other factors such as the developmental

environment (i.e., larval density and temperature) and parental age (Mousseau and Dingle 1991).

The most compelling studies of *Drosophila* found that larger males have a higher mating success than their smaller counterparts, both in laboratory and field conditions (Partridge et al. 1987, Santos et al. 1992, Hegde and Krishna 1997, Krishna and Hegde 2003). Male age is another trait known to influence female mating preferences: some studies found that females of *D. melanogaster* and *D. pseudobscura* prefer to mate with older-aged males (Moulin et al. 2001, Avent et al. 2008). One potential explanation is that females are likely to gain indirect benefits because older males are highly viable and have demonstrated their survival ability, and there is a general positive correlation between male longevity and genetic viability (Hansen and Price 1995, Kokko 1997, Beck et al. 2002). Little attention has been paid to the parental age effect in studies of *Drosophila* except for the work of Parsons (1964). Further,

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few *Drosophila* studies on age effects directly examined the effect of parental age on progeny fitness; instead they considered physiological changes associated with changes in parental age, molecular aspects, selection experiments, and comparisons of populations generated from individuals of different ages (Comfort 1953, Parsons 1962, Wattiaux 1968, Lints and Hoste 1974, Ganetzky and Flanagan 1978, Rose and Charlesworth 1981, Lubkinbill et al. 1984, Partridge and Fowler 1992, Roper et al. 1993, Chippindale et al. 1994, Orr and Sohal 1994). Very few attempts have been made to study the influence of male age on offspring fitness in *Drosophila* (Price and Hansen 1998, Avent et al. 2008). Even in those studies, a single fitness trait was studied, and therefore it is difficult to test the good-gene model of female preference for male age classes.

Herein, we approached this issue using *D. bipectinata*. In this species, females prefer to mate with old-aged males more frequently over young-aged males (Somashakar and Krishna 2011). Therefore in the present study, offspring (sons and daughters) of young- and old-aged males mated separately with 5-6-d-old virgin females were used to test the good-gene model (Arnold 1983) in low (LLD) and high larval density (HLD) situations.

## MATERIALS AND METHODS

### Experimental stock

An experimental stock of *D. bipectinata* established from progeny obtained from 3 isofemale lines collected at Mysore, Dharwad, and

Bellur was used. This stock was cultured using 40 flies (20 males and 20 females) in half-pint milk bottles (250 ml) containing wheat cream agar medium in  $21 \pm 1^\circ\text{C}$  and a relative humidity of 70% using a 12: 12-h light: dark cycle for 3 generations.

### Assignment of age classes

Before assigning age classes to males, the longevity of unmated males of the experimental stock was studied. Unmated males were transferred separately and individually into a vial containing wheat cream agar medium and maintained there in the same laboratory conditions as described above. These flies were transferred to a new vial containing wheat cream agar medium once a week, and this process was continued until the death of the fly. Fifty replicates were run, and the mean longevity of unmated males (number of days a male lived from the time of its eclosion) of *D. bipectinata* was calculated. It was found that the mean longevity of *D. bipectinata* was  $58 \pm 5$  d. Furthermore, males of *D. bipectinata* showed courtship activities within 11-12 h of their eclosion, and they remained sexually active for 50-55 d (Table 1). However, in nature, it is very difficult for the flies to live for even 50 d. Hence, we assigned male age classes as follows: young-aged males were 2-3 d old, and old-aged males were 46-47 d old. In our study, 90% of unmated males survived for more than 50 d.

The first emerging flies were aged for 47-48 d. When these flies reached 44 d old, the next set of flies was isolated and was aged for 2-3 d. These young- and old-aged males were separately mated with 5-6-d-old virgin females to obtain offspring

**Table 1.** Courtship activities (no.) of 11-12-h- and 50-55-d-old males of *D. bipectinata*

Parameter	Male age		t value
	11-12 h	50-55 d	
Male courtship activities			
Tapping	9.32 ± 0.31	14.28 ± 0.27	11.966**
Scissoring	9.68 ± 0.45	13.98 ± 0.61	5.594*
Vibrating	9.10 ± 0.26	14.40 ± 0.35	11.968**
Licking	3.76 ± 0.17	7.46 ± 0.18	14.976**
Circling	3.34 ± 0.26	5.88 ± 0.17	7.944*
Female courtship activities			
Ignoring	7.34 ± 0.23	3.60 ± 0.24	11.241**
Extruding	5.68 ± 0.27	2.42 ± 0.16	10.184**
Decamping	3.82 ± 0.16	1.86 ± 0.11	9.731*

\* Significant at  $p < 0.01$  and \*\*  $p < 0.001$ .

which were cultured in the same environment. This experimental design eliminated the problem of testing the flies at different times. However with this experimental design (horizontal variation), it is impossible to exclude differences in the histories of young- and old-aged males. Furthermore, 90% of published works studying horizontal variations used the same experimental design.

