climbing or motor effects in adult flies (not shown), indicating that the ingestion phenotype described here could be solely caused by visceral muscle dysfunction.

From a methodological stand point, we found that the protocol used for ingestion assays with the *mhc-Gal4* gives higher differences between genotypes, was more robust (smaller errors), easier to perform, and less time consuming.

In conclusion, the identification and characterization of sensitized and easy-to-score phenotypes is crucial in basic research to study the mechanism of pathogenesis of a disease and to perform genetic and chemical screens when a wide range of mutations or compounds are tested. In the current study, we describe three additional phenotypes in DM1 model flies; imaginal eye disc size reduction, decreased viability, and ingestion rate impairment, resulting from CTG repeat expression in eye, dopaminergic and cholinergic neurons, and visceral muscle, respectively. These phenotypes might prove useful in subsequent studies aimed at testing molecular defects in the pathogenesis pathways of the DM1.

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Remating behavior in a few closely related species of *Drosophila nasuta* subgroup.

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Introduction

In insects, successful copulation does not ensure paternity as competition between sperm coming from different males to fertilize the ovum continues even within the female reproductive tract. Hence, males have evolved various mechanisms to alter female's physiology and behavior to ensure his paternity. In *Drosophila*, the females that are once mated can store sperm in her storage organs that are enough to fertilize the eggs laid in her life time. Nevertheless, she often remates even in the presence of stored sperm in her reproductive tract (Lefevre and Jonsson, 1962) creating a competitive milieu for sperm coming from different males. In this conflict between interests of males and females during reproduction, female remating acts as a key determinant of the pattern of sexual selection (Singh and Singh, 2004) leading to the genetic heterogeneity and divergence. The time

required for female to regain her receptivity after mating once varies from a few minutes to weeks among different species of *Drosophila* (*cf*., Singh, *et al.* 2002). Most of these studies come from work on *D. melanogaster* and a few other species, which are prezygotically or postzygotically isolated from each other. In the present study we have analyzed the divergence in the female remating behavior among closely related species of *Drosophila*. The *D. nasuta* subgroup comprise of morphologically identical species and subspecies having varying levels of reproductive isolation among them (Nirmala and Krishnamurthy, 1974; Wilson, *et al.,* 1969). In the present study we have analyzed the remating behavior among the *D. nasuta* subgroup members and influence of age on female remating behavior.

Material and Method

Drosophila stocks that belong to different morphophenotypic complexes of *D. nasuta* subgroup used for the present work (Table 1) were obtained from Drosophila Stock Centre, University of Mysore, Mysore. These stocks were maintained in a vivarium on standard wheat cream agar medium at 22 ±1ºC, 70-80% humidity, and 12:12 light : dark cycle. The virgin females and unmated males were collected by isolating males and females within 3 hrs of their eclosion. Two age groups, *viz.,* 3 days old (young) and 7 days old (mature) flies, were used for the present experiment.

Table 1. List of *Drosophila* stock employed in the present experiment.

Species/Subspecies	Strain	Stock No.
Frontal sheen complex		
1. *Drosophila nasuta nasuta*	Coorg, India	201.001
2. *Drosophila nasuta albomicans*	Okinawa, Japan	202.001
3. *Drosophila nasuta kepulauana*	Sarawak	203.001
Orbital sheen complex		
1. *Drosophila sulfurigaster sulfurigaster*	Queensland, Australia	205.001
2. *Drosophila sulfurigaster neonasuta*	Mysore, India	206.001

Using 7 days old unmated males and virgin females of similar age group, pair mating was conducted in culture vials (8 cm × 2 cm) at room temperature (22-25°C). All matings were set up at 7 AM and were retained until 11 AM. After completion of mating the males were discarded. Every 24 hrs, an unmated male was introduced into vial containing mated female for 25 consecutive days until she remated. Females which do not remate within 25 days were discarded (Singh and Singh, 2004). The day on which female remating occurred was recorded as remating latency. Similarly, to analyze the influence of age on regaining receptivity, the remating experiments were conducted in young flies also. After first mating the females were provided with fresh age matched males for remating every day morning up to 15 days, since all the females undergo remating within 15 days after mating once.

The data were subjected to analysis of variance (ANOVA) followed by Tukey's HSD test and Chi-square test using SPSS software (Version 10.0).

