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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 F_1 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 males[1]. The morphology, physiology and behaviour associated with internal fertilization are characterized by rapid evolution[2]. 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 insects[3] are known to play a primary role in reproduction; in that their secretory products are essential for transfer, storage and utilization of the sperms[4]. 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 heterosis[5-11]. However, there are also instances wherein the hybrid is found to be inferior to its parents[12, 13]. Rajasekarsetty *et al.*[14] have demonstrated the occurrence of heterosis of fitness parameters in F_1 individuals of *D. n. nasuta* × *D. n. albomicans* followed by breakdown in the F_2 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 Delcour[15]. Eggs (50) were placed into each vial (8 cm × 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 F_1 and F_2 generations. Unmated parental, F_1 and F_2 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 before[16]. The quantity of protein present in 25 samples (one sample from one individual) was individually estimated following micromethod[17] 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

[Values are mean ± SE from 25 observations in each group]

Sl. No.		Number of cells[1]	Quantity of secretions[2] (μg)
1.	*D. n. nasuta*	1902 ± 10.64^{a}	13.00 ± 0.09^{a}
2.	*D. n. albomicans*	$1942 \pm 9.47^{a,b}$	20.00 ± 0.14^{b}
3.	F_1 hybrid ♂♂ (*D. n. nasuta* ♀♀ × *D. n. albomicans* ♂♂)	1995 ± 19.17^{b}	12.50 ± 0.43^{a}
4.	F_1 hybrid ♂♂ (*D. n. nasuta* ♂♂ × *D. n. albomicans* ♀♀)	2089 ± 25.10^{c}	11.50 ± 0.33^{c}
5.	F_2 hybrid ♂♂ (*D. n. nasuta* ♀♀ × *D. n. albomicans* ♂♂)	1828 ± 9.90^{d}	14.73 ± 0.09^{d}
6.	F_2 hybrid ♂♂ (*D. n. nasuta* ♂♂ × *D. n. albomicans* ♀♀)	1991 ± 13.00^{b}	11.77 ± 0.46^{c}
	F value	78.02	222.7

[1]Average number of cells in a single lobe of accessory gland (N = 25)
[2]Average protein quantities in secretions/individual (N = 25)
The members with similar letters in superscript are not significantly different at 5% level according to DMRT. df = (5, 144)

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

[Values expressed as number of eggs per individual are mean ± SE]

	Fecundity
D. n. nasuta	243.0 ± 4.22^{a}
D. n. albomicans	250.4 ± 3.75^{a}
F_1 females (of *D. n. nasuta* ♀♀ × *D. n. albomicans* ♂♂)	90.3 ± 2.56^{b}
F_1 females (of *D. n. nasuta* ♂♂ × *D. n. albomicans* ♀♀)	56.8 ± 2.82^{b}
F value	278.7

Note: The members with similar letters in superscript are not significantly different at 5% level according to DMRT. df = (3, 116)

To determine the number of cells present in the gland, the glands were isolated from a single 7 day old male fly, fixed in 1*N* HCl for 5 min and later transferred to 2% lactoacetoorcein. 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 F_1 females in comparison with their parental females was determined as per the standard procedure[18]. The data was subjected to statistical analysis by ANOVA followed by DMRT to determine the significance[19].

The variation in the number of cells among the glands of parents was found to be non-significant (Table 1). The cell number in all the hybrids differed significantly with that of *D. n. nasuta*. The differences in the quantity of secretions in the F_1 hybrid males of *D. n. nasuta* ♂♂ × *D. n. albomicans* ♀♀ were significant with their parents. While the quantity differences in F_1 males of *D. n. nasuta* ♂♂ × *D. n. albomicans* ♀♀ were significant only with *D. n. albomicans*. The F_2 males of *D. n. nasuta* ♀♀ × *D. n. albomicans* ♂♂ showed significantly more quantity of secretions than F_1 males; whereas F_2 males of reciprocal cross contained the quantity of secretions that are not significant with F_1 but with the parents. Perusal of Table 2 that embodies data on fecundity of *D. n. nasuta*, *D. n. albomicans* and their hybrids reveals that the fecundity of F_1 females was significantly less than their parental females irrespective of the direction of the cross.

Several investigators have analyzed F_1 and F_2 progeny for fitness parameters in comparison with respective parental populations in *D. melanogaster*[20] and *D. pseudoobscura*[21, 22]. In all these studies the F_1 progeny were found to flourish, while F_2 progeny showed breakdown for many of the fitness parameters. On the contrary, absence of F_1 heterosis and F_2 breakdown for fitness parameters in *D. subobscura*[23] forms an exception to these reports. In *D. ananassae* there is evidence for heterosis but absence of breakdown of heterosis[24]. Anderson[22] has opined that F_1 heterosis was due to increased heterozygosity for genes with overdominant effects while F_2 breakdown occurs through the reassortment of genes by recombination and consequent disruption of synergestic combination of genes. de Miranda and Eggleston[5] have

shown that the heterosis was due to either interchromosomal interaction, or the complementing action of haploid autosomes. Rajasekarasetty *et al.*,[14] have studied three fitness parameters namely fecundity, rate of development and viability in F_1 and F_2 hybrids of *D. n. nasuta* and *D. n. albomicans* wherein the F_1 generation was found to be heterotic while the F_2 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[25]. 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[26]. 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 F_1 and F_2 males was nearer to that of *D. n. nasuta* though the differences were significant when F_1 (that has more number of cells but less secretions than *D. n. nasuta*) and F_2 males (that have less number of cells and less 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[27]. As the secretions from accessory gland are involved in reproduction[2], 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[28] 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 F_1 females of *D. n. nasuta* ♀♀ × *D. n. albomicans* ♂♂, *D. n. nasuta* ♂♂×*D. n. albomicans* ♀♀ had a fecundity of only 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 F_1 males had accessory gland secretory protein quantities similar to that of *D. n. nasuta*, they produced significantly less number of eggs leading to F_2 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 F_1 heterosis for fitness parameters of these hybrids was attributed to the dissociation of these coherent system and F_2 breakdown to the destruction of these integrated and coherent genetic organizations through recombination[29]. The results of the present study supports the findings of Rajasekarasetty *et al.*,[14] with regard to breakdown in fecundity and differs from the findings of Hihara[28].

Among *Drosophila,* most hybrids from crosses between closely related species are viable but sterile[30]. Wu and Davis[31] 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[32] 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 luxuriance for non sexual traits of interspecific hybrids and observed that the sexual traits do not manifest luxuriance in the interspecific hybrids wherein the testes 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[33], they are genetically and biochemically distinct as far as the accessory gland secretory proteins are concerned.

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