### Male age influence on offspring pre-adult fitness

Eggs were collected from separately crossing young- and old-aged males with 5-6-d-old virgin females using Delcour's procedure (1969). One hundred eggs were seeded in a vial (7.6 × 2.5 cm) containing wheat cream agar medium to create an LLD. Similarly, 400 eggs were seeded in a vial (7.6 × 2.5 cm) containing wheat cream agar medium to create an HLD. These vials were cultured and maintained in laboratory conditions as described above. The number of eggs hatching into 1st instar larvae was counted. Then 100 1st instar larvae were transferred to a new vial containing wheat cream agar medium, and the number of progeny that emerged from these larvae was also counted. This was considered the larva-adult viability. In total, 50 replicates were created for offspring of young- and old-aged males. A two-way analysis of variance (ANOVA) of a general linear model was used on offspring egg-larval hatchability and larval-adult viability from the SPSS 10.0 program (SPSS, Chicago, IL, USA).

### Experiment 1: Male age influence on the son's mating success using a female choice experiment with LLD and HLD

#### Effect of paint on the son's mating success

The effect of paint on the son's mating success was tested before commencing the experiment by painting one of 2 sons of young- and old-aged males, and allowing them to mate

for 1 h. Fifty replicates were used, and the results indicated that painting one of the competing sons in a female-choice experiment had no effect on the performance of the flies (Table 2).

To study the influence of the male age on the son's mating success, in each trial, a 5-6-d-old virgin female (obtained from the main experimental stock) along with 2 unmated 5-6-d-old sons of young- and old-aged males were individually aspirated into an Elens-Wattiaux mating chamber (1964). The thorax of sons of the young-aged male was painted with Indian ink in 1 trial, while in the other trial, the thorax of sons of the old-aged male was painted with Indian ink following the procedure of Hegde and Krishna (1997), and then the males were observed for 1 h. When mating occurred, copulating pairs were aspirated out from the mating chamber. Sons rejected by females in the female mate-choice experiment were also individually transferred to new vials containing wheat cream agar medium. Both selected and rejected sons by females in the female mate-choice experiment were later used to measure wing length following the procedure of Hegde and Krishna (1997). Fifty replicates were used for each combination in the female mate-choice experiment, and a Chi-squared analysis was applied, a paired *t*-test was also run on the mean wing length of selected and rejected sons. Separate experiments were carried out for both LLD and HLD.

### Experiment 2: Male age influence on son's mating latency and copulation duration

A son at 5-6 d old and a 5-6-d-old virgin female (obtained from the main experiment stock) were individually aspirated into an Elens-Wattiaux chamber and observed for 1 h. Any pair that had not mated within 1 h was discarded. If mating occurred, we recorded the mating latency (time between the introduction of the male and female together into the mating chamber until initiation of copulation of the pair) and copulation duration (time

**Table 2.** Effects of paint on the mating success of sons of young- and old-aged males of *D. bipectinata*

Female	Sons of different male age classes	Mating success (%)		
		Painted male	Non-painted male	$\chi^2$ value
5-6 d	Young, Young	23 (46%)	27 (54%)	0.32 <sup>ns</sup>
5-6 d	Old, Old	28 (56%)	22 (44%)	0.72 <sup>ns</sup>

<sup>ns</sup>, non-significant.

between initiation of copulation to termination of copulation by the pair).

### Male age influence on progeny production

Soon after copulation, the mated female as in experiment 2 was individually transferred to a new vial containing wheat cream agar medium that was refreshed every 5 d, and the number of progeny obtained was recorded.

### Male age influence on the son's mating ability

Soon after mating as in experiment 2, a mated son was allowed to mate with a 2nd female (a 5-6-d-old virgin female obtained from the experimental stock). If mating occurred with the 2nd female, the pair was allowed to complete copulation, and then the female was checked for insemination as described above. This process was continued, and the number of females inseminated by each son in 1 h was recorded as the son's mating ability (number of females inseminated by each son in 1 h).

### Male age influence on son's longevity

Soon after recording the sons' mating ability, these sons were individually transferred to new vials containing food medium which was refreshed once a week until their death to record the longevity.

Fifty replicates were separately conducted for each of the sons of young- and old-aged males to examine such parameters as the son's mating latency, copulation duration, progeny production, mating ability, and longevity. Experiments were also carried out separately for LLD and HLD. A two-way ANOVA was used on these data using the SPSS 10.0 program.

### Experiment 3: Daughter fitness mating success and fecundity

#### Effect of painting on the daughter's mating success

The effect of paint on a daughter's mating success was tested before commencing the experiment by painting one of 2 daughters of young- and old-ages males and allowing them to mate for 1 h. Fifty replicates were used, and the results indicated that painting of one of the 2 daughters in the male-choice experiment did not have an effect on the performance of the flies (Table 3).

