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Male diet influence on male mating ability and progeny size in two sympatric species of *Drosophila*

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Abstract
The amount and quality of nutrients consumed by organisms have a strong impact on fitness, life-history traits and reproduction. The balance between energy acquisition and expenditure is crucial to the survival and reproductive success of animals. Male diet has been examined in two sympatric Drosophila species, *D.bipectinata* and *D.malerkotliana*, to see how it affects male mating ability and progeny quantity. It was shown that males fed a high-protein diet inseminated more females and produced more progeny than males fed a high-carbohydrate diet or a control diet. *D. malerkotliana* has a much higher male mating ability and offspring output than *D. bipectinata* among sympatric species. As a result of these investigations in sympatric Drosophila species, it appears that dietary regimes have a considerable impact on male mating capacity and progeny production. Further protein rich diet increases male fitness in species of *Drosophila*.

Keywords: male mating ability, *D. melanogaster*, male diet, progeny number

Introduction

Reproductive capacity is a mechanism by which organisms enhance their fitness since biological processes observable in an organism are directly tied to reproduction (Turner and Andersson, 1983) [18]. Mating latency, mating time, duration between mating, fertility, fecundity, productivity, viability, and longevity are all factors in fitness. These mechanisms, taken together, determined the fertility component of fitness, which is defined in the broadest meaning (Turner and Andersson, 1983) [18]. *Drosophila* is an excellent model organism for studying reproductive capability since its mating behavior, stimuli involved in courtship, and male-female contact during mating have all been thoroughly characterized (Singh and Singh, 2002). Under both natural and laboratory environments, remating is prevalent in many Drosophila species. In Drosophila, multiple mating has a direct relationship with fitness (Koref-Santibanez, 2001) [9]. Male and female remating times in many Drosophila species have been investigated. (Karif-Santibanez, 2001; Singh and Singh, 2002) [9]. Remating and sperm preservation are two distinct characteristics that can influence female fecundity and male mating success. Male remating capacity is a component of male fitness that is regulated by genetic and environmental variables (Partridge *et al.*, 1986; Gillot, 2003; Roopashree *et al.*, 2001) [13, 2, 15]

Male characteristics such as size and age have also been demonstrated to affect male mating ability and offspring production in *Drosophila* species in recent studies (Hegde and Krishna, 1997; Abholhasan and Krishna, 2014) [10, 1]. These investigations revealed that large males in drosophila species have a higher reproductive potential and can inseminate a greater number of females in a shorter period of time than small males. In the *D.melanogaster*, it has been discovered that young males inseminate more females than medium and older males. In nature, the availability of diet composition in terms of both quantity and quality can change greatly over an individual's life time; hence it is an essential element influencing an individual's reproductive capacity in a particular organism. The amount and quality of nutrients intake by organisms have a strong impact on life-history traits, such as disease vulnerability, fertility, reproduction, longevity and stress resistance (Hoffmann and Parsons, 1991; Rion and Kawecki,2007; Lee *et.al.*, 2008) [6, 14, 11]. Thus, rather than calorie quantity per se, it is diet composition, such as the protein-to-carbohydrate ratio, that is important. Males' capacity to attract females and obtain mating may be affected by nutritional state in several insects (Sisodia and Sing, 2002) [16]. As a result, two sympatric species of the *melanogaster* species group, *D. malerkotliana* and *D. bipectinata*, were used in the current study. The following are the objectives of this study: 1) to determine the impact of male feeding regimens on male mating ability and offspring production.

Materials and Methods

The experimental stocks of *D. bipectinata* and *D. malerkotliana* used in this work were created from Isofemale lines of wild-caught females collected in August 2018 in Mysore, India. These stocks were cultivated and maintained using 40 flies (20 males and 20 females) per quarter-pint milk bottles (250 ml) utilizing wheat cream agar medium at 21°C and 70 percent relative humidity in a 12:12 light: dark cycle. Delcour method was used to collect eggs from these flies. Twenty-five eggs were then placed in separate culture bottles with 7mL wheat cream agar medium (control diet) wheat cream agar medium + 60% casein (protein rich diet) as well as wheat cream agar media + 20% sucrose (Carbohydrate rich diet). Before adding water, prepare the carbohydrate enriched medium by mixing wheat cream agar media and sucrose in a 4:1 ratio (20 percent carbohydrate). Similarly, Casein and wheat cream agar media were mixed in a 3:2 ratio to make protein-enriched medium. In *Drosophila* culture vials containing 7ml of contol/protein-rich / carbohydrate-rich media were taken and utilized to cultivate the experimental flies. A dried yeast solution was pasted into all vials (Drosophila culture vials).

These culture vials were housed in the lab under the same circumstances as before. Unmated male and females these diets were isolated within 3hrs of their eclosion and were used in the present experiments.

