Groga sample was 33.70. A similar value was obtained for *D. subobscura* (34.97). Finally, in the study of species diversity the values obtained for *H'* (Shannon diversity index) and *J* (Shannon uniformity index) were 0.678 and 0.421, respectively. These estimates are very similar to those obtained in September 2009 in Montpellier by Calabria (2012), who reported *H'* = 0.679 and *J* = 0.422, but differ from those reported by the same author in a Font Groga sample of October 2007 (*H'* = 0.904 and *J* = 0.505).

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Evidence of selective mating in *D. malerkotliana*: greater reproductive success of wild flies than Spw mutant.

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Introduction

Mating behavior of *Drosophila* consists of specific actions which are accompanied by orientation movements. Such actions referred to as courtship displays are performed sequentially. Mating occurs only if the female responds by performing acceptance signals. In *Drosophila* mating behavior has been studied using various species, strains and mutants (Merrell, 1949; Reed and Reed, 1950; Rendel, 1951; Bastock, 1956; Petit, 1959; Barker, 1962). These studies have shown the genetic control of this behavior. Though mutants are rare events, they form the source of variations for evolution and through these mutants the functioning of many genes can be understood. Therefore more studies using mutants are warranted.

In *Drosophila* the pattern of mating has been tested using mutants (Merrell, 1949; Crossley and Saul, 1970; Rendel, 1951). Some of these studies showed the occurrence of selective mating while others found a lack of selective mating. The lack of selective mating could be due to changes in their behavior patterns causing them to provide sub-optimal courtship to reduce activity generally (Bastock, 1956; Kyriacou *et al.*, 1978) so that females reject more frequently than wild males. In almost all these studies mutants of *D. melanogaster* have been employed due to availability of mutants in this species. In contrast to this only a few mutants have been identified and described in other species of *Drosophila*, such as *D. hydei, D. virilis, D. subobscura, D. pseudoobscura, D. ananassae, D. bipectinata, D. nasuta*, and *D. malerkotlinana* (Lifechytz, 1974; Strursa, 1983; Mohanty *et al.*, 1988; Lozovskaya and Evengener, 1991; Singh and Sisodia, 1999). Therefore, in the present study Spw mutant of *D. malerkotliana* has been employed to study the role of Spw male and female in pre mating and post copulatory success in *D. malerkotliana*. *D. malerkotliana* is a member of the *bipectinata* species complex of the *ananassae* sub group of the *melanogaster* species group distributed in South East Asia. In the laboratory stock of this species, Spw spontaneous recessive autosomal mutation has been detected (Krishna and Hegde, 1998). Therefore, in the present study wild and mutant spread winged strains of *D. malerkotliana* were used to study the role

of spread winged mutant male and female behavior in pre mating success and post copulatory success in *D. malerkotliana*.

Materials and Methods

Stocks used in the present study were wild and Spw mutant (wings spread at 45°) strains of *D. malerkotliana*. When progeny appeared, flies were distributed to different culture bottles and were maintained under constant temperature (22 ± 1°C). For every generation, flies multiplied in different culture bottles were mixed together and eggs were collected using Delcour's (1969) procedure. Eggs (100) were seeded in fresh quarter pint milk bottles with 25 ml of wheat cream agar medium to avoid larval competition during development. From these culture bottles virgin females and males were isolated within three hours of their eclosion and maintained separately at 22 ± 1°C in fresh food vials containing yeast and aged for 5-6 days.

Virgin females and males (aged 5-6 days) were used to see whether there is any difference in mating success between mutant and wild strains of *D. malerkotliana*. In a multiple choice experiment, 10 females of each of the two strains were introduced into the mating chamber along with 10 males of respective strains and were observed for 60 min. When a pair commenced mating it was aspirated out and the type of individuals mated was recorded. Total 5 trials were run.

In a male choice experiment, a mutant male was introduced together with a mutant and a wild female into the mating chamber. A reciprocal cross was also made with wild male and two females, one mutant and one wild. Similarly, in the female choice experiment, we introduced a wild female together with a wild and a mutant male. Reciprocal crosses were also made here. Total 50 trials were run for each experiment.