In each trial, a 5-6-d-old unmated male (obtained from the main experimental stock) and 2 virgin 5-6-d-old daughters of young- and old-aged males were individually aspirated into an Elens-Wattiaux mating chamber (1964). The thorax of a daughter of a young-aged male was painted with Indian ink in 1 trial, while in the other trial, that of a daughter of an old-aged male was painted with Indian ink following the procedure of Hegde and Krishna (1997), and then the flies were observed for 1 h. When mating occurred, copulating pairs were aspirated out of the mating chamber. Fifty separate replicates were each used for sons of young- and old-aged males. Separate experiments were carried out for LLD and HLD. A Chi-square analysis was applied to the daughter's mating success data.

### Experiment 4: Male age influence on the daughter's fecundity

Daughters at 5-6 d old of young- and old-aged males and 5-6-d-old unmated males (obtained from the main experiment stock) were individually aspirated into an Elens-Wattiaux chamber and observed for 1 h. Any pair which did not mate within 1 h was discarded. If mating occurred, then soon after copulation, the mated female was

**Table 3.** Effects of paint on the mating success of daughters of young- and old-aged males of *D. bipectinata*

Male	Daughters of different male age classes	Mating success (%)		
		Painted male	Non-painted male	$\chi^2$ value
5-6 d	Young, Young	24 (48%)	26 (52%)	0.08 <sup>ns</sup>
5-6 d	Old, Old	27 (54%)	23 (46%)	0.32 <sup>ns</sup>

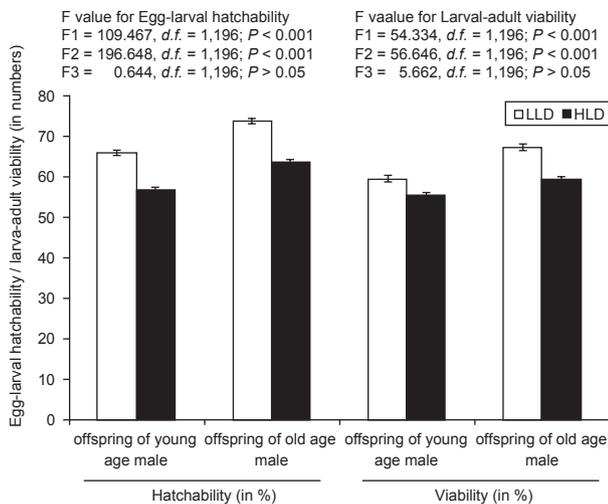
<sup>ns</sup>, non-significant.

individually transferred to a new vial containing wheat cream agar medium that was refreshed once every 24 h until her death. The number of eggs obtained was counted, and the daughter's longevity was recorded. Fifty separate replicates were used for daughters of young- and old-aged male classes. Experiments were carried out separately for LLD and HLD. A two-way ANOVA in the SPSS 10.0 program was used on the above data.

## RESULTS

### Male age influence on offspring pre-adult fitness

Figure 1 shows pre-adult fitness in terms of the mean egg to larval hatchability and larval to adult viability in offspring of young- and old-aged males. It was noted that the offspring of old-aged males had greater pre-adult fitness in both LLD



**Fig. 1.** Male age influence on offspring pre-adult fitness (in %) in low (LLD) and high larval densities (HLD) of *D. bipunctata*. F1,  $F$  value between age classes; F2,  $F$  value between larval densities; F3,  $F$  value for the interaction between age classes and larval densities.

and HLD than did offspring of young-aged males. Pre-adult fitness with the LLD was greater than that with the HLD. Significant variations in egg to larval hatchability and larval to adult viability were found between offspring of young- and old-aged males and also between the LLD and HLD. But an insignificant variation was found for the interaction of male age and larval density by a two-way ANOVA of the general linear model using the SPSS 10.0 program.

### Male age influence on the son's mating success

Females of *D. bipunctata* chose to mate with sons of old-aged males more frequently than sons of young-aged males (Table 4). The mating success of sons of old-aged males was 80% ( $n = 50$ ) in an LLD and 76% ( $n = 50$ ) in an HLD, while mating success of sons of young-aged males was 20% ( $n = 50$ ) in an LLD and 24% in an HLD when sons of young- and old-aged males were involved in the crosses. A Chi-squared analysis applied to the mating success data showed significant variations between sons of young- and old-aged males and also between the LLD and HLD.

Mean wing lengths of selected and rejected sons by females in LLD and HLD of *D. bipunctata* are given in table 5. In both LLD and HLD for all combinations, the mean wing length of rejected sons was slightly longer than that of selected sons, but the difference was insignificant between the mean wing lengths of selected and rejected sons.

