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Effect of rearing temperature on offspring fitness in *Drosophila bipectinata*

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Abstract

The thermal environment experienced by an individual during development has a direct and indirect effect on the female preference for their mate. A great diversity of male traits such as size, colour, pigmentation, courtship song, mating call are known to be used by female in mating decision to obtain direct and indirect fitness benefits. Male age is one such male trait that has received a lot of attention as a possible cue for female mating decision. In *D. bipectinata*, offspring of females mated with young, middle aged and old-aged males reared at three different temperatures 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. Adult fitness traits are important components of fitness traits that a father can give to his offspring. Our results in the present study suggest that sons of old-aged males had greater reproductive success than sons of young and middle-aged males.

Key words : Rearing temperature, *D. bipectinata*, female preference, offspring fitness

INTRODUCTION

Fitness of an individual not only reflect its genotype, parental age, immediate impact of the environment and other factors such as larval crowding, temperature encountered in early development can also contribute to it [1]. In such situation it is not known whether the parental age effect resulted in the offspring fitness is dependent of offspring rearing condition encountered early in development i.e. temperature.

The thermal environment experienced by an individual during development has a direct effect on final size, body weight increasing as temperature decreases [2]. Life history traits such as age specific survival and fecundity are of particular evolutionary interest because of their very close relationship of fitness itself [3]. Temperature often has a large environmental effect on life history traits of ectotherms [4]. Partridge et al., [5] showed that, at a given temperature, the flies from populations maintained at that temperature showed the highest longevity and fecundity, as expected from an adaptive response. The trade-off between longevity and early fecundity in outbred laboratory-adapted populations of *D. melanogaster* has been proposed as a possible example of a fundamental physiological constraint on life history evolution [6]. Leroi et al., [7] reported a role for genotype x environment interaction in the long term laboratory evolution of a genetic life history trade-off in *D. melanogaster*. Betran et. al [8] reported a statistically significant contribution of the second-chromosome inversions to the phenotypic plasticity of body size and developmental time in *D. buzzatii*. Further, these inversions make a positive contribution to the total genetic correlation between the traits, as expected by the suggested trade-off.

A great diversity of male traits such as size, colour, pigmentation, courtship song, mating call are known to be used by female in mating decision to obtain direct and indirect fitness benefits [9]. Male age is one such male trait that has received a lot of attention as a possible cue for female mating decision [10].

Various models have been proposed for female preference to male of different age classes. Good gene model suggests that old males are preferred as mates [13,14]. One potential explanation is that females are likely to gain indirect benefits, because older males higher viable and have demonstrated their survival ability and generality of the positive correlation between male longevity and genetic viability [10, 16], on which this explanation relies. Some model advocates the selection of middle aged males as mates [17, 18]. Potential explanations of this are that younger males will have accumulated fewer germ line mutations [19] that the presence of negative genetic correlations between traits results in tradeoffs between early and late fitness components [20] or in long lived organisms, that offspring born to younger parents are better adapted to current environmental conditions because their parents were exposed to recent selection [10]. However these models also predicted that preferences for younger males are equally possible, depending upon the conditions specified. The difference in conclusions of these models is a probable result of their contrary approaches towards the change in the physiological state of male with age and the existence of life history trade-offs.

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 [21]. 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.

Little attention has been paid to the parental age effect in studies of *Drosophila* except for the work of Parsons [22]. Further, 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 [23]. Very few attempts have been made to study the influence of male age on offspring fitness in *Drosophila* [14, 21]. 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 [15]. Therefore in the present study, sons of young, middle aged and old-aged males mated separately with 5-6-d-old virgin females were used to test the good-gene model [24] the three different varying environments like 15±1°C, 22±1°C, and 29±1°C situations.

MATERIALS AND METHODS

Male age influence on offspring mating success at different temperatures.

