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Body size, mating success and advantage of large flies in *Drosophila bipectinata* species complex

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Mating success of large and small flies of *Drosophila malerkotliana* and *D. bipectinata* was studied using multiple, male and female choice methods. In multiple choice method the large male mated with large female and small male paired with small female. In female choice method, large male was successful in mating with female irrespective of its size while in male choice method, preferential mating occurred between large male and large female as well as small male and small female. The consequence of such non-random mating in the presence of male rivarly and preferential mating has been discussed.

A positive correlation between body size and male mating success has been a common finding in many insects. Size has been an important theme in many investigations of *Drosophila* mating system[1-3]. Partridge *et al* [4] and Santos *et al* [3] have shown the influence of body size on mating success. Body size also influences mating speed, fecundity and other fitness characters [5,6,2,7,8]. Laboratory studies have demonstrated male mating success is related to male size in a number of *Drosophila* species [4,1]. Very few studies of *Drosophila* male mating success have been conducted in the field but those which have been reported also support the importance of male size in *D. melanogaster* [4] *D. nigrospiracula* [1] *D. buzzatii* [2,7] and *D. testacea* [9].

In nature, mating males are larger than non-mating[4] and the advantage of large size is only for males and mating females are less variable than non-mating one [4]. The reasons suggested for higher success of large males are intrasexual selection in the form of aggression or scramble type of competition for receptive females or because large males are more active, can move faster, they not only encounter more receptive females than do small males, but better able to track females when they move during courtship[4]. Higher mating success also seems to be related to the rate of encounter of females, their receptivity and vigour of males. Though most of these studies highlight the importance of large males, there are a few reports showing that body size is an important component of male mating success but it is not the largest male who is always successful. For example Santos *et al* [7] showed that both males and females of *D. buzzatii* can outlive and outmate small flies. They showed linear increase in mating success with increasing thorax length. Steele [10] showed that large *D. subobscura* males have greater courtship success with starved females and this is probably because they produce larger drops of food during courtship. If all males are prevented from producing drops then the small males have greater courtship success than the large males.

Thorax length has been used as an index of body size in *Drosophila* [4,2,7]. Apart from thorax length other morphological traits such as wing length, wing width, face width have also been used as index of body size. Wing is another phenotypic trait which can be used as an index of body size [11,12]. In *Drosophila* wing plays an important role in courtship because species specific auditory signals (courtship song) are produced by male's wing vibration [13]. Aspi and Hoikkala [14] showed the importance of male song on mating success of *D. littoralis* and *D. montana*. A male with long wings produces more effective courtship song than those with short wings. Using wings as an index of body size Monclus and Prevosti[15] and Naseerulla and Hegde [12] have shown that fast mating flies have longer wings than slow and non-mating *D. subobscura* and *D. malerkotliana* in multiple choice situation. Thus most of these studies have highlighted the advantage of large size in males but not the largest of the two competing males (example the advantage of large size was noticed when competing male was small, if the competing male is larger then which is advantageous is the question). The present study therefore, is aimed at understanding (1) the role of male and female size on mating success to study whether preferential mating exists for body size or not (using multiple, male and female choice methods; and (2) bigger is best in *Drosophila* or not.

Materials and Methods

Two species of *Drosophila bipectinata* complex namely *Drosophila malerkotliana* and *D. bipectinata* were used. All experiments were made separately for each of the two species. The stocks used in the present study originated from 150 naturally inseminated females from Mysore, Karnataka. When progeny appeared, flies were distributed to different culture bottles and were maintained under constant temperature (22°± 1°C). In every generation flies multiplied in different culture bottles were mixed together and eggs were collected using Delcour's procedure[16]. Eggs(100) were seeded in fresh quarter pint milk bottles with 25 ml of wheatcream-agar medium to avoid larval crowding during development (this procedure allow us to reduce environmental variation in size). After 10 generations, when adults emerged, virgin females and males were isolated within 3 hrs of their eclosion and maintained separately at 22°±1°C. Wing lengths of male and female flies were measured separately when they reached required age. Each fly was etherized individually, the intact left wing kept in horizontal plane was measured from humeral crossvein to the tip with an occular micrometer at X 100 magnification. Wing length was measured in unit of 1/10mm. After measuring the wing length, each fly was placed separately in fresh food vial to study mating success.

