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X-chromosomal linkage of larval secretion fractions in the *Drosophila nasuta* subgroup.

In earlier studies we have analysed the patterns of larval secretion protein fractions in various members of the *Drosophila nasuta* subgroup. These members are taxonomically closely related and exhibit different degrees of reproductive isolation. By employing alternatively 13.4% and 15% SDS-Polyacrylamide separating gels with 5.6% stacking gels, we could show that: (1) Homologous secretion fractions in individual *Drosophila nasuta* subgroup members (Figure 1) differ by their electrophoretic mobility. By this, we grouped them into five (I - V) domains (Ramesh and Kalisch, 1989). (2) Domain I - III fractions are glycosylated (Zajonz *et al.*, 1996b). (3) Domain II and III fractions are X-chromosomal linked. Data are based on the F1 progeny from parents, which are cross fertile subgroup members indicating different electrophoretic mobilities of homologous fractions (Ramesh and Kalisch, 1989). (4) By puff analysis of polytene chromosomes and recombination analysis of F1 and F2 patterns we could assume that synthesis of domain II and III fractions probably is clustered in the huge puff of salivary gland X-chromosome division 10 (Ramesh and Kalisch, 1988a). (5) Chromosomal location of domain I fractions so far was hampered by almost the same electrophoretic mobility in all subgroup members (Figure 1). However, recently we got the first hint for X-chromosomal linkage of domain I fractions from the F1 progeny of *Drosophila nasuta nasuta* females and *Drosophila sulfurigaster sulfurigaster* males by using an elongated 5.6% stacking gel together with our standard 13.4% separating gel (Zajonz *et al.*, 1996a).

In the present study, we have analysed domain I fractions by using gradient gels for pattern analysis of cross fertile subgroup members *Drosophila nasuta nasuta* (wild type Mysore I) and *Drosophila nasuta albomicans* (Okinawa; No. 155112-1751.0, stock list 1990 of Bowling Green) as well as for *Sandhya* (*Drosophila nasuta nasuta*^{Sa}), a dominant