Experimental stock:

Progenies of three isofemale lines collected at Mysore were used to establish experimental stock. The stock was cultured using 40 flies in quarter pint milk bottles (250 ml) containing wheat cream agar medium and maintained them at 21 ± 1^{0}C, relative humidity of 70 % and 12: 12 L: D cycle for three generations.

Assigning of age classes:

Longevity of unmated male of this strain was studied by transferring separately and individually into a vial containing wheat cream agar medium once in a week and maintained them in the same laboratory condition until their death to assign male age class. Fifty replicates were made to calculate mean longevity (number of days a male lived from the time of its eclosion). Mean longevity of *D. bipectinata* was found to be 58 ± 5 days. Therefore young, middle and old age male were selected as follows {Young age male (2-3 days), middle aged male (24-25 days) and old aged male (46-47 days)}.

At 4th generation, eggs were collected separately by crossing young, middle and old age males separately with 5-6 days old virgin females using Delcour's procedure [25].One hundred eggs from these crosses were separately seeded in a vial (3″x1″) containing wheat cream agar medium and were placed them in different temperatures (15±1°C, 22±1°C and 29±1°C separately).

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- middle aged and old males were separately mated with 5-6-d-old virgin females to obtain offspring 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 the son's mating success using a female choice experiment reared at three different temperatures

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 the 2 sons of young, middle aged and old 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. 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 males were individually aspirated into an Elens-Wattiaux mating chamber [26]. 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 [27], 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 [27]. Fifty replicates were used for each combination in the female mate-choice experiment, and a Chi-square 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 all three rearing temperatures.

Male age influence on son's 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 [26] and observed for 1 h. Any pair that had not mated within 1 h was discarded. If mating occurred, we recorded the copulation duration (time between initiations of copulation to termination of copulation by the pair). Separate experiments were carried out for all three rearing temperatures.

Male age influence on progeny production

Soon after copulation, the mated female as in the above experiment 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. Experiments were carried out separately for all three rearing temperatures.

RESULTS

Male age influence on son's mating success in three different temperatures

Figure 1a-c shows male age influence on mating success of sons obtained from three different temperatures of *D. bipectinata* using female choice method. It was noticed generally that females of *D. bipectinata* chose to mate with sons of old age male more frequently than sons of either young or middle aged males. The son's mating success of old age male in crosses involving sons of young male and old aged male was 64% (n=50) (χ^2 = 3.91, df=1, P< 0.05) at 15±1°C; 68% (n=50) (χ^2 = 6.48, df=1, P< 0.001) at 22±1°C; and 62% (n=50) (χ^2 = 2.88, df=1, P< 0.05) at 29±1° C.

Among crosses involving sons of old aged male and middle aged male was 62% (n=50) (χ^2 = 2.88, df=1, P< 0.05) at 15±1°C; 70% (n=50) (χ^2 = 8.00, df=1, P< 0.001) at 22±1° C; and 60% (n=50) (χ^2 = 2.00, df=1, P>0.05) at 29±1° C.

The mating success of sons of middle age males was 68% (n=50) (χ^2 = 6.48, df=1, P< 0.001) at 15±1° C; 78% (n=50) (χ^2 = 15.68, df=1, P< 0.001) at 22±1° C; and 66% (n=50) (χ^2 = 5.12, df=1, P< 0.05) at 29±1° C in crosses involving sons of young and middle age males. Chi square analysis applied on mating success data showed significant variation.

Male age influence on son's copulation duration in three different temperatures

Son's copulation duration of young, middle and old age males are provided in Figure 2. Sons of old age male had copulated