Wing size and mating success—Five to six days old flies with long or short wings of the same species (see Tables 1 and 2 for chosen wings size) were used to study whether there is any preferential mating between large and small flies. Multiple, male and female choice methods were adapted for this purpose. In multiple choice experiment, 10 pairs (males and females) of long and short winged flies were introduced into the mating chamber. In male choice (male preferential) experiment, 10 males with long wings were introduced along with 10 females with long wings and 10 females with short wings in to the mating chamber.

A reciprocal cross was made with 10 males with short wings and 20 females, 10 with long wings and 10 with short wings. Similarly, in female choice (female preferential) mating experiment, 10 females with long wings were introduced into the mating chamber along with 10 males with long wings and 10 males with short wings. Reciprocal cross was made for female preferential mating experiment also.

To identify the males or females with long or short wings, in multiple choice experiment both males and females with long wings were painted with Indian ink. In male choice method, females with long wings and in female choice males with long wings were painted with Indian ink on the scutellum. The effect of painting was tested before commencing the experiment by painting the small flies in one set and large flies in another and allowing them to mate. The results indicated that painting has no effect on the performance of these flies.

Table 1 - Wing length and mating success in *D. malerkotliana* analysed by female and male choice methods

A-Female choice method

	Males: Large	Males: Small	Total no. of pairs mated	
Females				
Small female	68(77)	30(20.5)	98	χ^2=14.73*
Large female	86(77)	11(20.5)	97	χ^2=57.98**
Total	154	41		

B-Male choice method

	Females: Large	Females: Small	Total no. of pairs mated	
Males				
Small male	23(51.5)	60(38.5)	83	χ^2=16.49*
Large male	80(51.5)	17(38.5)	97	χ^2=40.92**
Total	103	77		

Expected numbers in the contigency χ^2-square test are given in parentheses.
P values: * < 0.001; ** < 0.0001
Small female-(15.88 units) Large female-(18.39 units)
Small male-(14.34 units) Large male-(17.06 units)
Wing lengths of large and small flies is given in parentheses

When mating occurred pairs in copulation were removed with an aspirator without disturbing mating. Observation was made for 1 hr and number of flies mated was recorded. Flies which did not mate within 1 hr were considered as unmated. A total of 10 trails were made for each set in separate mating chamber.

Influence of male or female size on mating success and fertility—Five to six days old virgin females and bachelor males were used to study the influence of male or female size on mating success and fertility. The male and female choice methods were employed here. In female choice method, two males with known size difference (0.1, 0.13, 0.15, 0.18, 0.25, 0.28, 0.35, 0.40, 0.50, 0.53, 0.75, and 0.78 units) and a female of unknown size were introduced into the Elens- Wattiaux mating chamber (eg. cross 1; ♀ x 1st ♂ + 2nd ♂ : difference in wing size between two males was 0.1 units; cross 2; ♀x 1st ♂ + 2nd ♂ : difference in wing size between the two males was 0.13 units etc). Similarly in male choice method two females with known size difference (0.1, 0.13, 0.15, 0.18, 0.25, 0.28, 0.35, 0.40, 0.50, 0.53, 0.75, and 0.78 units) and a male of unknown size were introduced into the Elens-Wattiaux mating chamber (eg. cross 1; ♂ x 1st ♀ + 2nd ♀ : difference in wing size between two females was 0.1 units : cross 2; ♂x 1st ♀ + 2nd ♀ : difference in wing size between the two females was 0.13 units etc). Observation was made for 1 hr. Flies which did not mate within 1 hr were considered as unmated. In the female choice experiment number of females mated by large males was recorded. Similarly in the male choice experiment number of large mated females were recorded. Total 50 trials were run for each cross and χ^2-square test was carried out.

