Ravi Ram, K., and S.R. Ramesh. 2002. Male accessory gland secretions in *D. nasuta* subgroup: Qualitative and quantitative correlations. *Dros. Inf. Serv*. 85: 1-3.

Male accessory gland secretions in *D. nasuta* subgroup: Qualitative and quantitative correlations.

Ravi Ram, K., and S.R. Ramesh. Drosophila Stock Center, Department of Studies in Zoology, University of Mysore, Mysore-570 006. India.

The male accessory gland in *Drosophila* is a bilobed structure, each of which opens into the proximal part of vas deferens that holds mature sperm (Bairati, 1968). Each accessory gland has a simple structure composed of a single layer of cells surrounding a lumen that are in turn surrounded by a muscle sheath. It synthesizes a complex mixture of proteins, carbohydrates, lipids and amino acids, which are transferred to the female during copulation (Chen, 1984; Wolfner, 1997). These glands have been studied to determine the number of cells/gland and their relation to the quantity of secretions (Bairati, 1968; Bertram *et al.*, 1992; Ravi Ram and Ramesh, 2002a,b). Present investigations were undertaken to understand the relationship between accessory gland cell size and the quantity of secretory protein synthesized.

Seven members that belong to the *Drosophila nasuta* subgroup, namely *D. nasuta nasuta* (Coorg, India; Stock Number 201.001), *D. n. albomicans* (Okinawa, Japan; Stock Number 202.001), *D. n. kepulauana* (Sarawak, Stock Number 203.001), *D. kohkoa* (Thailand, Stock Number 204.001), *D. sulfurigaster sulfurigaster* (Queensland, Australia; Stock Number 205.001), *D. s. albostrigata* (Cambodia; Stock Number 207.001), and *D. s. neonasuta* (Mysore, India; Stock Number 206.001), were employed for the present study. These stocks were obtained from Drosophila Stock Centre, University of Mysore, Mysore, India. Into each culture vial (8 × 2.5 cms) containing standard wheat cream agar medium, 50 synchronized eggs were placed, having been collected from the stock cultures by following the modified method of Delcour (Rama-chandra and Ranga-nath, 1988). Care was taken to maintain the constancy of temperature, moisture and quantity of food in these cultures, which otherwise would influence the larval development and ultimately the size of the adults. Unmated males isolated within 3 hr of their eclosion from the pupal case were kept in fresh media vials. All the stocks and experimental cultures were maintained at 22 ± 1°C.

Table 1. Size of the male accessory gland cell and quantity of secretions in different members of *D. nasuta* subgroup.

	Size of the cell (μm^2)*	Quantity of secretions/pair of glands (µg)*
D. n. nasuta	6.04 ± 0.14^{a}	13.00 ± 0.03^{a}
D. n. albomicans	2.55 ± 0.05^{b}	20.00 ± 0.05^{b}
D. n. kepulauana	5.13 ± 0.09^{c}	10.50 ± 0.05^{c}
D. kohkoa	2.39 ± 0.06^{b}	9.50 ± 0.04^{d}
D. s. sulfurigaster	3.16 ± 0.09^{bd}	13.00 ± 0.09^{a}
D. s. albostrigata	3.53 ± 0.15^{d}	13.50 ± 0.06^{a}
D. s. neonasuta	2.89 ± 0.08^{b}	15.20 ± 0.01^{e}
F value	231.4	1297

The strains with the same alphabet in superscript are not significantly different at 5% level according to DMRT.

Note: df = * (6, 168)

For the present investigations, seven-day-old unmated males were utilized and the samples were prepared by dissolving accessory gland secretions of a pair of glands (from single individual) in 25µl of sample buffer (see Ravi Ram and Ramesh, 2001). The protein quantity in these samples was determined by the micromethod (Neuhoff, 1985). For the determination of cell size (in terms of area), the accessory glands dissected out from seven-day-old unmated male flies in Medium A (Ashburner, 1970), were fixed in 1N HCl for 5 min and later transferred to 2% lactoaceto-orcein.

After 20 minutes, the glands were gently opened up with the help of the fine entomological needles and squashed between a slide and cover glass in 45% acetic acid so as to spread the cells in a single layer. Under high magnification (400×), the length and breadth of the cell was measured by micrometry. The area thus calculated was taken as the size of the cell as it is not possible to obtain cell volume through micrometry. Twenty five such preparations were used to determine the average size of the cell. This value was divided by magnification to get actual size of the cell. The data were subjected to ANOVA followed by DMRT to determine the significance of differences. Further, the correlation coefficient was calculated for the comparison between size of the cell and the quantity of secretions and the Student t-test was applied to test the significance of the correlation coefficient.

Among different members analyzed, the amount of secretions was maximum in the case of *D. n. albomicans*, while *D. kohkoa* had the least. The differences in the quantity of secretions are non-significant only among *D. n. nasuta, D. s. albostrigata* and *D. s. sulfurigaster*. In all other comparisons, the differences were significant (Table 1). Further, cell size was maximum in *D. n. nasuta*, while those of *D. kohkoa* are the smallest. The differences in the cell size were non-significant among *D. n. albomicans, D. kohkoa, D. s. sulfurigaster*, and *D. s. neonasuta*. Cell size in *D. s. albostrigata* was found to be significantly different when compared with that of all the members studied except *D. s. sulfurigaster*. The size of accessory gland cells in *D. n. nasuta* and *D. n. kepulauana* differed significantly not only among themselves but also with others (Table 1). The coefficient of correlation was (-) 0.29 for the comparison between the quantity of secretions and the cell size that was found to be non-significant when subjected to Student t-test (Table 2).

