Male accessory gland secretions in hybrids of Drosophila nasuta nasuta and D. n. albomicans neither show luxuriance nor breakdown

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Male accessory gland secretions, which have a role to play in reproduction have been investigated. The number of cells that make-up the gland, the quantity of secretions synthesized and the influence of these secretions on fecundity of the female have been studied in D. n. nasuta, D. n. albomicans and their F1 progeny. The results revealed that the hybrid males show a trend towards D. n. nasuta in the synthesis of male accessory gland proteins and the fecundity of the female is influenced more by its genetic constitution rather than the quantity of accessory gland secretions.

The evolution of internal fertilization in higher organisms gave rise to a multitude of opportunities for strong selective interactions between males and females and between males1. The morphology, physiology and behaviour associated with internal fertilization are characterized by rapid evolution2. Among insects, there is a fascinating diversity of adaptations in which the male contribution goes far beyond the mere transfer of sperms and that includes the transfer of accessory gland secretions. The accessory glands of male Drosophila, as in many other insects3 are known to play a primary role in reproduction; in that their secretory products are essential for transfer, storage and utilization of the sperms4. An event that can easily occur because of restrictions in population size or area is mating between relatives or inbreeding. Another event is cross-breeding which occurs between two different strains/sub-species/species which usually yields more vigorous hybrid offspring than either of the parent strains considered separately and this superiority of the hybrid is known as heterosis. Several studies in Drosophila have provided the evidence for heterosis5-11. However, there are also instances wherein the hybrid is found to be inferior to its parents12-13. Rajasekarsetty et al. 14 have demonstrated the occurrence of heterosis of fitness parameters in F1 individuals of D. n. nasuta ×D. n. albomicans followed by breakdown in the F2 progeny. Present report deals with part of investigations on the dynamics of male accessory gland protein synthesis and its influence on fecundity of the hybrid females arising out of reciprocal crosses between D. n. nasuta and D. n. albomicans.

Two members of Drosophila nasuta subgroup namely D. n. nasuta (Coorg, India; Stock No. 201.001) and D. n. albomicans (Okinawa, Japan; Stock No. 202.001) were employed. Both these stocks were obtained from Drosophila Stock Center, University of Mysore, Mysore, India. Uniformity was maintained with regard to temperature, space, amount of food, moisture and the larval population density in the cultures that are used in the present analysis. Synchronized eggs were collected from both the cultures through modified method of Delcourt15. Eggs (50) were placed into each vial (8 cm x 2.5 cm) containing wheat cream agar medium seeded with yeast. All the experimental cultures were maintained at 22°±1°C. Unmated males and virgin females were isolated from above mentioned cultures within 3 hr of their eclosion from the pupal case. They were transferred to vials containing fresh media and aged for 5 days. Reciprocal crosses were conducted between D. n. nasuta and D. n. albomicans to get F1 and F2 generations. Unmated parental, F1 and F2 males were isolated within 3 hr of their emergence. They were transferred to separate vials containing fresh medium. After aging them for 7 days, the accessory glands were dissected, secretions were precipitated, isolated and the samples were prepared as described before16. The quantity of protein present in 25 samples (one sample from one individual) was individually estimated following micromethod17 using bovine serum albumin (BSA) as the standard.

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Table 1 — Number of main cells and quantities of accessory gland proteins/secretions in *D. n. nasuta* and *D. n. albomicans* and their hybrids

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Experimenter</th>
<th>Number of cells</th>
<th>Quantity of secretions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>D. n. nasuta</em></td>
<td>1902 ± 10.64*</td>
<td>13.00 ± 0.09*</td>
</tr>
<tr>
<td>2.</td>
<td><em>D. n. albomicans</em></td>
<td>1942 ± 9.47*</td>
<td>20.00 ± 0.14*</td>
</tr>
<tr>
<td>3.</td>
<td><em>F₁ hybrid</em></td>
<td>2089 ± 25.10*</td>
<td>11.50 ± 0.33*</td>
</tr>
<tr>
<td>4.</td>
<td><em>F₂ hybrid</em></td>
<td>1991 ± 13.00*</td>
<td>11.77 ± 0.46*</td>
</tr>
</tbody>
</table>

Note: The members with similar letters in superscript are not significantly different at 5% level according to DMRT. 

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Table 2 — Fecundity in *D. n. nasuta*, *D. n. albomicans* and their hybrids

<table>
<thead>
<tr>
<th>Experimenter</th>
<th>Fecundity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. n. nasuta</em></td>
<td>2430 ± 4.22*</td>
</tr>
<tr>
<td><em>D. n. albomicans</em></td>
<td>2504 ± 3.75*</td>
</tr>
<tr>
<td><em>F₁ hybrid</em></td>
<td>90.3 ± 2.56*</td>
</tr>
<tr>
<td><em>F₂ hybrid</em></td>
<td>56.8 ± 2.82*</td>
</tr>
</tbody>
</table>

F value = 278.7

Note: The members with similar letters in superscript are not significantly different at 5% level according to DMRT. 