Table 2 - Wing length and mating success in *D. bipectinata* analysed by female and male choice methods

A-Female choice method

	Males: Large	Males: Small	Total no. of pairs mated	
Females				
Small female	69(78)	29(20.5)	98	χ^2=16.33*
Large female	87(78)	12(20.5)	99	χ^2=56.82**
Total	156	41		

B-Male choice method

	Females: Large	Females: Small	Total no.of pairs mated	
Males				
Small male	25(50.5)	61(39.5)	86	χ^2=15.07*
Large male	76(50.5)	18(39.5)	94	χ^2=35.79**
Total	101			

Expected numbers in the contigency χ^2 -square test are given in parentheses.
P values : * < 0.001 ; ** < 0.0001
Small female - (16.32 units) Large female - (19.36 units)
Small male - (14.40 units) Large male - (17.28 units)
Wing lengths of large and small flies is given in parentheses

To study fertility of large males in female choice method and large females in male choice method, flies mated as above were separately transferred to individual food vials containing wheatcream-agar medium. These flies were again transferred to fresh vials containing food on every 24 hr without etherization up to 15 days. Total number of flies emerged from each vial was recorded. One way analysis of variance was carried out on mean fertility of large males in female choice and large females in male choice methods.

Results

Mating success of long and short winged flies of *D. malerkotliana* and *D. bipectinata* under multiple choice situation are given in Fig. 1. It is clear

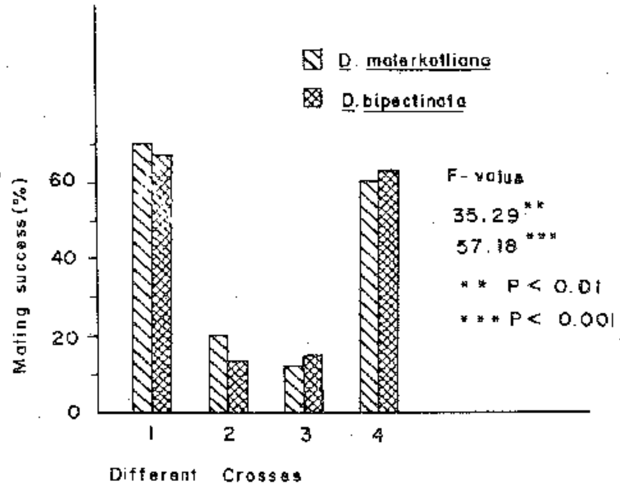

Fig. 1 - Preferential mating in *Drosophila*. [Different crosses: 1. Large male with large female; 2. Large male with small female; 3. Small male with large female; 4. Small male with small female].

that in *D. malerkotliana* when both males and females of both sizes were present long winged males preferred to mate long winged females (70) and small winged males preferred small winged females (60). Even in *D. bipectinata* (67) large males mated large females and (63) small males paired with small females as against (13) large males with small females and (15) small males with large females.

Data on wing length and mating success in the male and female choice methods of *D.malerkotliana* are given in Table 1. In female choice method, the female with short wings was mated by a male with long wings. Out of 98 matings, 68 matings were of this type. In the reciprocal cross out of 97 large females 86 mated with large males. In both the crosses male with long wings was successful to mate. In male choice method, out of 83 short winged males, 60 mated with short winged females and the remaining males mated with long winged females. In reciprocal cross, out of 97 long winged males, 80 mated with long winged females while remaining 17 mated with short winged females.