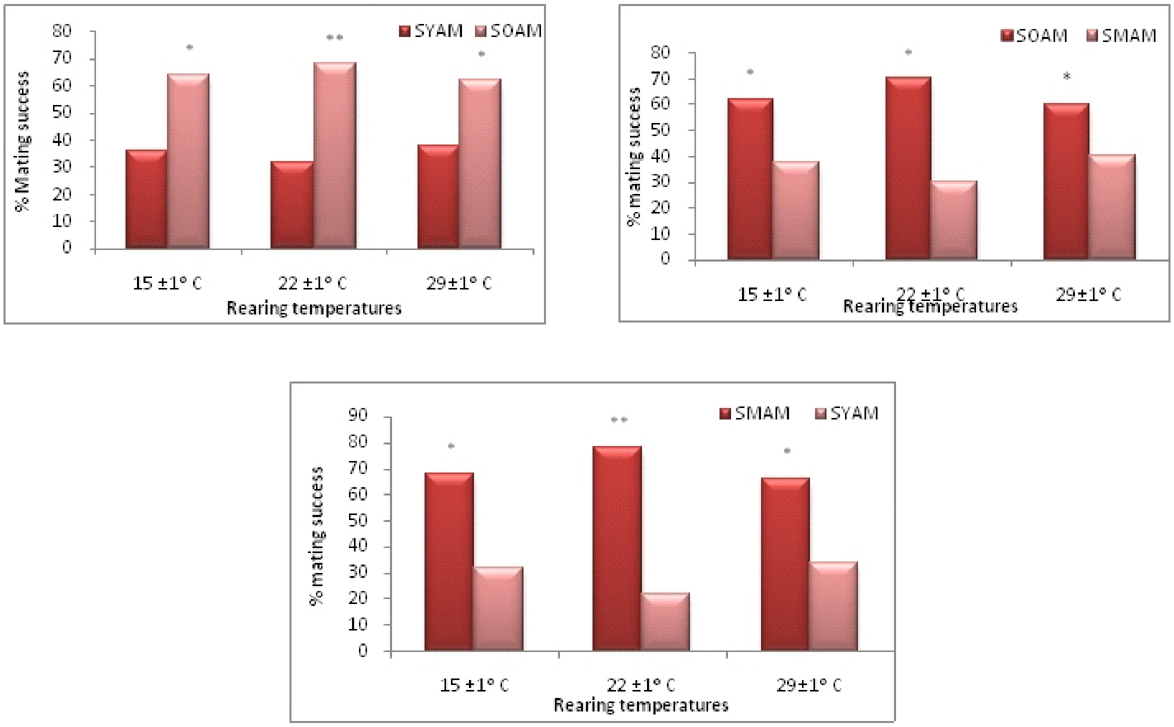

Figure 1a-c. Age effect of male on son's mating success reared at different temperatures in *D. bipectinata*

* Significant at 0.05 level by Chi Square test; ** Significant at 0.01 level by Chi Square test
SYAM- Sons of young male; SMAM-Sons of middle aged male; SOAM-Sons of old male

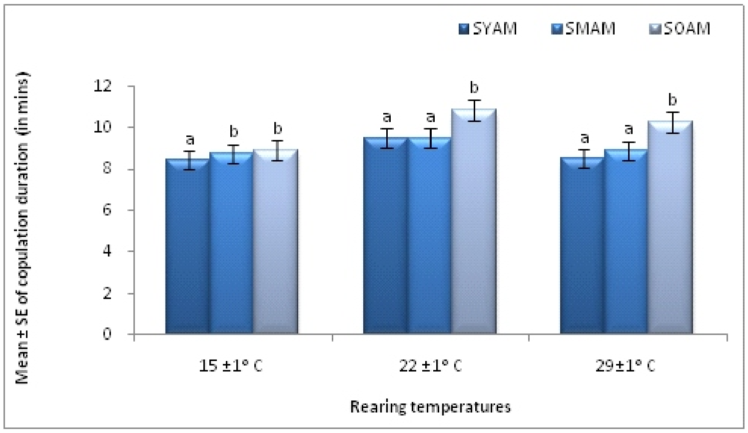

Figure 2. Age effect of male on son's copulation duration at different temperatures in *D. bipectinata*

Different letters on the bar graphs indicate significant difference by Tukeys post hoc test at 0.05 level
SYAM- Sons of young male; SMAM-Sons of middle aged male; SOAM-Sons of old male

longer compared to sons of young and middle age males. Among temperatures, sons obtained from 22±1° C copulated longer than flies in 15±1° C and 29±1° C. Two way ANOVA followed by Tukey's test carried out on son's copulation duration data (Table 1) showed significant variation between sons of different male age classes, between temperatures and also in the interaction between male age and temperatures.