To determine the number of cells present in the gland, the glands were isolated from a single day old male fly, fixed in 1N HCl for 5 min and later transferred to 2% lactic acid. After 20 min the glands were gently squashed in 45% acetic acid between a slide and cover glass so as to spread the cells in a single layer. These slides after sealing were used for counting the number of main cells. The cell number was counted under low magnification with the help of a tally counter. Only one lobe from a pair of glands was considered for counting. Twenty five such preparations were used to determine the average number of main cells.

Fecundity of *F₁* females in comparison with their parental females was determined as per the standard procedure. The data was subjected to statistical analysis by ANOVA followed by DMRT to determine the significance.
shown that the heterosis was due to either interchromosomal interaction, or the complementing action of haploid autosomes. Rajasekarasetty et al., have studied three fitness parameters namely fecundity, rate of development and viability in F₁ and F₂ hybrids of *D. n. nasuta* and *D. n. albomicans* wherein the F₁ generation was found to be heterotic while the F₂ showed breakdown. All the investigations listed here are similar in approach in one way or the other as they included either the analysis of only fitness parameters or inversions.

If the performance of the hybrid is higher than the mid parental value, then it is considered as heterotic and if the performance of the hybrid is less than the least parent then it is considered as hybrid breakdown. There are two types of secretory cells in the accessory glands. The predominant type are main cells which are hexagonal and binucleate. The other type includes secondary cells, which are spherical binucleate cells having large vacuoles and are scattered near the distal tip of the gland. Perusal of Table 1 reveals that there was a significant difference between *D. n. nasuta* and hybrids with respect to cell number, while the quantity of secretions in the accessory glands of F₁ and F₂ males was nearer to that of *D. n. nasuta* though the differences were significant when F₁ (that has more number of cells but less secretions than *D. n. nasuta*) and F₂ males (that have less number of cells and secretions than *D. n. nasuta*) of *D. n. nasuta* × *D. n. albomicans* were compared with their parents. This suggests that the observed quantity differences are probably due to differences in synthetic activity of the cells. The results of cell count and quantitative estimation thus suggest that there is neither breakdown nor heterosis with respect to these accessory gland proteins. Some models of speciation have predicted that mating and fertilization traits remain well buffered within species and that the escape from such selective constraints will be achieved by founder event strong enough to disrupt previous genetic balances. As the secretions from accessory gland are involved in reproduction, it is probable that their levels are buffered and hence, they reach the level of parent (one with least quantity-as in case of *D. n. nasuta*).

Hihara has shown that in *D. melanogaster*, the number of eggs laid is closely associated with the quantity of accessory gland secretions in the adult male about 70% of which are transferred to the female during mating. *D. n. nasuta* is found to have a fecundity of 243 eggs/individual and *D. n. albomicans* has a fecundity of 250 eggs/individual. However, the F₁ females of *D. n. nasuta* × *D. n. albomicans* and *D. n. nasuta* × *D. n. albomicans* × *D. n. nasuta* × *D. n. albomicans* had a fecundity of 90.3 eggs/individual, 56.8 eggs/individual respectively. When compared to parents, these values are significantly less (see Table 2). Thus, it is evident that though F₁ males had accessory gland secretory protein quantities similar to that of *D. n. nasuta*, they produced significantly less number of eggs leading to F₂ breakdown. The results suggest that the genetic constitution of an individual has a bearing on the fecundity of the female rather than the quantity of accessory gland secretions in the adult male that are secreted and transferred to the female during mating. The genotypes of *D. n. nasuta* and *D. n. albomicans* represent an integrated and co-adapted genetic system. The F₁ heterosis for fitness parameters of these hybrids was attributed to the dissociation of these coherent system and F₂ breakdown to the destruction of these integrated and coherent genetic organizations through recombination. The results of the present study supports the findings of Rajasekarasetty et al., with regard to breakdown in fecundity and differs from the findings of Hihara.

Among *Drosophila*, most hybrids from crosses between closely related species are viable but sterile. Wu and Davis have shown that this trend is not only a consequence of the heterogametic condition of the male but may also be influenced by faster evolution of the male reproductive system. Civetta and Singh have shown that there is higher divergence of sexual than non sexual traits between species of *Drosophila melanogaster* complex and sexual traits were better predictors of species distinctness than non sexual traits. Further, they have shown the existence of luxuri ance for non sexual traits of interspecific hybrids and observed that the sexual traits do not manifest luxuriance in the interspecific hybrids wherein the tests showed an average additive effect with a trend towards paternal dominance. However, such phenomenon was not encountered in the present study but as far as quantity of accessory gland secretory proteins are concerned, there was a trend towards *D. n. nasuta* in all the cases analyzed (see Table 1). Further, the accessory gland proteins did not manifest the phenomena of luxuriance in the interspecific hybrids. Thus, though *D. n. nasuta* and *D. n. albomicans* have open genetic system, they are genetically and biochemically distinct as far as the accessory gland secretory proteins are concerned.
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