Different crosses were made (wild male × wild female; wild male × mutant female, wild female × mutant male; mutant male × mutant female) to study the various components of sexual selection. Twenty five pairwise matings were done for each combination. Courtship latency (time between introduction of male and female together in mating chamber until orientation of male to female), mating latency (time between introduction of male and female together in mating chamber until initiation of copulation of each pair), and copulation duration (time between initiation of copulation to termination of copulation of each pair). Courtship acts such as tapping, scissoring, vibration, licking, circling, ignoring, extruding, and decamping were measured following the procedure of Hegde and Krishna (1997). Soon after mating, mated males were transferred individually into a separate vial to study remating ability of male and mated females. They were then transferred into fresh food vials every 24 h without anesthesia to study fertility. Total number of progeny emerged in such vials were counted over a period of 15 days.

To study remating ability of male, mated males were placed separately in the vials and were provided with virgin females (wild/mutant female depending on crosses). Soon after mating the mated female was aspirated out and replaced with another virgin female, and the same procedure was repeated. The observation was made for 2 h and the number of females mated by a single male was recorded. If there was no mating within 2 h, then the pairs were discarded and mean remating ability of male was calculated.

To study female remating, 25 mated females obtained as above were transferred individually into Elens-Wattiaux mating chamber containing 5-6 days aged male. Each female was allowed to stay with this male for 2 hours. Then the female was aspirated out from the Elens-Wattiaux mating chamber and placed back in its marked food vial. This procedure was repeated every day until the

female mated with a second male. The total number of females remated and the interval in terms of days in each cross was recorded.

To measure longevity, different crosses were made using virgin males and females. The pairs were placed in a fresh food vial containing wheat cream agar medium. Active yeast was also added to vials 48 h prior to use. The vials were renewed daily during measurement of longevity. All cultures were maintained at 22 + 1°C and at 12:12 light/dark cycle.

Results

Mating success of wild and mutant flies of *D. malerkotliana* in multiple choice method showed that in 62 crosses wild males mated with wild females, and in 54 mutant males mated with mutant females as against 44 matings of wild males with mutant females and 32 matings of mutant males with wild females ($F = 78$, df = 3, 188; $P < 0.0001$). This shows homogamic matings were more than heterogamic matings. Even in male choice situations, out of 50 wild males, 37 mated with wild females and the remaining males mated with mutant females ($\chi^2 = 11.52$, df = 1, $P < 0.01$). In reciprocal crosses, out of 50 mutant males, 36 mated with mutant females while remaining 14 mutant males mated with wild females ($\chi^2 = 9.68$, df = 1, $P < 0.001$). In female choice method, wild females mated with wild males. Out of 50, 41 wild females paired with wild males while the remaining 7 females mated with mutant males ($\chi^2 = 20.48$, df = 1, $P < 0.001$). In 50 reciprocal crosses, 44 mutant paired with wild males and remaining 6 mutant females mated with mutant males ($\chi^2 = 28.88$, df = 1, $P < 0.001$). In both the crosses wild males were more successful than mutant males.

Table 1. Mating behaviour and fitness characters of wild and spread winged mutant strains of *D. malerkotliana.*

Parameters	Crosses				
	Wild Male x Wild Female	Wild Male x Mutant Female	Mutant Male x Wild Female	Mutant Male x Mutant Female	F-Value
No. of Pairs	25	25	25	25	
Pairs copulating(%)	92	84	56	60	
Courtship latency	4.15 ± 0.41	5.02 ± 0.45	11.05 ± 0.37	9.00 ± 0.31	99.24**
Mating latency	4.36 ± 0.33	5.08 ± 0.33	11.72 ± 0.27	9.52 ± 0.36	106.02***
Tapping	14.56 ± 0.41	13.00 ± 0.43	9.15 ± 0.43	8.51 ± 0.65	68.00**
Scissoring	18.35 ± 0.56	18.02 ± 0.31	14.31 ± 0.45	12.52 ± 0.61	71.00**
Vibration	10.35 ± 0.35	10.41 ± 0.39	9.12 ± 0.31	8.81 ± 0.26	9.68*
Licking	6.35 ± 0.42	6.02 ± 0.31	5.42 ± 0.16	5.12 ± 0.19	12.15**
Circling	19.38 ± 0.51	18.48 ± 0.26	15.15 ± 0.39	15.85 ± 0.41	56.10**
Ignoring	6.31 ± 0.26	4.49 ± 0.26	7.39 ± 0.36	4.81 ± 0.31	14.12**
Extruding	21.51 ± 0.42	17.21 ± 0.35	25.42 ± 0.51	18.21 ± 0.32	59.37**
Decamping	18.97 ± 0.24	14.31 ± 0.35	21.42 ± 0.62	15.32 ± 0.61	50.37**
Compulation duration	11.52 ± 0.30	9.84 ± 0.32	6.60 ± 0.21	8.28 ± 0.29	51.35**
Fertility	285.00 ± 2.53	243.60 ± 5.64	195.60 ± 3.67	222.24 ± 4.82	71.84**
Male mating ability	4.60 ± 0.23	5.00 ± 0.27	2.56 ± 0.14	3.60 ± 0.28	21.03**
Female remating (%)	63.23	54.51	95.27	61.29	39.21**
Female remating interval (in days)	11.51 ± 0.21	14.32 ± 0.42	4.21 ± 0.162	7.18 ± 0.29	195.21**