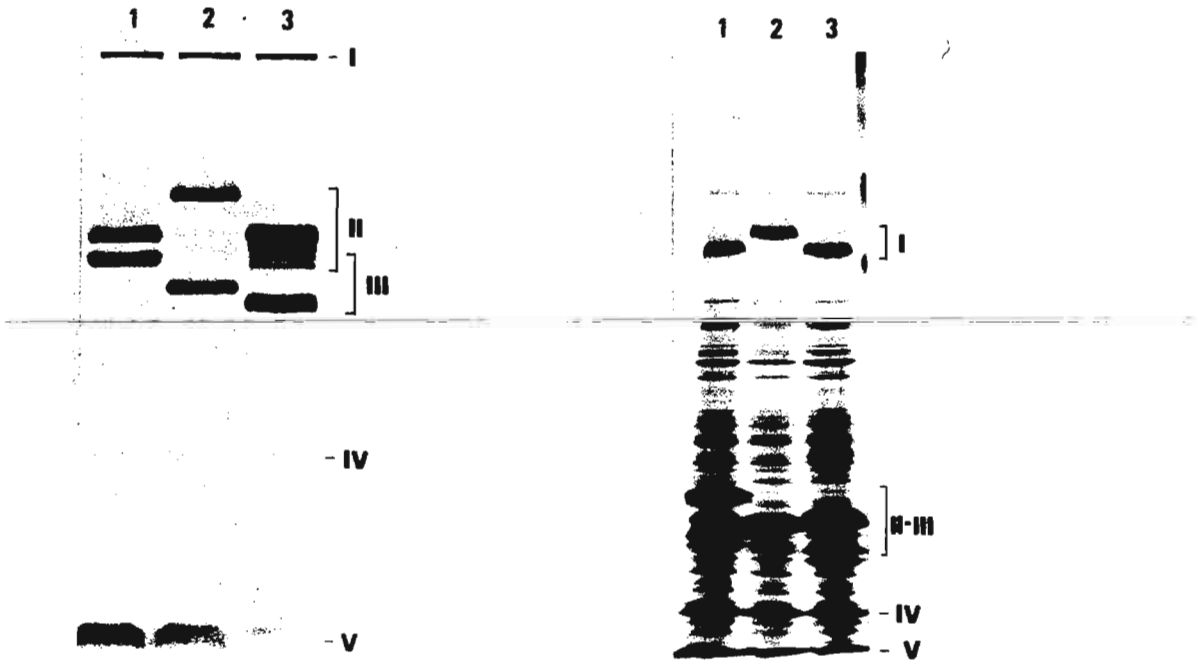

Figure 1 (above, left). Larval secretion fractions in (1) wild type Mysore I mutant *Drosophila n. nasuta*^{Sa}, (2) *Drosophila n. nasuta* (wild type Mysore I), (3) *Drosophila n. albomicans* (Okinawa). 15% SDS-Polyacrylamide separating gel and 5.6% stacking gel (not shown); CBB-staining; two secretion plugs used for each lane; depiction of domain I - V according to Ramesh and Kalisch (1989).

Figure 2 (above, right). Different electrophoretic mobility of domain I fractions in (1) *Drosophila n. nasuta* (wild type Mysore I), (2) wild type Mysore I mutant *Drosophila n. nasuta*^{Sa} (3) *Drosophila n. albomicans* (Okinawa). 4 - 20% SDS-Polyacrylamide gradient gel with 3% stacking gel (not shown); CBB-staining; six salivary glands were used for each lane. Note that domain II and III fractions can not be separated in lane (2) by the technique used.

and spontaneous secretion protein mutant from the Mysore I wild type strain (Kalisch and Ramesh, 1988b). Furthermore, we checked domain I fractions in *Drosophila nasuta kepulauana* (Brunei, Borneo; 15112-1761.1), *Drosophila kohkoa* (Bon Chakkrarat, Thailand; 15112-1771.0), *Drosophila sulfurigaster albostrigata* (Ari Ksatr, Kambodia; 15112-1811.1) and *Drosophila sulfurigaster sulfurigaster* (Kavieng I, New Ireland; 15112-1831.1).

Figure 1 provides a comparative picture of salivary gland secretion protein fractions obtained after electrophoresis on a 15% SDS-Polyacrylamide gel. For more details concerning double band character of domain II and III fractions as well as methodological details see Ramesh and Kalisch, 1988a. Note that homologous domain I, IV, and V fractions in individual strains indicate almost the same electrophoretic mobility.

Figure 2 indicates differences of domain I fraction mobility, using a 4 - 20% SDS-Polyacrylamide gradient gel together with a 3% stacking gel (not depicted). In comparison with Figure 1 the increased number of fractions is due to the use of whole salivary glands instead of only secretion plugs. Domain II and III fractions can hardly be separated in Figure 2 (lane 1 and 3) and can not be separated in (2) by use of the 4 - 20% gradient gel.

