

Several deletions in the 38-39 region were mapped in the study. The new data on the break points of the deletions are given below.

Df(2) DS8: 39A6-7 – 39D3

Df(2) DS9: 38D1-2 – 39B

Df(2) TW1: 38B3 – 39D

Df(2) prA14: 37D – 39B2

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Ontogenetic profiles of male accessory gland secretory proteins in a few species of *nasuta* subgroup of *Drosophila*.

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Seminal fluid molecules critical for many of the mating induced changes in *Drosophila* are produced in the male's accessory gland (Chen *et al.*, 1988; Kalb *et al.*, 1993; Harshman and Prout, 1994; Chapman *et al.*, 1995). These secretory proteins are sex specific, stage specific, and tissue specific (Chapman and Wolfner, 1988). Their transcripts and translational products can be detected right from the time of adult eclosion (Chen, 1985).

Stumm-Zollinger and Chen (1985) have shown that the accessory gland secretory proteins in *D. melanogaster* comprise 40 fractions in one-dimensional gels and 85 in two dimensional gels. It has been documented that in unmated males 7 days after eclosion, the secretory proteins that accumulated in the gland lumen amounts to nearly three quarters of the total soluble protein (Chen, 1991).

The *nasuta* subgroup of *Drosophila immigrans* group consists of a cluster of morphologically almost identical, related species having varying degrees of reproductive isolation (Wilson *et al.*, 1969). Shivanna and Ramesh (1995) working with the male accessory gland secretory proteins in two *nasuta* subgroup species, namely, *D. n. nasuta* and *D. s. neonasuta*, have shown that the patterns are much simpler and the accumulation of secretory proteins reaches maximum in about a week of post eclosion in the unmated males. However, the information pertaining to developmental changes in the male accessory gland secretions of both *D. melanogaster* and *nasuta* subgroup is confined to quantitative measurements. Thus the qualitative studies involving the developmental patterns of the secretory protein fractions are lacking. Present investigations were undertaken to study the pattern of accessory gland secretory protein profiles during the development of the imago in seven species of *D. nasuta* subgroup, to compare the extent of variation if any, in this tissue specific, sex specific, and stage specific protein among closely related species.

In the present study, we have used *D. nasuta nasuta* (201.001), *D. n. albomicans* (202.001), *D. n. kepulauan* (203.001), *D. kohkoa* (204.001), *D. sulfurigaster sulfurigaster* (205.001), *D. s. albostrigata* (207.001) and *D. s. neonasuta* (206.001). All these stocks were obtained from *Drosophila* stock centre, University of Mysore, Mysore, India. All these cultures were maintained at $22 \pm 1^\circ\text{C}$ under uniform conditions. For the experimental purposes, synchronized eggs were collected following the modified method of Delcour (Romachandra and Ranganath, 1988). 50 eggs thus collected were allowed to develop in $3'' \times 1''$ vials containing equal quantities of food medium, maintained at a temperature of $22 \pm 1^\circ\text{C}$. The adult males, within 3 hours of their emergence from such cultures, were isolated from females and placed in fresh culture vials and were also maintained under the said environmental conditions.

Accessory glands were isolated by dissecting the males of specific age. The isolated glands were fixed in 95% ethanol to precipitate the secretions, which later were separated from the gland with the help of fine entomological needles. Samples were prepared from isolated secretions by putting them into 20 μ l of sample buffer (0.625 M Tris HCl pH 6.8, 2% SDS, 5% β -Mercaptoethanol and 10% Glycerol). Samples from 1 to 12 day old unmated males were prepared, processed separately and analyzed by way of SDS-polyacrylamide gels (T = 13.4%; C = 3.5%). These gels were then stained with Coomassie brilliant blue R-250 and the patterns were documented.

The major protein fractions of accessory gland secretions in various members of *nasuta* subgroup could be arbitrarily categorized into 3 groups, based on the intensity of CBB staining and homology of their SDS-PAGE mobility. When the ontogenetic patterns of accessory gland secretory proteins were analyzed, it was found that all the three major group fractions appear on day one and there is a progressive increase in their quantity from day one to day twelve (Figure 1). However, a minor fraction having an approximate molecular weight of 70 kD appears on day one and its quantity increases up to day eight and then gradually diminishes (Figure 1a). At present, the functional significance of this 70 kD fraction is not known. Further, it is observed that the rate of accumulation of all the three groups of fractions is not the same. It is observed that the group III fractions, the majority of which are produced by X-chromosomal genes (Ravi Ram and Ramesh – communicated for publication), accumulate to the maximum extent as evidenced by the thickening of bands (see Figure). Thus, the synthesis of this tissue specific and sex specific protein is characterized by progressive increase of all the secretory protein fractions, except the 70 kD fraction.