### Male age influence on the son's mating activities and longevity

Mean values of the mating latency and copulation duration of sons of old- and young-aged males are given in figure 2. Sons of old-aged males showed less time for mating latency and copulated longer compared to sons of young-aged males. Among larval densities, flies from the LLD

**Table 4.** Mating success (%) of sons of young- and old-aged males in low (LLD) and high larval densities (HLD) of *D. bipunctata*

Larval density	Sons of young-aged males	Sons of old-aged males	$\chi^2$ value
LLD	20%	80%	18.0**
HLD	24%	76%	12.5**

\*\* Significant at  $p < 0.001$ .

took a shorter time to begin mating and copulated longer than flies from the HLD. A two-way ANOVA using the general linear model was carried out on the above parameters and showed significant variations between sons of young- and old-aged males, between LLD and HLD, and also for the interaction between male age classes and larval densities.

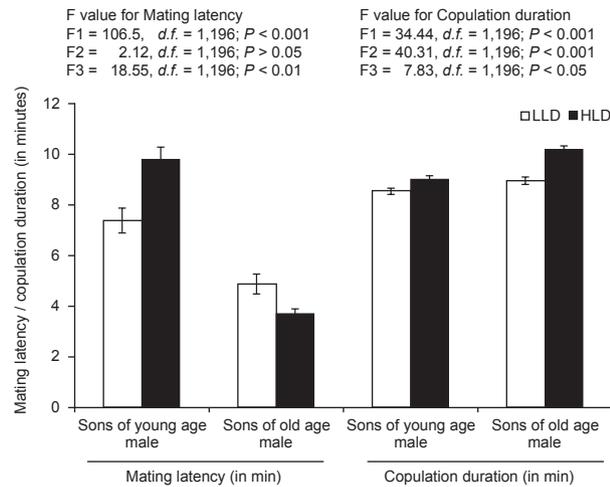
Figure 3 shows the mean sons' mating abilities of young- and old-aged males of *D. bipectinata*. It was noted that sons of old-aged males inseminated a greater number of females in a given unit of time than sons of young-aged males. The sons' mating ability data subjected to a two-way ANOVA using the general linear model showed significant variations between sons of young- and old-aged males and between larval densities, but an insignificant variation was noted for the interaction between male age classes and larval densities.

Mean longevity data of sons of young- and

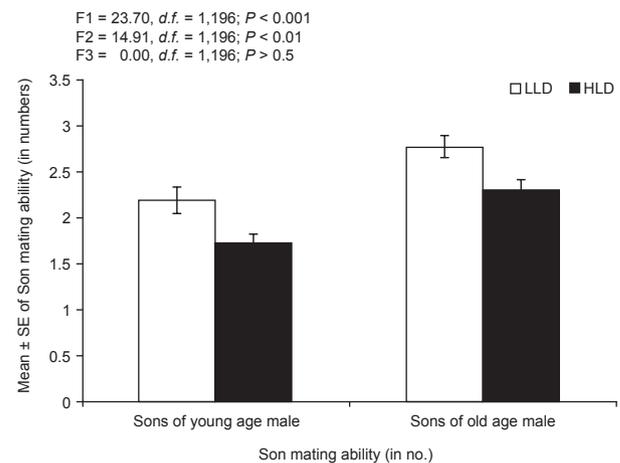
old-aged males are given in figure 4. Sons of old-aged males lived longer compared to sons of young-aged males. A two-way ANOVA using the general linear model on the sons' longevity data showed significant variations between sons of young- and old-aged males and between larval densities, but an insignificant variation was found for the interaction between male age classes and larval densities.

**Male age influence on fecundity and fertility of females mated with sons of young- and old-aged males**

Mean values of fecundity and fertility of females mated with sons of young- and old-aged males in the LLD and HLD of *D. bipectinata* are given in figure 5. The fecundity and fertility of females mated with sons of old-aged males were found to be greater than those of females mated with sons of young-aged males. The fecundity



**Fig. 2.** Male age influence on the son's mating latency (in min) and copulation duration (in min) in low (LLD) and high larval densities (HLD) of *D. bipectinata*. F1, F value between age classes; F2, F value between larval densities; F3, F value for the interaction between age classes and larval densities.



**Fig. 3.** Male age influence on the son's mating ability (in no.) in low (LLD) and high larval densities (HLD) of *D. bipectinata*. F1, F value between age classes; F2, F value between larval densities; F3, F value for the interaction between age classes and larval densities.

**Table 5.** Mean wing length (mm) of selected and rejected sons of young- and old-aged males in low (LLD) and high larval densities (HLD) of *D. bipectinata*

Larval density value	Females	Males	Wing length of selected sons (mm)	Wing length of rejected sons (mm)	t
LLD	5-6 d old	Young, Old	1.596 ± 0.013	1.597 ± 0.015	0.103 <sup>NS</sup>
HLD	5-6 d old	Young, Old	1.46 ± 0.009	1.47 ± 0.009	1.42 <sup>NS</sup>

<sup>NS</sup>, non-significant.

and fertility of flies from the LLD were found to be greater than those of flies from the HLD. A two-way ANOVA using the general linear model applied to the fecundity and fertility data showed significant variations between young- and old-aged males and between the LLD and HLD. However, an insignificant variation was found for the interaction between sons of male age classes and larval densities.