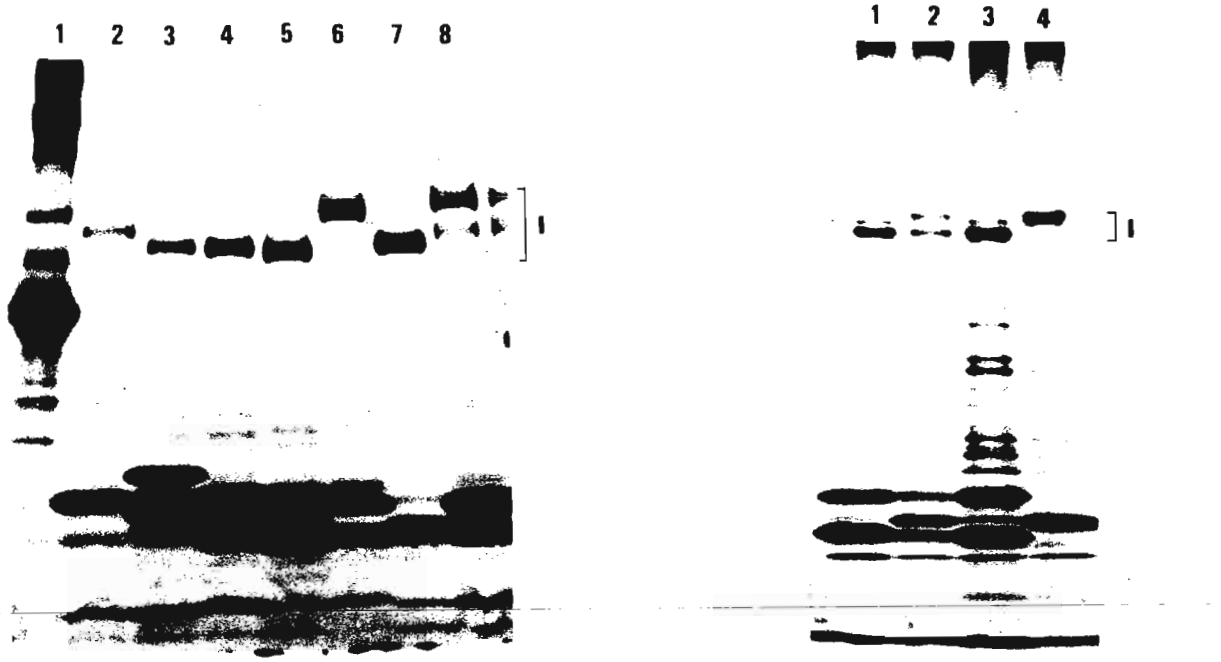

Figure 3 (above, left). Electrophoretic mobility of domain I fractions in different *Drosophila nasuta* subgroup members. (1) Phosphorylase b marker protein, SIGMA, P 8906; (2) *Drosophila n. nasuta*^{Sa} females, 4 secretion plugs; (3) *Drosophila n. nasuta* males, 5 plugs; (4) *Drosophila n. albomicans* females, 5 plugs; (5) *Drosophila n. kepulauana* females, 6 plugs; (6) *Drosophila kohkoa* females, 6 plugs; (7) *Drosophila s. albostrigata* males, 5 plugs; (8) *Drosophila s. sulfurigaster* females, 4 plugs. 4 - 20% SDS-Polyacrylamide gradient gel with 3% stacking gel (not depicted); CBB-staining.

Figure 4 (above, right). X-chromosomal linkage of the domain I fraction. (1) *Drosophila n. nasuta* female larvae, (2) F1 female larvae of the crossing *Drosophila n. nasuta* females x *Drosophila n. nasuta*^{Sa} males, (3) F1 male larvae of the same crossing, (4) *Drosophila n. nasuta*^{Sa} male larvae. Five plugs were used for each lane; 4 - 20% SDS-Polyacrylamide gradient gel with 3% stacking gel (not depicted); silver-staining (Ansorge, 1985).

Surprisingly, domain I fractions in *Drosophila nasuta nasuta* and *Drosophila nasuta albomicans* are almost identical (158 kD) in the 4 - 20% gradient gel of Figure 2, whereas *Sandhya* indicates a value of 178 kD for the homologous fraction. However, matching of kD values in *Drosophila nasuta nasuta* and in *Drosophila nasuta albomicans* are incidental. In an additional study (in preparation) we have found various kD values even for domain I fractions in several wild type strains of *Drosophila nasuta albomicans*.

Additional data of remaining subgroup members in Figure 3 also indicate significantly different electrophoretic mobility of domain I fractions. Based on these results, we assume that subgroup member-specific patterns of domain I

fractions could be used in the same way for taxonomic identification as it has already been done with domain II and III fractions (Ramesh and Kalisch, 1989).

A comparison (Figure 4) between the patterns of P-generation *Drosophila nasuta nasuta* females and *Drosophila nasuta nasuta*^{Sa} males as well as F1 males and females proves that domain I fractions are X-chromosomal linked. We also obtained comparable data for remaining subgroup members (not depicted). However, as already mentioned in Zajonz *et al.* (1996a), the second (and smaller) domain I fraction in *Drosophila sulfurigaster sulfurigaster* [(8) in Figure 3] is autosomally linked (data not depicted).

Note that in Figure 4 we used the very sensitive silver-staining by which: (1) the glycosylated domain I (and II) fractions are stained yellow in the gel and, therefore, indicate a lower contrast; (2) additional fractions become visible on top of each lane (Kalisch and Ramesh, 1997); (3) one additional fraction (brown color in the gel) becomes visible on top of the domain I fraction in *Drosophila nasuta nasuta* [lane (1) and (3)].