(Mean values are reported with standard error)
Df = 3.69; * P<0.05; ** P<0.01; *** P<0.001

Table 1 shows courtship and mating activities of different crosses. Highest mating was noticed in pairs involving wild males and females while lowest mating was observed in crosses involving mutant males and wild females. Courtship and mating latency were shortest in crosses of wild males and females while they were highest in crosses of mutant males and wild females indicating that wild males oriented quickly and mated faster than mutant males. Even in male courtship patterns, such as tapping, scissoring, vibration, and circling, were greater in wild males than mutant males. Rejection responses of females, such as ignorance, extruding, and decamping, were greater in crosses involving mutant males and wild females while these responses were lower in crosses involving wild males and mutant females. Highest copulation duration and fertility were noticed in pairs of wild males and females while lowest copulation duration and fertility were observed in pairs of mutant males and wild females. Therefore, copulation duration and fertility were positively related. Highest male remating ability was noticed in crosses of wild males and mutant females while lowest male remating ability observed in pairs of mutant males and wild females suggesting that wild males inseminated more females than mutant males. All parameters were significantly varied by one-way ANOVA.

Female remating frequency and female remating interval in different crosses showed that the highest percentage of female remating was noticed in crosses involving wild females and mutant males while the lowest percentage of female remating was observed in crosses involving wild males and mutant females. Longevity of wild and mutant flies is provided in Table 1. Wild flies had greater longevity than mutant flies suggesting that wild flies live longer than spread winged mutant.

Discussion

It is known that male activity and female receptivity are the main factors responsible for successful mating in *Drosophila* (Bastock, 1956). It is evident from the present study that in both multiple and male choice situations preferential mating was noticed between wild and mutant flies of *D. malerkotliana* suggesting occurrence of selective matings between spread winged mutant and wild flies *D. malerkotliana*. This agrees with the work of Morpurgo and Nicoletti (1955) and Barker (1962) in *D. melanogaster*. They also found strong selective mating between the white and wild type flies and also between yellow mutant and wild type in *D. melanogaster*. However, in female choice situation wild males were more successful than mutant males suggesting that under competitive condition wild males were more successful in pre-mating than spread winged mutant. This shows that wild males were more active and vigorous than mutant males during pre mating behavior. This agrees with the work of Faugers (1971), who while studying the components of sexual selection in *D. melanogaster* has also suggested that genotype having no influence whatsoever the more active and vigorous males being likely to win their contests. Even in the present study it was noticed that wild males inseminated more females than mutant males (Table 1). This clearly shows greater sexual vigor of wild males than mutant males.

Furthermore, studies have also indicated that variation is more pronounced in males than in females (Spiess, 1970; Parsons, 1973; Gilbert *et al.*, 1983). According to Smith (1956) in males differences in sexual vigor due to genetic difference must exist in natural population enabling selection to operate. In the present study even in no choice situation where there is no competition, wild male crossed with wild female had greater mating percentage, oriented quickly and mated faster than mutant males crossed with wild/mutant females. This is due to greater sexual vigor of wild males than mutant males; therefore, they oriented quickly and mated faster hence their courtship and mating latency were shorter. On the other hand spread winged mutant males have showed sub-optimal behavior to female as seen in their promoting behavior. In *Drosophila* studies have also