### Male age influence on the daughter's mating success

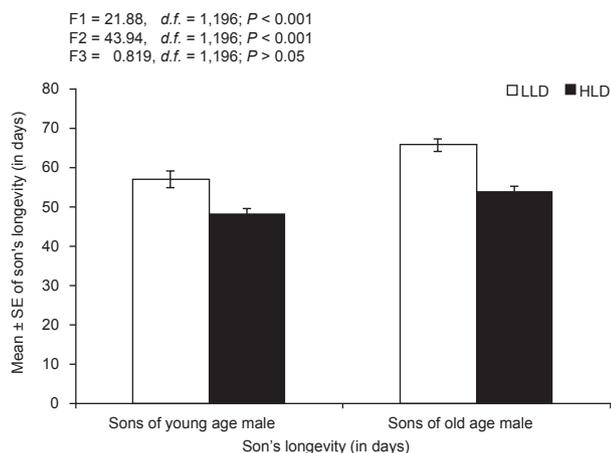
Table 6 gives the mating success data of daughters of young- and old-aged males. Daughters of old-aged males had greater mating success than daughters of young-aged males. The mating success rates daughters of old-aged males were 74% in the LLD and 70% in the HLD ( $n = 50$ ), while those of young-aged males were 26% in the LLD and 30% in the HLD. Mating success data of

daughters of young- and old-aged males showed significant variations between daughters of young- and old-aged males and also between the LLD and HLD.

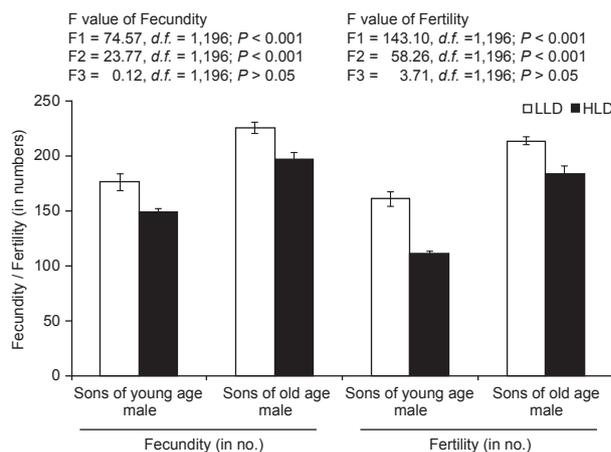
### Male age influence on the daughter's fecundity

Table 7 and figure 6 shows the mean fecundity of daughters of young- and old-aged males in the LLD and HLD. It was noted that daughters of old-aged males had greater fecundity compared to daughters of young-aged males. Among larval densities, flies from the LLD had greater fecundity compared to those from the HLD. Significant variations were found in the fecundity of daughters of young- and old-aged males and between the LLD and HLD. Insignificant variations were found in fecundity from the interaction between sons of male age classes and larval densities.

Similar results was also found in mean longevity of daughters of different male age



**Fig. 4.** Male age influence on the son's longevity (in d) in low (LLD) and high larval densities (HLD) of *D. bipectinata*. F1, F value between age classes; F2, F value between larval densities; F3, F value for the interaction between age classes and larval densities.



**Fig. 5.** Fecundity (in no.) and fertility (in no.) of females mated with sons of young- and old-aged males in low (LLD) and high larval densities (HLD) of *D. bipectinata*. F1, F value between age classes; F2, F value between larval densities; F3, F value for the interaction between age classes and larval densities.

**Table 6.** Mating success (%) of daughters of young- and old-aged males in low (LLD) and high larval densities (HLD) of *D. bipectinata*

Larval density	Daughters of young-aged males	Daughters of old-aged males	$\chi^2$ value
LLD	26%	74%	11.52**
HLD	30%	70%	8.0**

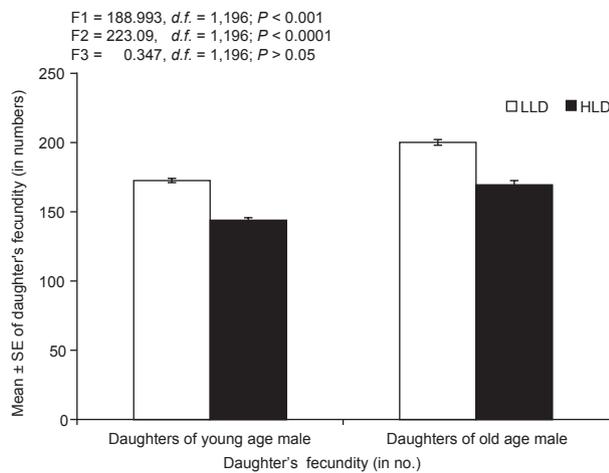
\*\* Significant at  $p < 0.001$ .

classes in the LLD and HLD (Table 7 and Fig. 7).