Table 2 shows wing length and mating success of *D.bipectinata* using male and female choice methods. In female choice method, the female with short wings was mated by a male with long wings

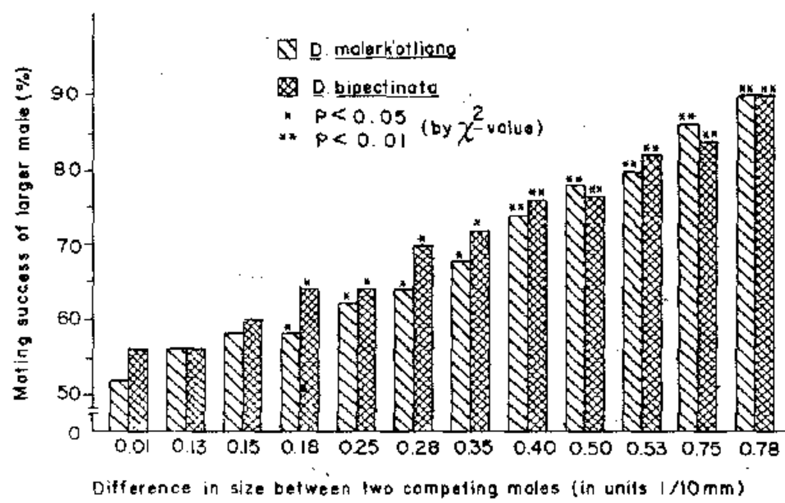

Fig. 2- Body sige related mating success in *Drosophila* (using female choice method).

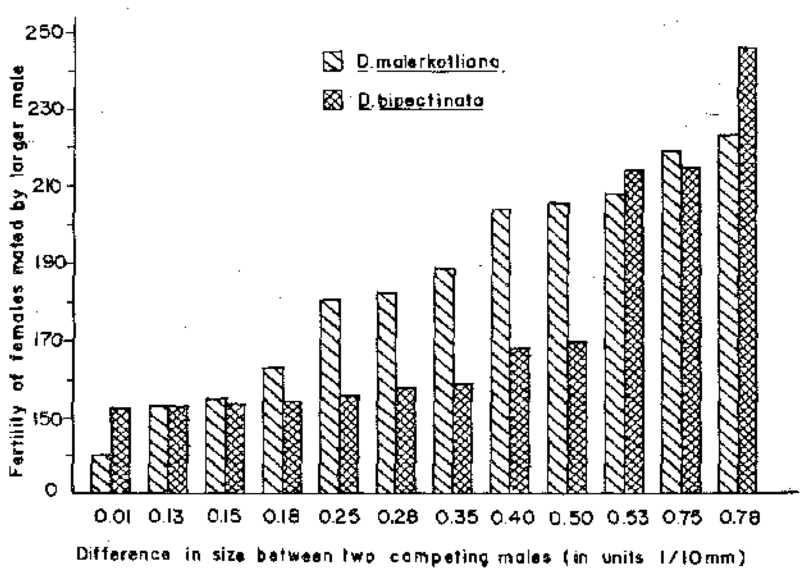

Fig. 3- Body sige related mating success in *Drosophila* (using female choice method).

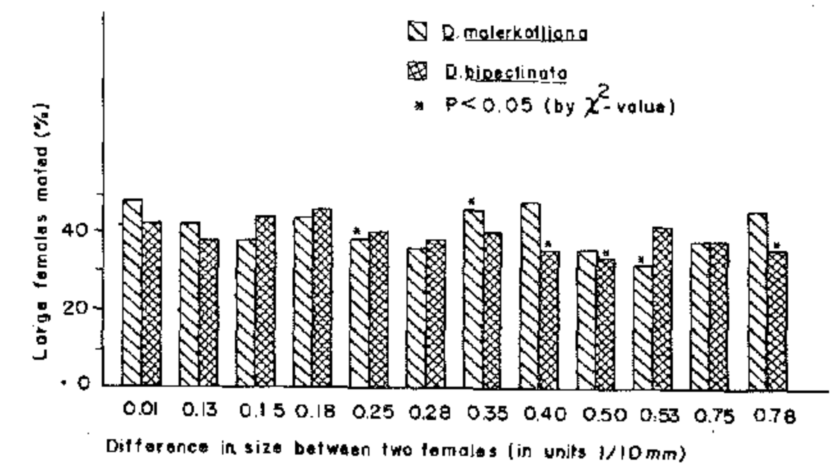

Fig. 4- Body sige related mating success in *Drosophila* (using male choice method).