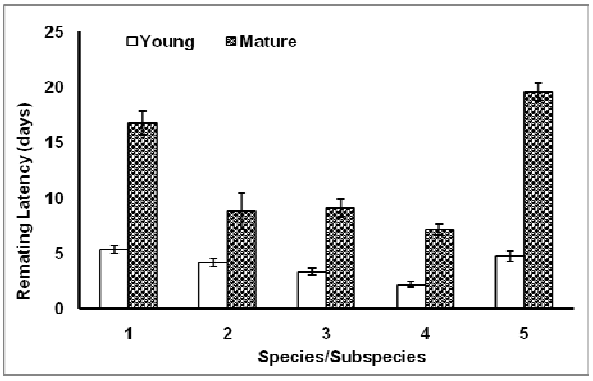

Figure 1

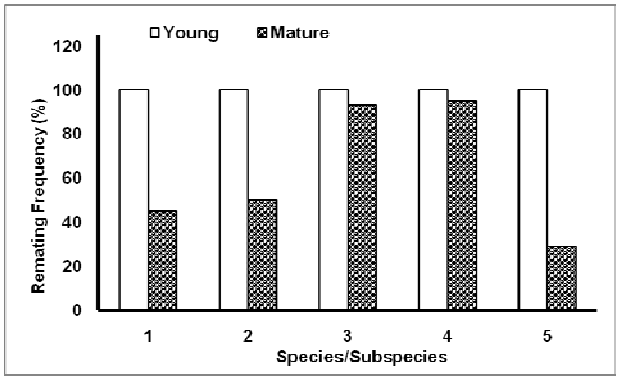

Figure 2

Figure 1. Remating latency (days), and Figure 2. Remating frequency (%) in both the age groups of *D. nasuta* subgroup. (1) *D. n. nasuta*, (2) *D. n. albomicans*, (3) *D. n. kepulauana*, (4) *D. s. sulfurigaster* and (5) *D. s. neonasuta*.

Results and Discussion

Remating latency in young flies ranges form 2.16 days (*D. s. sulfurigaster*) to 5.34 days (*D. n. nasuta*), where as in mature flies it ranges from 7.16 days (*D. s. sulfurigaster*) to 19.50 days (*D. s. neonasuta*). ANOVA shows significant difference in the female remating latency among young (P = 0.0001) and mature flies (P = 0.0001). According to Tukey's HSD test young *D. n. nasuta* differs significantly from *D. n. kepulauana* and *D. s. suflurigaster*; while *D. s. suflurigaster* differs from all the other members except *D. n. kepulauana.* Further, among mature flies *D. n. albomicans, D. n. kepulauana,* and *D. s. suflurigaster* differ significantly from *D. n. nasuta* and *D. s. neonasuta. D. n. nasuta* and *D. s. neonasuta* do not, however, differ significantly from each other (Figure 1).

Young females of all the species/subspecies under study show 100% remating frequency; hence, they do not differ in their remating frequency (χ^2 = 0.000, df = 4, P = 1.000). 16% *D. n. nasuta* and 31.25% *D. s. neonasuta* females accept males 48 hrs after first mating. 40% of *D. n. albomicans*, 54% of *D. n. kepulauana,* and 70% *D. s. sulfurigaster* females become receptive by 24-48 hrs after mating. (Figure 2).

The remating frequency of mature females ranges from 29% in *D. s. neonasuta* to 93% in *D. n. kepulauana,* and they differ significantly in their remating frequency (χ^2 = 57.231, df = 4, P = 0.0001). In mature flies the majority of *D. n. nasuta* females remain non-receptive until the 11th day after first mating and by the 20th day 83.7% females remate. 13.33% of *D. n. albomicans* females show receptivity after 24 hrs after mating, and 46.62% of them remate within 6 days after mating. In *D. n. kepulauana* 8.1% females show receptivity in 24 hrs after mating; however, 59.4% of them remate between 6 to 14 days after first mating. 8.10% of *D. s. sulfurigaster* females become receptive in 3 days, while peak remating frequency of 35.1% is seen on the 6th day after first mating, and *D. s. neonastua* are receptive only after 15 days, though 50% of them remate on the 19th day after first mating (Figure 2).