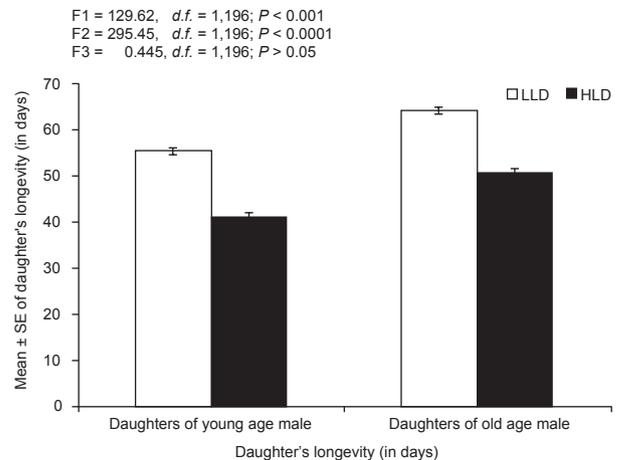
### DISCUSSION

In *D. bipectinata*, offspring of females mated with young- and old-aged males reared in LLD and HLD were analyzed to test the good-gene model associated with female preference for male age on the one hand and whether or not a father produces successful offspring on the other. Life-history traits such as pre-adult traits (egg to larval hatchability and larva to adult viability) and adult fitness traits (son's mating success, mating ability, progeny production, and longevity and daughter's mating success, fecundity, and longevity) are important components of fitness traits that a father can give to his offspring. Figure 1 reveals that in both LLD and HLD, offspring of old-aged males

had significantly greater egg to larval hatchability and larval to adult viability compared to offspring of young-aged males. This suggests that offspring pre-adult fitness increases with increasing male age. Our results also support the work of Pervez et al. (2004), who while studying a predatory ladybug, demonstrated a positive effect of male age on egg viability, and they found that eggs sired by 20-30-d-old males had significantly higher viability than those sired by younger males. In contrast to this, in *D. melanogaster*, Price and Hansen (1998) found decreased larval viability in eggs sired by older males compared to younger males. Similarly, the negative effects of male age on offspring fitness were also shown in other species. Serre and Robaire (1998) reported a significantly higher neonatal death rate in progeny of older male Norway rats. Jones et al. (2000) observed a higher egg hatching success in lekking



**Fig. 6.** Male age influence on daughter's fecundity (in no.) in low (LLD) and high larval densities (HLD) of *D. bipectinata*. F1, F value between age classes; F2, F value between larval densities; F3, F value for the interaction between age classes and larval densities.



**Fig. 7.** Male age influence on daughter's longevity (in d) in low (LLD) and high larval densities (HLD) of *D. bipectinata*. F1, F value between age classes; F2, F value between larval densities; F3, F value for the interaction between age classes and larval densities.

**Table 7.** Fecundity (no.) and longevity (d) of daughters of young- and old-aged males in low (LLD) and high larval densities (HLD) of *D. bipectinata*

Parameter value		Daughters of young-aged males	Daughters of old-aged males	t
Fecundity (no.)	LLD	171.52 ± 1.88	199.74 ± 2.03	9.80**
	HLD	143.28 ± 1.61	169.18 ± 2.27	10.45**
	t value	11.32**	10.10**	
Longevity (d)	LLD	55.34 ± 0.69	64.02 ± 0.71	8.49**
	HLD	40.88 ± 0.75	50.64 ± 1.03	7.86**
	t value	13.76**	9.98**	

\*\* Significant at  $p < 0.001$ .

sandfly females mated to young- and middle-aged than to older males. In the present study, greater egg to larval hatching ability and larval to adult viability of eggs sired by female mated with old male may reflect the higher quality of zygotes sired by older fathers. However, like in Jones et al. (2000), better egg hatchability might be a result of fertilization success.

The reproductive success of offspring of young- or old-aged males depends on their pre-adult fitness and also on adult traits, i.e., son and daughter success in mating, mating ability, progeny production, and longevity (Krishna and Hegde 2003). Table 4 shows that females of *D. bipectinata* were able to discriminate sons of young- and old-age classes, and they preferred to mate more frequently with sons of old-aged males over sons of young-aged males. This result was found to be similar in both LLD and HLD suggesting that the inheritance of successful mating traits from father to son was not influenced by larval density. The observed greater mating success of sons of old-aged males could be due to differences in the attractiveness of sons of young- and old-aged males. Studies showed that females that mated with attractive males provided better fitness benefits to their offspring such as greater longevity (Norris 1993, Petrie 1994), faster growth rate (Reynolds and Gross 1992), increased fecundity of daughters (Reynolds and Gross 1992, Moore 1994), and increased attractiveness of sons (Wedell and Tregenza 1999). In species in which male attractiveness is not associated with many morphological features, male attractiveness can be measured using components involved in courtship behavior, i.e., mating latency, copulation duration, and levels of activities during courtship, which can be used to measure the attractiveness of the male (Hegde and Krishna 1997). Males which show greater activities during courtship are more attractive (Hegde and Krishna 1997). Species of the genus *Drosophila* do not show body color, pigmentation, or morphological feature variations with male age or attractiveness. Hence the activity level of courting males may be used to indicate the attractiveness of males. In *D. bipectinata*, sons of old-aged males showed greater activity; they mated faster and copulated longer than sons of young-aged males during courtship, suggesting that sons of old-aged males were more attractive than sons of young-aged males. Even in the parental generation of *D. bipectinata*, old-aged males had greater courtship activities compared to young-aged males suggesting that old-aged

