

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*^{5a} 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*^{5a} mothers. However, additional experimental work is needed (so far hampered by the absence of suitable genetic markers) to prove our assumption 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. Ramesh². ¹Ruhr-Universität Bochum, FR Germany and ²University of Mysore, India. ¹e-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 existence 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).

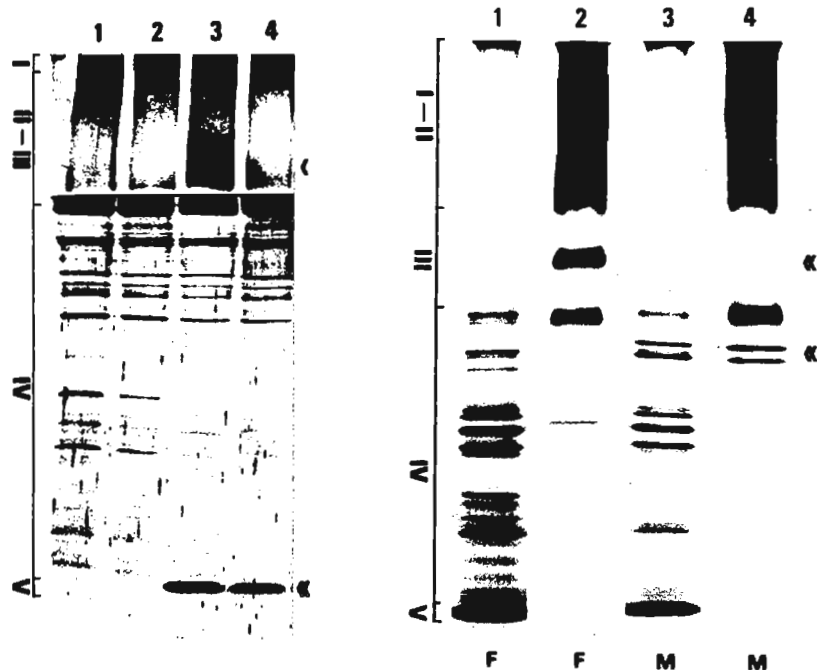


Figure 1 (above, left). Larval secretion fractions from the two salivary gland plugs of an individual *Drosophila hydei* wild type larva: (1) Münster female, (2) Münster male, (3) Zürich female, (4) Zürich male. Note that Zürich shows a strain-specific fraction [«] as well as a sex-specific fraction in the female [<]. 13.4% SDS-Polyacrylamide gel with 5.6% stacking gel; silver-staining. Minor fractions belong, at least in part, to salivary gland cell fractions which remain attached to the plugs in various amounts by the preparation technique used (Ramesh and Kalisch, 1988).

Figure 2 (above, right). Sex-specific salivary gland fractions in *Drosophila hydei* wild type Tübingen larvae comparing CBB-staining (lanes 1 and 3) and additional silver-staining (2 and 4) in 4 - 20% SDS-Polyacrylamide gradient gel with 3% stacking gel (not shown). Five salivary glands were used in each sex; M = males and F = females. « = fractions with different gene expression in females and males. Note the difference of CBB- and silver-staining (Ansorge, 1985) of domain I and II fractions.

Additive silver-staining of Coomassie Brilliant Blue R 250 stained patterns in Figure 2 needs additional information: (1) Domain I and II fractions are hardly to depict by the standard CBB-staining used, but get overstained by the silver-staining (by which divisioning into domain I and II, compare Figure 3, is no longer possible). The biochemical details of these different staining behaviours are still unknown. (2) CBB stained fractions in lane 1 and 3 represent secretion fractions and salivary gland cell fractions (most of the smaller domain IV fractions shown). These fractions have not lost their prominent contrast during the additional silver-staining in lane 2 and 4, but have been reduced in photographic contrast not to overexpose domain I - III fractions in the same lanes.

In additional experiments we used PAS-staining (not depicted), but we failed to find any glycosylated secretion fraction in *Drosophila hydei*.

For a more detailed staining of domain I and II fractions, we used plugs instead of whole salivary glands (Figure 3) and conducted a more intensive (2 hour) CBB staining. By this, we found that in contrast to Figure 2 (lanes 1 and 3) at least individual domain I and II fractions of 288 kD and 182 kD could be shown by CBB-staining.