So far, we have not found any recombinant pattern (concerning the domain I and II fractions) in thirty F1 males from heterozygous *Drosophila nasuta nasuta/Drosophila nasuta nasuta*^{Sa} mothers. However, additional experimental work is needed (so far hampered by the absence of suitable genetic markers) to prove our assumtion that even the synthesis of the domain I fraction is clustered within the salivary gland X-chromosome division 10 puff.

Nevertheless, differences of domain I - III fractions in individual *Drosophila nasuta* subgroup members as well as individual wild type strains on one side and differences of domain I - III fractions in the *Sandhya* mutant on the other have prompted us to assume that DNA sequencing of the X-chromosomal division 10 puff could give some interesting insights on evolutionary genetic processes, which have occurred within the *Drosophila nasuta* subgroup.

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References: Ansorge, W., 1985, J. Biochem. Biophys. Methods 11: 13-20; Kalisch, W.-E. and S.R. Ramesh 1997, Dros. Inf. Serv., this issue; Ramesh, S.R. and W.-E. Kalisch 1988a, Biochem. Genetics Vol. 26, Nos. 7/8: 527-541; Ramesh, S.R. and W.-E. Kalisch 1988b, Dros. Inf. Serv. 67: 51-52; Ramesh, S.R. and W.-E. Kalisch 1989, Genetica 78: 63-72; Zajonz, M., S.R. Ramesh, and W.-E. Kalisch 1996a, Dros. Inf. Serv. 77: 47-48; Zajonz, M., S.R. Ramesh, and W.-E. Kalisch 1996b, Dros. Inf. Serv. 77: 76-78.

Kalisch, W.-E.,[1] and S.R. Ramesh[2]. [1]Ruhr-Universität Bochum, FR Germany and [2]University of Mysore, India. [1]e-mail: wolf.kalisch@rz.ruhr-uni-bochum.de Salivary gland secretion fractions in *Drosophila hydei.*

In earlier studies (Zajonz *et al.*, 1996a and b), we could show that patterns of secretion fractions in *Drosophila hydei* using SDS-Polyacrylamide gels are: (1) species-specific and completely different from *Drosophila melanogaster*; (2) wild-type-specific by different electrophoretic mobility of homologous fractions; (3) wild-type-specific by the presence of individual fractions; (4) sex-specific by individual fractions in several wild type strains (Figure 1).

Our earlier experiments were conducted by using our standard 13.4% SDS-Polyacrylamide separating gels in combination with elongated 5.6% stacking gels and analysed by silver-staining. In the present paper we have checked *Drosophila hydei* strains by using 4 - 20% SDS-Polyacrylamide gradient separating gels with 3% stacking gels to spread the prominent fractions depicted in Figure 1. We used CBB-staining as well as CBB/silver-staining.

Domains I - V indicate to what extent the 4 - 20% gradient gels in Figures 2 and 3 depict more details in comparison with the 13.4% gel in Figure 1. Domains are based on so far comparative pattern analyses of homologous fractions in different *Drosophila hydei* wild type strains. Otherwise, the domains are arbitrary. Thus, individual domain fractions in *Drosophila hydei* and, for instance, *Drosophila nasuta* (Kalisch and Ramesh, 1997) probably lack any homology concerning functional or biochemical aspects.

Comparison between CBB-staining (Figure 2, lane 1 and 3) and additional silver-staining (lanes 2 and 4) in male and female patterns depict that sex-specific fractions obviously are present in both sexes of the Tübingen wild type, but indicate sex-specific differences concerning the gene expression. Even if one considers that the patterns of individual lanes in CBB/silver-stained SDS-Polyacrylamide gradient gels can be different by: (1) the quantity of proteins extracted from the glands, (2) by staining conditions, and/or (3) by photographic parameters, one can´t ignore sex-specific differences between lanes 2 and 4 by their prominence and by the reproduction of experimental data. The interpretation of sex-specific fractions in *Drosophila hydei* larvae is still missing. But their existance probably could contradict the proposition so far made, that secretion fractions are exclusively used for fixing the pupa to a substratum (Fraenkel and Brookes, 1953; Shirk *et al.*, 1988; Riddiford, 1993).