males were more attractive than younger-aged males (Somashekar and Krishna 2011). This attractiveness was also passed on to their sons.

Our results support the argument of Fisher (1950) who while working on *D. melanogaster*, pointed out that successful fathers could produce successful sons and pass a legacy of greater mating success on to their offspring. Our results in *D. bipectinata* also support studies of females which mated with attractive males: they provided fitness benefits to their offspring in the form of greater longevity (Norris 1993, Petrie 1994), faster growth rates (Reynolds and Gross 1992, Moore 1994, Welch et al. 1998), increased fecundity of daughters (Reynolds and Gross 1992, Moore 1994), and increased attractiveness of sons (Wedell and Tregenza 1999).

Since 5-6-d-old sons of young- and old-aged males were cultured and maintained in the same temperature and environmental conditions, the observed greater mating success cannot be attributed to difference in mating history, experience, or maintenance of sons of different male age classes; instead, it could be due to the difference in the male age classes. Another study of *Drosophila* also showed that male flies which inseminate more females in a given time also produce more progeny than male flies which inseminate a smaller number of females (Krishna and Hegde 2003). In *D. bipectinata*, we noted that sons of old-aged males inseminated more females in a given time and lived longer than sons of young-aged males (Figs. 3, 4). This suggests that sons of old-aged males had greater reproductive success than sons of young-aged males.

In *D. bipectinata*, the observed indirect genetic benefits might have been achieved either by passing good genes on to their offspring, thus ensuring more-viable sons and daughters or the heritability of male attractiveness, i.e., more-attractive fathers sired more-attractive sons. In contrast to this in *D. pseudobscura*, Avent et al. (2008) found that females of *D. pseudobscura* could not discriminate sons of young- or old-aged males. Even in *D. melanogaster*, the mating ability of old-aged males was found to be less than that of sons of young- and intermediate-aged males (Hansen and Price 1995). This suggests that species-specific differences may exist with regard to the influence of male age on offspring fitness. We also noted that females that mated with sons of old-aged males had significantly greater fecundity and fertility than those mated with sons of young-aged males (Fig. 5). Thus, these studies on *D.*

*biplectinata* confirm that successful fathers produce successful sons.

Daughters of old-aged males had greater mating success, fecundity, and longevity in both LLD and HLD than daughters of young-aged males (Tables 6, 7, Figs. 6, 7). This confirms that in *D. biplectinata*, females select old-aged males in their parental generation to obtain better offspring. The notion that mating with aged males may impose costs to the female somehow contributes to the view that male age is an honest signal of male genetic quality, because older males have proven their superior ability to survive (Manning 1985). This was not true in our study, as *D. biplectinata* females mating with old-aged males might not incur costs to the female; instead, females preferred to mate with old-aged male than young-aged males. Therefore in *D. biplectinata*, male age is an honest signal of male genetic quality. A female preference for old males was demonstrated in several species.

Among larval densities, offspring of old- and young-aged males in an LLD had significantly greater egg to larval hatchability and larval to adult viability compared to offspring in an HLD (Fig. 1). This suggests that rearing larval density has a significant influence on egg to larval hatchability and larval to adult viability. This supports earlier studies on the influence of larval density on egg-adult viability in *Drosophila* (Barker 1973, Barker and Podger 1970). Thus in *D. biplectinata*, although food shortages due to larval rearing densities influence offspring pre-adult fitness, it did not influence the inheritance of characters from father to offspring.

Thus pre-adult and adult fitness traits of *D. biplectinata* in LLD and HLD suggest that offspring fitness increases with increased male age. In contrast to results of Hansen and Price (1995), our results in *D. biplectinata* support the good-gene model because preferred old age males provide indirect benefits through the production of higher-quality offspring. Hence in *D. biplectinata*, females can use age as a reliable signal of heritable variations in male quality.

The experimental design eliminated potential maternal effects by mating 5-6-d-old females to young- and old-aged males. The results of our experiments in *D. biplectinata* are not in accordance with mutation accumulations in the germ line as the sole cause of genetic differences between ages, as there was no evidence for reduced pre-adult or adult fitness traits. Instead, we found increased offspring fitness. Furthermore,

this increased offspring fitness may also be attributed to the removal of deleterious mutation as male age increases. On the other hand beneficial mutations might have increased with increasing male age.