Progeny produced by sons of different male age classes in three different temperatures

Figure 3 shows mean values of fertility of females mated to sons of young, middle and old age males in three different temperatures of *D. bipectinata*. Fertility of female mated to sons of old age male was found to be greater than female mated to sons of young or middle age male. Among temperatures, fertility of female mated to sons of different male age classes had greater fertility in 22±1° C than in 15±1° C and 29±1° C. Fertility data subjected to two way ANOVA followed by Tukey's test (Table 1) showed significant difference in female mated to sons of different male age classes, between temperatures and also interaction between male age classes and temperatures.

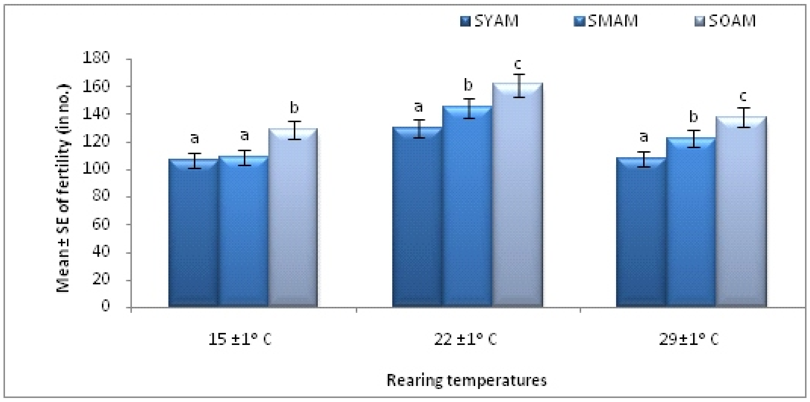

Figure 3. Age effect of male on fertility of females mated to sons of different age classes at different temperatures and larval densities in D. bipectinata

Different letters on the bar graphs indicate significant difference by Tukeys post hoc test at 0.05 level
SYAM- Sons of young male; SMAM-Sons of middle aged male; SOAM-Sons of old male

Table 1: ANOVA of age effect of male on son's copulation duration and fecundity at different temperatures in *D. bipectinata*

Parameter	Dependent	Type III Sum of Squares	df	Mean Square	F
Copulation duration	Rearing Temperature	86.157	2	43.078	56.153**
	Sons of different male age classes	122.048	2	61.024	79.545**
	Rearing Temperature * Sons of different male age classes	62.843	4	15.711	20.479**
	Error	338.318	441	0.767	
	Total	39620.501	450		
Fertility	Rearing Temperature	203078.004	2	101539.002	241.695**
	Sons of different male age classes	170658.151	2	85329.076	203.110**
	Rearing Temperature * Sons of different male age classes	73505.396	4	18376.349	43.742**
	Error	185269.560	441	420.112	
	Total	8659200.000	450		