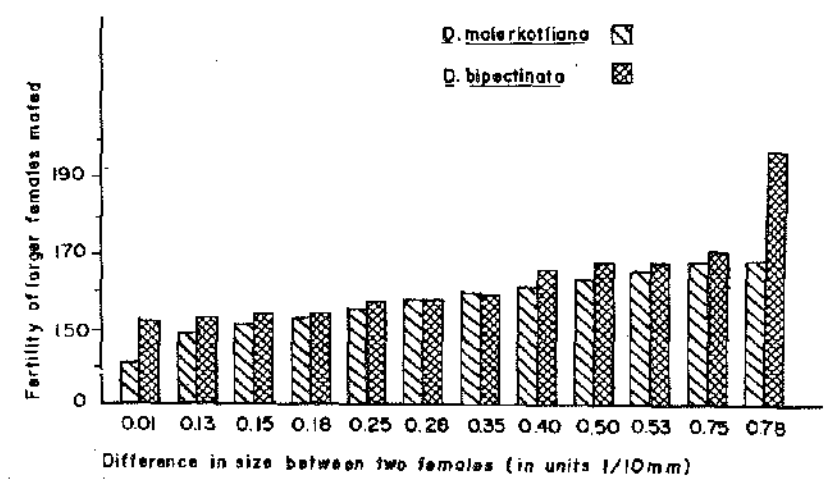

Fig. 5- Body sige related mating success in *Drosophila* (using male choice method).

in 69 out of 98 pairs mated. In the reciprocal cross, out of 99 pairs mated 87 large females mated with large males. In male choice method, out of 86 short winged males mated, 61 were mated with short winged females remaining 25 mated with long winged females. In reciprocal cross, out of 94 long winged males mated, 76 were mated with long winged females and remaining 18 long winged males paired with short winged females. The overall results obtained on wing length and mating success using multiple choice, male choice and female choice method of *D. malerkotliana* and *D. bipectinata* are same.

Body size related mating success of male in *D. malerkotliana* and *D. bipectinata* are given in Fig. 2. This data is obtained on the basis of series of female choice methods between a female and two males with known size difference. Mating success of large male increases with increasing difference in wing lengths of two males. Further mating was random when difference in size between two males was less than 0.25 mm. When the difference in size between two males was greater than 0.28 mm then mating was non-random (preferential). Even in *D. bipectinata* same observations were made (Fig. 2).

Body size related fertility of male *D. malerkotliana* and *D. bipectinata* are given in Fig. 3. Fertility of female mated by large male increases with increasing difference in wing length between two males. One way analysis of variance carried out on mean fertility of large male showed significant variation in fertility (*D. malerkotliana* F = 114.91, $P < 0.001$, *D. bipectinata* F = 286.68, $P <$0.0 0001).

Fig. 4 shows body size related mating success of female *D.malerkotliana* and *D. bipectinata*. Percentage of mating of large female did not increase with increased difference in wing length of two females. However, body size related fertility of female *D. malerkotliana* and *D. bipectinata* (Fig. 5) show that fertility increased when the size difference between the two females was higher. One way analysis of variance applied on mean fertility data showed significant variation (*D. malerkotliana* F = 128.16, $P < 0.001$; *D. bipectinata*, F = 113.44, $P < 0.001$).

Discussion

In the present study preferential mating of large male with large female and small male with small female under multiple choice situation was observed in both *D. malerkotliana* and *D. bipectinata*.

According to Santos *et al* [2] males or females could enhance their reproductive success if they are able to prefer their mates by means of "male choice" (male preferential mating) method: If males prefer from a variety of females and mate with those which are large and consequently have higher fecundity or "Female choice" (female preferential mating) method: If females prefer from a variety of males and mate with those which confer higher quality to the offspring. Further the male or female choice tests would also indicate the role of male or female in sexual selection.