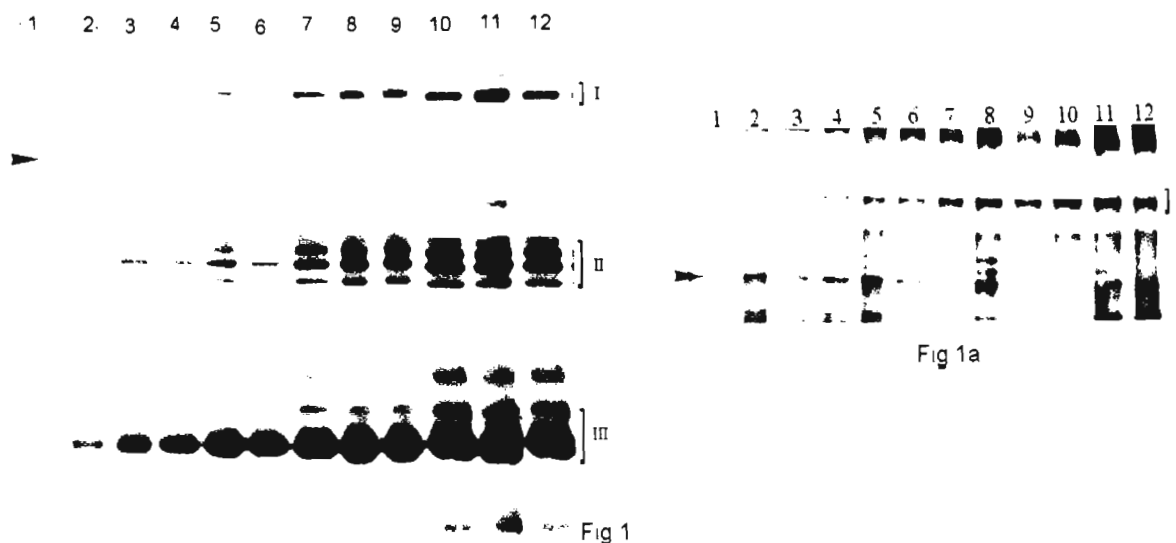


Figure 1. SDS-PAGE patterns of male accessory gland secretory proteins during development. 1-12: accessory gland samples from one to twelve day old unmated adult males (CBB R-250 staining). Figure 1a. Showing the disappearance of 70kD (arrow head) fraction (Silver staining).

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Studies on the genotoxicity of cypermethrin in *Drosophila melanogaster*.

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Cypermethrin (CAS No. 52315-07-8) is a synthetic pyrethroid insecticide used against a wide range of crop pests. It is reported to decrease the activities of acid and alkaline phosphatases in the liver and muscle of fishes (Bhatnagar *et al.*, 1995). It also depletes blood cholesterol, free fatty acids and mitochondrial enzymes in different tissues of fishes (Ghosh, 1990).

According to Pluijmen *et al.* (1984) and Klopman *et al.* (1985) this compound failed to induce gene mutations in *Salmonella typhimurium*. In *E. coli*, cypermethrin did not induce gene mutations (Brooks, 1976). Atale *et al.* (1993) observed a reduction in mitotic index and induction of chromosomal aberrations in *Capsicum annum*. It also induced chromosomal aberrations in the spermatogonial cells of grasshopper *Poecilocus pictus* (Shyin and Usha Rani, 1994). According to Amer and Aboul-ela (1985) this compound induced micronuclei in high frequencies in the bone marrow cells of mouse. The present communication describes the results obtained in the *Drosophila* wing spot as well as sex-linked recessive lethal tests following larval exposures.

Wing mosaic test is a fast one-generation test which assays several genetic end points induced by a mutagen in the wing primordial cells of *Drosophila*. The *mwh* $+/+$ *flr*³ trans-heterozygous larvae were obtained from the cross of high bioactive strains of *mwh* females and *flr*³/TM3, *Ser* males. The allele *mwh* (*multiple wing hairs*, 3-0.3) and *flr*³ (*flare*, 3-38.8) are recessive genetic markers expressed autonomously as multiple trichomes or thick, misshapen trichomes on an otherwise normal adult wing. For details of the markers please refer to Lindsley and Zimm (1992). Third instar larvae were exposed to the LD₅₀ (1×10^{-4} %) and lower doses of the test compound in instant food for the rest of the larval life, *i.e.*, for 48 h (Graf *et al.*, 1984). The wings of the eclosing flies were mounted and observed under a compound microscope to record the size and frequency of the mosaic spots (clones). Each experiment was repeated and the data were pooled and are represented in Table 1. For each experiment a concurrent control experiment was run where the larvae were exposed to the solvent (distilled water). The data were statistically evaluated following the conditional binomial test (Frei and Wurgler, 1988).

The sex-linked recessive lethal (SLRL) test, although time consuming as it involves more than one generation, is regarded as the best validated genotoxicity test in *Drosophila*. In this test, Oregon R larvae of same age were exposed to similar doses of cypermethrin as in the wing mosaic assay. The adult males, on eclosion, were crossed with 3 *Basc* homozygous females for 3 days. The resulting *Basc*/Y males were mated to their *Basc*/Ore-R sibs at a ratio of 1:1 in individual vials. The F₂ progeny were checked for the presence/absence of males with wild type eyes. The data on the frequency of lethal induction (Table 2) were evaluated statistically following Kastenbaum and Bowman (1970).

The frequency of small singles (*mwh* or *flr*³) with 1-2 cells, large singles with 3 or more affected cells and twin (*mwh/flr*³) spots were evaluated separately and the statistical outcomes were inconclusive for all types of spots. In this assay single spots originate due to the induction of gene mutations or gene conversions in the corresponding wild-type genes, deletion of chromosome parts carrying the wild-type alleles (Graf *et al.*, 1984) or induction of mitotic recombination in the chromosome region between the *mwh* and *flr*³ loci (Garcia-Bellido and Dapena, 1974). Twin spots with *mwh* and *flr*³ subclones, on the other hand, stem from the induction of mitotic recombination in the chromosome region between the *flr*³ locus and the centromere (Becker, 1976). In the present experiments, since the frequencies of different wing spots were not significantly higher than the control frequencies, it is concluded that cypermethrin is nongenotoxic in the wing primordial cells of *Drosophila*.

In the SLRL test, the frequency of lethals although higher than the control frequency was not significantly different from the control. Normally the sex-linked recessive lethals arise due to induction of