shown that successful matings are also dependent on the activities of the courting pairs (Hegde and Krishna, 1997). Further, courtship behavior of one male may also change the behavior of other males (Rendel, 1951). In the present study, we noticed that wild males recognized wild/mutant females more quickly and displayed different courtship acts such as tapping, scissoring, vibration, licking, and circling more frequently than mutant males. Through these courtship acts males not only transmit sexual signals but also stimulate the female (Spieth and Ringo, 1983). On the other hand, wild females showed more rejection responses such as ignorance, decamping, and extruding to mutant males than wild males. Therefore, wild male paired with wild or mutant females had greater mating success than mutant males paired with wild/mutant females. This agrees with the work of Soudergard (Smith, 1956), who found several changes in mating behavior between ebony mutant and wild males of *D. melanogaster*. The ebony males exhibit less licking than wild type and show more frequent breaks in courtship (Crossley and Zuill, 1970). Also the courtship song is changed in ebony males.

According to Elens (1973) the behavior of the males varied according to the genotype of the female they were courting. In the present study in order to test the female behavior we have studied female remating. Female remating frequency and female remating internal varied significantly between wild and mutant males of *D. malerkotliana* (Table 1). Wild/mutant female initially mated with mutant male remated more rapidly and more frequently than it initially mated with wild male. This is because in *Drosophila* females remate only when sperms in the spermatheca are exhausted. According to Gromko and Pyle (1978) the female remating interval seems the result of selection on both sexes. Sexual selection would favor any adaptations that lower female receptivity to remating or that insure most or all of their sperm is used. However, females suffer a decrease in fitness when the amount of sperm stored drops to a low level (Gromko and Pyle, 1978) and selection on females for the maintenance of high reproductive output would favor their remating before the sperm stored from a prior mating is exhausted. In the present study we also found that females (wild/mutant) copulated longer time with wild males than with mutant males. Increased copulation duration increases the number of ejaculations (Hegde, 1979). Hence females initially mated with wild males remated more slowly than it initially mated with mutant males.

Sexual selection is common among animals, because the females reproductive success is limited by the number of eggs she can produce in her lifetime and a male's reproductive success is limited by the number of females he can inseminate. In the present study fertility varied significantly between crosses. Wild male mated with wild female had greater fertility than mutant male mated with wild or mutant female.

According to Krishna and Hegde (1997) higher reproductive success can also be accounted for its longevity. In the present study we found wild flies had higher longevity than mutant flies and varied significantly. Wild male with higher longevity, greater sexual vigor, and fast mating ability could inseminate more females than could mutant males. As a result the total number of progeny they produce is greater. On the other hand, wild female with greater longevity and remating ability could receive more sperms and produce more fertile offspring in their lifespan than mutant females. Thus, in *D. malerkotliana* present observation clearly suggests there is an evidence that selective mating exists between spread winged mutant and wild flies and greater reproductive success of wild flies.

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A novel mechanism underlying axon guidance phenotypes: Indirect disruption of embryonic axon guidance by unspecified cells.

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Abstract

During characterization of the *Netrin* locus, we uncovered a background maternal effect mutation in which a small number of presumptive mesoderm cells fail to express the mesoderm determinant *twist* (*twi*). In contrast to *twi* mutants in which the mesoderm cells adopt alternative cell fates, the affected cells did not appear to differentiate. The cells failed to invaginate with other mesodermal cells and remained at the central nervous system (CNS) midline physically blocking cell migration and axon outgrowth. Due to low penetrance we were unable to map the mutation, but propose that the gene is required for coordination of gene expression throughout the cells of a tissue. We believe that the phenotype represents a novel way for cell fate alterations to disrupt axon guidance, distinct from alterations in neuronal or target cell identity.

Introduction

Like all animals, early development of *Drosophila* embryogenesis is controlled by gene products deposited by the mother in the oocyte (Tadros and Lipshitz, 2009). One of the first zygotic genes to be activated is the *twist* (*twi*) gene, a basic helix-loop-helix (bHLH) transcription factor required for mesodermal cell fate (Thisse *et al*., 1987). In *twi* mutants, the presumptive mesoderm fails to invaginate during gastrulation and adopts alternative cell fates, mainly neurectodermal (Leptin and Grunewald, 1990; Rao *et al*., 1991). The failure of a large number of cells to invaginate leads to an increase in the length of the embryo which, as the embryo is constrained by the egg membranes, leads to twisting of the embryo giving the mutant its name (Simpson, 1983).

The *Drosophila* central nervous system (CNS) is formed by the juxtaposition of neurectodermal tissue from opposite sides of the embryo after the presumptive mesoderm has