In the female choice methods (Table 1) in *D.malerkotliana* when both large and small males were available, the small female was mated by large male. In the reciprocal cross also the large male was successful. It is evident that large males could succeed in both the occasions because they could win the competition with small males. This is in agreement with the work of Krebs and Barker[17] who while studying male size difference and mating success in *D. buzzatii* showed that when two males that differed in thorax length by atleast 100 units (approximately 0.04 mm) were placed in a mating vial with single virgin female, the larger of the two mated with the female. Dow and Schilcher[20] while studying aggression and mating success in *D. melanogaster*, showed that large male was successful to mate the female. Even in *D. bipectinata* (Table 2) large male was successful in the female choice showing that large male can win over the smaller. In *D. ananassae*, flies with high number of sternopleural bristles which are larger in size are more successful in mating than those with low number of bristles (medium size)[18,19].

However in the male choice method (Tables 1 and 2) of *D. malerkotliana* and *D. bipectinata* where there was no competition between different types of males, and females of both sizes were available the large female was mated by large male. Similarly a small female preferred small male. Thus this assortative mating with in the species of *D. malerkotliana* and *D. bipectinata* also indicates that the females can descriminate the males during mate selection. This agrees with the finding of Singh and Chatterjee[21] who while studying intraspecific sexual isolation in *Drosophila* found that females are more important in sexual selection than males.

Figures 2 and 3 show the body size related male mating success and fertility in *D. malerkotliana* and *D. bipectianata* analysed through "female choice" method. In these methods the size of the two competing males was different in different sets. When the difference in wing length increases between the two rival males the larger will succeed leading to non-random mating. In otherwords the larger male could win over the smaller in getting the female partner. Though there is no physical fight in *Drosophila*, larger male by virtue of his higher vigour would be successful in obtaining the mate. This agrees with the work of Butlin *et al* [22] who while studying the effect of a chromosomal inversion on adult size and male mating success in the seaweed fly (*Coelopa frigida*) have shown that when the data are grouped in terms of size difference between two competing males, the mating is random if the difference in size is less than 0.2 units otherwise the mating is non-random. Contrary to the above data, Fig. 4 show that in females the mating success does not depend on body size. According to this figure, in the male choice method when two females of differing sizes were present the male mated with one of the females (because size of male was unknown). Though the size of the two available females was different, the male was of medium size. There was no choice for the females except to mate with the available male hence mating was random. Fig. 5 provides information on mean fertility of females mated in the above experiments. When the difference in size between the two rival males was higher, the larger succeeded and the female thus mated had higher fertility. Therefore it is clear that the large male not only has higher mating success but also has higher fertility. In *D. ananassae*, males and females with high number of sternopleural bristles are more successful in mating than those with low number of bristles[18]. Flies with high number of bristles are larger in size than those with low numbers of bristles. Further *D. ananassae* flies with high number of bristles show greater fertility than those with low number of bristles [19].

Though mating was random in the male choice experiment (Fig.4) when females of differing sizes were present, the mated large females had higher fertility than smaller (Fig. 5). The fertility increased with increased difference in female size. Thus it is evident that the female size though does not influence the mating success, the larger female by virtue of high fertility can produce more offsprings than smaller. This agrees with the work of Tantaway and Vetukhiv[23] who found positive relationship between body size and fecundity.

Natural population can be compared to a multiple choice situation where a variety of males and females are available. Under natural conditions large males seem to have higher mating success by virtue of their aggression to mate with females and large females may have higher success because they can discriminate the size of their mate. The finding of Ewing[24] that small males are less preferred than large males when both are present would confirm the above statement. In the present study on mating success of large and small flies (Fig. 1) under multiple choice situation also showed preferential matings of large male with large female and small male with small female. The female choice method here, which partially resembles natural condition, when multiple males are available large male was successful to the mate female. The male choice experiment has demonstrated the preferential mating; the large female prefers large male and small female to small male. Perhaps in nature, both large males and females have higher mating success than small individuals by virtue of large females preference to mate with large male, and large male's aggression to mate with more females. Thus it is evident that preferential mating exist for body size in *Drosophila* and largest flies are advantageous.

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