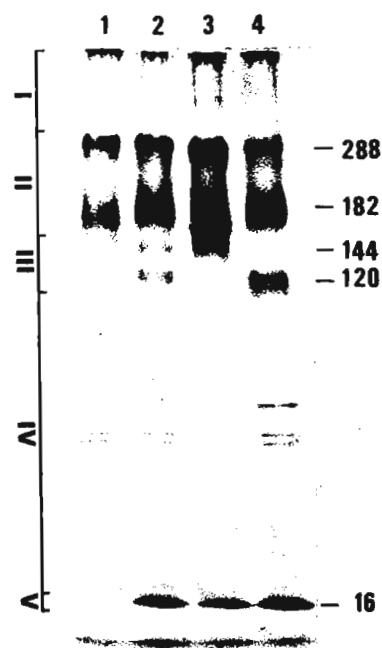
The Münster and the Alicante wild types (which indicate no sex-specific fractions) differ by the electrophoretic mobility of the domain III fraction and by the presence of the 16 kD domain V fraction in the Münster wild type.

From data of Figure 3 and the reciprocal crossing (not depicted) we conclude that domain III fractions are gonosomally linked, whereas the domain V fraction is autosomally linked. Experiments are in progress to localize the gonosomal and autosomal genes by a technique so far not used in *Drosophila* genetics: Recombination analysis of phenotypic markers are combined with recombination analysis of secretion patterns in always one and the same recombinant. This is possible by secretion analysis of single pupae after hatching of the flies; a technique which we have already established earlier (Kalisch and Ramesh, 1988).

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References: Ansorge, W., 1985, J. Biochem. Biophys. Methods 11: 13-20; Fraenkel, G., and V.J. Brookes 1953, Biol. Bull. (Woods Hole) 105: 442-449; Kalisch, W.-E., and S.R. Ramesh 1988, Dros. Inf. Serv. 67: 51-52; Kalisch, W.-E., and S.R. Ramesh 1997, Dros. Inf. Serv., this issue; Ramesh, S.R., and W.-E. Kalisch 1988, Biochem. Genetics, Vol.

Figure 3. Chromosomal linkage of secretion protein fractions in *Drosophila hydei* larvae. (1) wild type Münster females, (2) F1 females of the crossing: wild type Münster female \times wild type Alicante male, (3) F1 males of the same crossing, (4) Alicante males. Five plugs were used for each lane of the 4 - 20% SDS-Polyacrylamide gradient gel covered by a 3% stacking gel (not shown); CBB-staining. kD values are based on phosphorylase b marker protein (P 8906, Sigma). Note that domain III fractions indicate X-chromosomal linkage, whereas domain V fraction indicates autosomal linkage.



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Stain-specific characterization of larval secretion fractions.

In several studies concerning the biochemical genetics of salivary gland secretion fractions in the *Drosophila nasuta* subgroup we could show that the major fractions could be grouped into five domains (I - V) which are characterized by variations in the electrophoretic mobility of homologous secretion fractions in: different subgroup members, different wild

type strains, and mutants (Ramesh and Kalisch, 1988; Kalisch and Ramesh, 1997). In a recent paper we could show that at least two additional fractions (which are named domain 0 fractions in the following) could be localized when the larval secretion plugs of *Drosophila nasuta* subgroup members and other species were electrophoresed on 13.4% SDS-Polyacrylamide gels with elongated 5.6% stacking gels. To visualize these fractions, one has: (1) to fix and eluate the salivary glands with ethanol (instead of a TCA and ethanol/chloroform mixture) and (2) to use silver-staining instead of the common Coomassie Brilliant Blue staining technique (Zajonz *et al.*, 1996).

In the present paper we have checked these domain 0 fractions by using alternatively 4 - 20% and 4 - 12% SDS-Polyacrylamide gradient gels covered by 3% stacking gels. Furthermore, we have used various staining techniques to characterize the differences between the individual fractions.

We checked larval secretion fractions of *Drosophila nasuta nasuta* [wild type Mysore I, No. 15112-1781.0 of the species stock list (1990) from Bowling Green], *Drosophila repleta* (No. 15084-1611.0), *Drosophila rubida* (No. 15115-1901.0), and *Drosophila simulans* (wild type Ethiopia). Staining techniques used are: (CBB) Coomassie Brilliant Blue R 250 (Diezel *et al.*, 1972); Silver (Ansorge, 1985); (PAS) Periodic Acid Schiff (Jay *et al.*, 1990); Alcian Blue 8GX (Krueger and Schwarz, 1987); and Sudan black B (Andrews, 1986).

Figure 1 (A and B) indicates that the domain 0 fractions are not visible by standard CBB-staining in 4 - 20% SDS-Polyacrylamide gradient gels. The reason probably is the use of TCA to fix the tissue in the standard CBB technique. A more detailed description of the *Drosophila nasuta* pattern in a 4 - 20% SDS-Polyacrylamide gradient gel is given in Kalisch and Ramesh (1997).