According to Hansen and Price (1999), age and sex variations in the mutation load are very dependent on the average effect of new mutations, and they argued that the mutation load most likely increases with age. Our results in *D. biplectinata* do not agree with this; instead deleterious mutation appeared to have been weeded out as males aged. Therefore offspring of old age males had greater fitness.

Jones et al. (2007), while studying hide beetles, found that sperm viability and sperm transfer vary with male age but were smaller than those of an intermediate age. It is not known whether the observed greater egg to larval hatchability and larval to adult viability in *D. biplectinata* can account for the total sperm viability or quantity of sperm transferred with male age.

Studies of *D. melanogaster* found that the sex ratio of offspring produced by females was biased with respect to the age of males to which they were mated (Long and Pischedda 2005). They showed that females mated to old males produced a greater proportion of daughters than did females mated to young males. In the present study in *D. biplectinata*, we did not count the numbers of daughters and sons produced by females mated with sons of either young- or old-aged males. It is not known whether the sex ratio of offspring produced by females of *D. biplectinata* was biased with respect to the age of males.

In hide beetles, Jone and Elgar (2004) found that intermediate-aged males had greater mating success, and females mated to intermediate-aged males had greater fecundity and fertilization success, but they did not study offspring fitness. In contrast in *D. biplectinata*, females mated to old-aged males had greater fitness traits (i.e., fecundity and fertility) than females mated to young-aged males. In the present study, even the offspring of old-aged males had greater fitness. This suggests that the female preference for male age varies among different species and genera. Furthermore, Liu et al. (2011) also showed that female cabbage beetles did not discriminate their partners on the basis of age classes.

In the Mexican fruit fly, Perez-Staples et al. (2010) found that females did not receive direct benefits by mating with old and sexually experienced males but may have obtained indirect

benefits. In contrast in *D. bipectinata*, females mating with old-aged males received direct benefits in term of greater fecundity and fertility and also indirect benefits in terms of greater offspring fitness.

It was suggested that if deleterious mutations accumulate in the germline with age, they will decrease the genetic quality of sperm and impose a cost on female fitness. If these mutations also affect sperm's competitive ability or production, then females will benefit from polyandry instead of a preference for male age (Radwan 2003, Gasparini et al. 2010). Female multiple mating is also common in *Drosophila*; however, it is not known whether females of *D. bipectinata* which first mate with old-aged males will undergo multiple mating more frequently or not. In contrast to our study, Prokop et al. (2007) found decreased offspring fitness of female bulb mites mated with old-aged males compared to females mated with sons of young-aged males.

In sexual selection, it was noted that many sexual displays, i.e., song traits, had a great influence on female mate preferences and were found to vary with male age (Verburgt et al. 2011). But it is not known whether or not offspring of different male age classes show variations in secondary sexual characters that are paternally inherited.

In the present study, both pre-adult and adult fitness components were found to be significantly greater in offspring of old-aged males compared to offspring of young-aged males. This suggests that there was an increased in breeding values of all the life-history traits of old-aged males studied. Our result do not support the work of Price and Hansen (1998) who suggested that the breeding value of *D. melanogaster* was reduced with an increasing male age. However, Price and Hansen (1998) studied only 3 different characters of egg-adult viability, son mating ability, and daughter fecundity. Two of the 3 characters showed decreased values in offspring of old-aged males compared to offspring and intermediate-aged males. The Hansen and Price (1995) model was based on quantitative genetics and did not consider genetic details. However in 1999 while studying age and sex distributions of the mutation load, Hansen and Price assumed that mutations have an overall deleterious effect on the total fitness components.

In *Drosophila*, mutations with a large effect were excluded as only 2%-5% of all *Drosophila* zygotes carry a new lethal mutation (Crow

and Simmons 1983). Kondrashov and Houle (1994) showed that the deleterious effects of mutations can be elevated in harsh environments. Furthermore, the majority of mutations in *Drosophila* may be caused by transposable elements (Green 1988), and these mutations may typically have weak deleterious effects (Keightley 1996).

Fitness distributions with male age in *D. bipectinata* also showed that fitness parameters increased with increasing age.

Life-history theories or tradeoff models which predict negative genetic correlations between fitness components may be common (Charlesworth 1990, Houle 1991). In the present study, we tested 11 different fitness components (four of sons, three of daughters, and two of pre-adult fitness) in offspring of young- and old-aged males. In *D. bipectinata*, we noted that there was no tradeoff in 8 different fitness components between offspring of young- and old-aged males. Therefore, our results do not agree with the tradeoff model, and instead support the view of Manning (1985) and other hypotheses of increasing fitness of offspring with increased male age. The present study also supports an earlier study of Partridge (1980) who found increased larval viability among offspring of females that were allowed to choose their mates. Thus in *D. bipectinata*, females select old-aged males to obtain indirect genetic benefits, which supports the good-gene model.

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