The influence of age on female remating was assessed by comparing the remating latency and remating frequency in flies of both the age groups. To find the differences in these parameters between the two age groups in all the 5 species/subspecies, the data were analyzed by chi-square test and two-way ANOVA by considering species (factor 1) and age group of flies (factor 2). Chi-square

test shows a significant difference in the mating frequency among both the age group of flies (χ^2 = 17.643, df = 4, P = 0.0001). There is significant effect of interaction between factor 1 and factor 2 on remating latency (P= 0.0001); according to Tukey's HSD test *D. n. nasuta* and *D. s. neonasuta* differ significantly from all other members, whereas they do not differ significantly among themselves, and also other members do differ from each other significantly. Flies of both the age groups differ in their remating frequency (χ^2 = 21.990, df = 4, P = 0.0001). As age advances, there is a three-fold increase in remating latency and decrease in remating frequency. There is, however, no effect of interaction between factor 1 and factor 2 on duration of copulation during remating (P = 0.082).

In *Drosophila* the sperm received during mating are stored in the storage organs. The sperm received during mating once are enough to fertile eggs for few weeks. But females often remate even in the presence of sperm in her storage organs. This act of females increases the heterogeneity of a population. The major bulk of information on female remating in *Drosophila* comes from studies conducted in *D. melanogaster* (Gromko and Pyle, 1978), and there have been preliminary investigations on remating in a few other species, such as *D. pseudoobscura* (Snook, 1998), *D. ananassae* (Singh and Singh, 2004), and *D. pavani* and *D. gaucha* (Koref-Santibanez, 2001). Presently a study has been conducted on taxonomically closely related species with varying levels of reproductive isolation. In this context the *D. nasuta* subgroup forms an interesting model system to understand the dynamics of speciation (Ranganath, 2002). It is an assemblage of closely related, morphologically almost identical species and subspecies, showing varying levels of reproductive isolation. The remating behavioral studies in various species of *Drosophila* have shown that the time required for a female to regain her receptivity and the frequency of remating varies. In the present study, *D. n. nasuta* and *D. n. albomicans* differ in their remating latency, however show similar remating frequency. *D. n. kepualuana* and *D. n. albomicans* though reproductively isolated show similar remating latency although differ in their remating frequency. *D. s. sulfurigaster* shows lowest remating latency after first mating among all the members with highest remating frequency. In contrast, *D. s. neonasuta,* which is intercrossable with *D. s. sulfurigaster,* shows the highest remating latency after first mating with least remating frequency. Present investigation has revealed that even though the members of the *D. nasuta* subgroup are closely related and few among them share open genetic system, they differ significantly in their remating behavior. Hence, like any other sexual trait, the female remating behavior also shows high divergence even among closely related species/ subspecies that have varying level of reproductive isolation among them.

The female remating behavior is influenced by various factors like sperm load received during first mating (Kalb *et al.,* 1993), the quantity of seminal proteins received during mating (Hihara, 1981; Sirot *et al.,* 2009), the nutritional status of female (Chapman *et al*., 1994), density of population (Singh and Singh, 2001), and age of the flies (VanVianen and Bijlsma, 1993; Koref-Santibanez, 2001; Mack *et al.,* 2003). In the present study we have assessed the influence of age on remating behavior assessed by employing two age groups of flies that are 7 days and 3 days old. The 7 days old males have maximum quantity of accessory gland proteins and contribute maximum amount of accessory gland proteins to female during mating (Ravi Ram and Ramesh, 1999). On the basis of this, the 7 days old flies are considered as mature flies. In nature, the flies do not wait up to 7 days for mating, and hence we have employed 3 days old flies and are considered as young flies to analyze and compare the age related differences in reproductive behavior among the members of *D. nasuta* subgroup. The young flies show 100% remating frequency with much shorter remating latency, about three fold less than aged flies. The results of the present study differ from that of Van Vianen and Bijlsma (1993), who have shown that the young *D. melanogaster* flies (1-2 days old) are reluctant for both mating and remating compared to aged flies (4-6 days old). The closely related members of the *D. nasuta* subgroup differ in their reproductive biology; however, these differences do not come in the way of open genetics system exhibited among few members of the subgroup.

Hence, *D. nasuta* subgroup offers an excellent model system to understand the evolutionary divergence in reproductive behavior in closely related species.

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The importance of identification of the *willistoni* subgroup of *Drosophila* at the species level: the first evidence of *D. equinoxialis* in the Northeast region of Brazil.

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Introduction

A problem inherent in the ecological study of drosophilids is that parts of these organisms are composed of various sibling species, and this renders the identification of the specimens more difficult. Mayr (1963) defines sibling species as "natural populations which, although morphologically identical or very similar, are isolated reproductively". This is one of the most serious restrictions to the morphological concept of species. While the sibling species represent a considerable problem to many taxonomists, particularly those who hold that species should be