Male fitness and mating ability are influenced by male diet

Virgin females and a male (control/carbohydrate rich/protein rich media) were aspirated into an-Wattaiux mating chamber and watched for an hour. Flies that did not mate within an hour of being released were discarded. The flies were allowed to complete copulation if they mated, and the duration of copulation was recorded (time between initiations to termination of copulation of each pair). Every 24 hours after mating, the mated female was put into a vial containing wheat cream agar media. This was carried on till the female died. Mated male was given a 5-6 day virgin female once more, and observation was continued for one hour. This process was repeated and total numbers of females copulated were recorded. Mated females were moved to a vial containing wheat cream, medium once every 24 hours, and this process was continued until the mated female died. The total number of progeny they produced was recorded. For each male diet, fifty trails were made. One way ANOVA followed by Tuckey,s post hoc test was carried out male mating ability and progeny size.

Results and Discussion

The most compelling studies of mating in various drosophila species have shown that each mating provides an opportunity for drosophila males to inseminate and transfer sperm to mating females, implying that high male mating rates are typically associated with high male reproductive success in species. Further, because male *Drosophila* do not offer parental care to progeny following female insemination, males must inseminate more sperm to mated females in each mating and prevent females from re-mating in order to maximize fitness (Thornhill and Alcock 1983; Parker 1970, Jones and Elgar 2004, Jones *et al.*, 2007) [12, 7, 8]. In organisms that go through repeated cycles of fast population increase, male mating ability is a particularly strong indicator of fitness, and it has developed as a mechanism for species to maximize their chances for survival. Female reproductive success is determined by the number of eggs laid, while male reproductive success is determined by the number of females inseminated. The present study provided the opportunity of evaluating and comparing the effects of resource quality on male fitness in two sympatric species of *Drosophila* such as *D. malerkotliana* and *D. bipectinata*. Table 1 reveals that male fitness potential in *D. malerkotliana* and *D. bipectinata* may be strongly affected by diet quality and quantity. In both the species males fed a protein-rich diet inseminated more females in a given unit of time than males fed a carbohydrate-rich diet. This shows that the amount of nutrients in a drosophila's diet has a major impact on his ability to reproduce. This research also verifies the impact of male nutrition on mating activity and fitness in a variety of insects, including *Drosophila* species. Furthermore, *D.malerkotliana* males were able to inseminate more females in a given unit of time than *D. bipecinata* in the current study, indicating that there are species differences in male mating ability in *Drosphila* species. In their study of *D.melanogaster*, Abholhasan and Krishna (2014) [10] found that there was inter-stock variance in male mating capacity, with the outbred stock inseminating more females in a given unit of time than the Conton-S stock. Similarly, Guru Prasad *et al.* (2008) [4] discovered inter population variability in male mating ability in *D.bipectinata*. Male mating ability differences among different geographic populations experimental stock demonstrate interactions of these stocks with various surroundings, implying that male mating ability is a fitness feature. It was also noticed females that mated with males at young age had produced greater number of progeny than females that mated with either middle aged or old males. In the present study females mated with males fed a protein rich food had significantly greater progeny size than those females mated either with males fed with a carbohydrate rich diet or control diet in *D.bipectinata* and *D.malerkotliana* (Table 2). This suggests that there is positive relation between male diet, male mating ability and progeny size in species of *Drosophila*.Our study supports widely accepted view that males which inseminate more females produces more progeny than those that of males that inseminate less number of females (Gromko, 1992) [3]. Our study also confirms the earlier works that male remating increases male fitness in different species of Drosophila (Krishna and Hegde, 1997; Guru Prasad *et al.,* 2008) [10, 4]. In *D.melanogaster* young males were able to inseminate more females in a given unit of time and produces greater progeny size than older flies showing that male age has a significant impact on male mating ability progeny size in (Abolhasan and Krishna, 2014) [1]. Thus these studies in sympatric species of *Drosophila* suggests that dietary regimes has significant influence on male mating ability and progeny production. Further protein rich diet increases male fitness in species of *Drosophila*.

Table 1: Influence of male diet on male mating ability in two sympatric species of Drosophila

Dietary regimes	Male mating ability (in no.)	
	D. bipectinata	***D. malerkotliana***
Control	1.21+ 0.18^a	1.23+ 0.62^a
Protein rich	1.42+0.16^b	1.52 +0.71^b
Carbohydrate rich	1.18 + 0.10^c	1.19+0.17^c
F-value	19.521**	18.62**

**P<0.0001 level

Table 2: Influence of male diet on progeny size (fertility) in two sympatric species of *Drosophila*

Dietary regimes	Progeny size (in no.)	
	D. bipectinata	***D. malerkotliana***
Control	142+ 1.18^a	149+ 1.62^a
Protein rich	201+1.16^b	215 +1.71^b
Carbohydrate rich	139 + 1.10^c	141+1.17^c
F-value	42.825**	43.42**

**P<0.0001 level

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