** Significance at 0.001 level (p<0.001)

DISCUSSION

In *D. bipectinata,* offspring of females mated with young, middle aged and old-aged males reared at three different temperatures 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. 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 1a-c shows that females of *D. bipectinata* were able to discriminate sons of young, middle aged 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 all the three rearing temperatures suggesting that the inheritance of successful mating traits from father to son was not influenced by temperatures reared. The observed greater mating success of sons of old-aged males could be due to differences in the attractiveness of sons of different male age classes. Studies showed that females that mated with attractive males provided better fitness benefits to their offspring such as greater longevity [28], faster growth rate, and increased fecundity of daughters [29] and increased attractiveness of sons [30]. 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 [27]. Males which show greater activities during courtship are more attractive [27]. 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 by copulating longer than sons of young-aged males and middle aged males during courtship, suggesting that sons of old-aged males were more attractive than sons of either young or middle aged males. Even in the parental generation of *D. bipectinata*, old-aged males had greater courtship activities compared to young and middle-aged males suggesting that old-aged males were more attractive than younger-aged and middle aged males [15]. This attractiveness was also passed on to their sons. Our results support the argument of Fisher [31] who while working on *D. melanogaster*, pointed out those 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 [28], faster growth rates [29], increased fecundity of daughters [29], and increased attractiveness of sons [30]. Since 5-6-d-old sons of young, middle aged 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 [32]. In *D. bipectinata*, we noted that sons of old-aged males copulated longer than sons of young and middle-aged males (Figure 2 and Table 1). This suggests that sons of old-aged males had greater reproductive success than sons of young and middle-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. [14] 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 [10]. 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 produced significantly greater number of progeny than those mated with sons of young and middle aged males (Figure 3 and Table 1). Thus, these studies on *D. bipectinata* confirm that successful fathers produce successful sons.

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 [13]. This was not true in our study, as *D. bipectinata* females mating with old-aged males might not incur costs to the female; instead, females preferred to mate with old-aged male than young or middle aged males. Therefore in *D. bipectinata*, male age is an honest signal of male genetic quality. A female preference for old males was demonstrated in several species. Thus in *D. bipectinata*, although rearing environment like temperature has influences on the development of flies, it did not influence the inheritance of characters from father to offspring. In contrast to results of Hansen and Price [10], our results in *D. bipectinata* support the good-gene model because preferred old age males provide indirect benefits through the production of higher quality offspring. Hence in *D. bipectinata*, 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, middle aged and old-aged males. The results of our experiments in *D. bipectinata* are not in accordance with mutation accumulations in the germ line as the sole cause of genetic differences between ages, as there was 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 [10], 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. bipectinata* 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. [33], while studying hide beetles, found that sperm viability and sperm transfer vary with male age but were smaller than those of an intermediate 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 [34]. 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. bipectinata*, we did not count the numbers of daughters and sons produced by females mated with sons of either young, middle aged or old-aged males. It is not known whether the sex ratio of offspring produced by females of *D. bipectinata* was biased with respect to the age of males. In hide beetles, Jone and Elgar [35] 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. bipectinata*, females mated to old aged males had greater fitness traits (copulation duration and fertility) than females mated to young and middle-aged males. In the present study, even the offspring of old-aged males had greater fitness; it suggests that the female preference for male age varies among different species and genera. Furthermore, Liu et al. [36] 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. [37] 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 preference for male age [38]. 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. [39] 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 [40]. But it is not known whether or not offspring of different male age classes show variations in secondary sexual characters that are paternally inherited. This suggests that there was an increased in breeding values of all the life-history traits of old-aged males studied. Our result does not support the work of Price and Hansen [21] who suggested that the breeding value of *D. melanogaster* was reduced with an increasing male age. However, Price and Hansen [21] 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 [10] 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 [41]. Kondrashov and Houle [42] 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 and these mutations may typically have weak deleterious effects. 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 [42]. In the present study, we tested different fitness components in offspring of young, middle aged and old-aged males. In *D. bipectinata*, we noted that there was no tradeoff in fitness components between offspring of young, middle aged and old-aged males. Therefore, our results do not agree with the tradeoff model, and instead support the view of Manning [18] and other hypotheses of increasing fitness of offspring with increased male age. The present study also supports an earlier study of Partridge [43] 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.

CONCLUSION

In *D. bipectinata* the thermal environment experienced by an individual during development has a direct and indirect effect on the female preference for their mate. Our study shows that male age is one of the important traits that have a significant and possible cue for female mating decision. Our results in the present study suggest that sons of old-aged males had greater reproductive success than sons of young and middle-aged males. Thus in *D. bipectinata*, females select old-aged males to obtain indirect genetic benefits, which supports the good-gene model.

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