Table 2. Correlation coefficient for different comparisons and their significance.

Comparison	Correlation coefficient (r)	t	P
Cell size *vs* quantity of secretions	(-) 0.29	0.14	> 0.05
Protein fractions of group I *vs* quantity of secretions	0.00	0.00	> 0.05
Protein fractions of group II *vs* quantity of secretions	0.14	0.3	> 0.05
Protein fractions of group III *vs* quantity of secretions	0.94	18.05	> 0.05

Note: df = 5

Drosophila nasuta subgroup of *immigrans* group is a congerie of closely related morphologically almost identi-cal members with differential levels of reproductive isolation (Wilson *et al.*, 1969; Niramala and Krishnamurthy, 1974). Working on accessory gland proteins in the *D. nasuta* subgroup, recently we have shown absence of correlation between the quantity of secretions and the number of cells/gland indicating that it is the level of gene activity in the secretory cells which determines the quantity of secretions (Ravi Ram and Ramesh, 2002b). However, in the present study no correlation could be found between the quantity of secretions and the cell size. This indicates that the secretions are poured into the lumen of the gland immediately after their synthesis. This is contrary to the pattern of glue protein production by larval salivary glands of *Drosophila* wherein the secretions are retained within the secretory vesicles of the salivary gland cell and are extruded at once into the lumen just prior to pupation (Lane *et al*., 1974). The fact that there is an increase in the quantity of secretions in the gland lumen from day 1 to day 7 (Ravi Ram and Ramesh, 1999) supports the present finding of the release of secretions into the gland lumen immediately after their synthesis.

The SDS-PAGE patterns of accessory gland proteins in various members of the *D. nasuta* subgroup are simple, consisting of 4-8 major fractions (Ravi Ram and Ramesh, 1999a) as compared with 40 fractions in *D. melanogaster*. Based on mobility they are categorized into group I, II and III, with one protein fraction in group I, 2 to 4 in group II, and 1 to 3 protein fractions in group III (Ravi Ram and Ramesh, 1999a). By analyzing the peak area to determine their relative proportions with the

help of Molecular Analyst software of Gel Doc 1000 (Bio-Rad, USA) we have shown that the group III fractions constitute maximal part of the secretions that ranges from 65% (in *D. s. albostrigata*) to 74% (in *D. kohkoa*), whereas group II consisting of 2-4 fractions among the members analyzed constitute 14% to 25% with minimum in *D. n. nasuta* and maximum in *D. s. albostrigata*. Group I constitutes only 8 to 12% of the major fractions (Ravi Ram and Ramesh, 2001). In the present study, we have made an attempt to find out the relationship between the number of accessory gland protein fractions in different groups among seven members of *D. nasuta* subgroup with their quantity of the secretions. Correlation analysis has shown a significant positive correlation only between group III fractions and the quantity of secretions synthesized; but not with the fractions of groups I and II (Table 2). This clearly shows that bulk of the accessory gland secretions are formed by group III fractions. This also supports the finding that there is a substantial increase only with respect to group III fractions during aging of the fly (Ravi Ram and Ramesh, 1999b).

Acknowledgments: The authors thank Prof. H.A. Ranganath, Chairman, Department of Studies in Zoology, for the facilities and encouragement, Dr. Vishnu Hebbar, Department of Statistics, University of Mysore for statistical help.

References: Ashburner, M., 1970, Chromosoma, 31: 356-376; Bairati, A., 1968, Monit. Zool. Ital. 2: 105-182; Bertram, M., G.A. Akerker, R.L. Ard, C. Gonzalez and M.F. Wolfner, 1992, Mech. Dev. Biol. 38: 33-40; Chen, P.S., 1984, Ann. Rev. Entomol. 29: 233-255; Lane, N.J., Y.R. Carter, and M. Ashburner, 1972, Wilhelm. Roux. Archiv. 169: 216-238; Neuhoff, V., 1985, in *Modern Methods in Protein Chemistry*, edited by H. Tschesche, Vol. 2, Walter de Gruyter, Berlin, pp 1-62; Nirmala, S.S., and N.B. Krishnamurthy, 1974, J. Mysore Univ. 26: 162-167; Ramachandra, N.B., and H.A. Ranganath, 1988, Genome 30: 58-62; Ravi Ram, K. and S.R. Ramesh, 1999, Dros. Inf. Serv. 82: 65-67; Ravi Ram, K. and S.R. Ramesh, 2001, Biochem.Genet. 39, 99-115; Ravi Ram, K. and S.R. Ramesh, 2002a, Indian J. Exp. Biol. 39 (in press); Ravi Ram, K. and S.R. Ramesh, 2002b, Zool. Sci. (in press); Wilson, F.D., M.R. Wheeler, M. Harget, and M. Kambysellis, 1969, Univ. Texas Publ. 6918: 207-253; Wolfner, M.F., 1997, Insect Biochem. Mol. Biol. 27: 179-192.