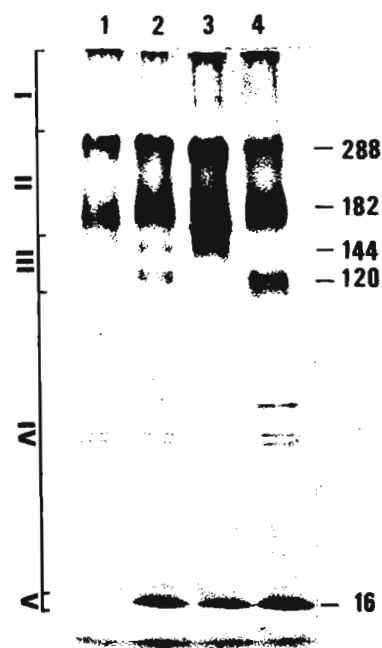


Figure 3. Chromosomal linkage of secretion protein fractions in *Drosophila hydei* larvae. (1) wild type Münster females, (2) F1 females of the crossing: wild type Münster female \times wild type Alicante male, (3) F1 males of the same crossing, (4) Alicante males. Five plugs were used for each lane 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.



26, Nos. 7/8: 527-541; Riddiford, L.M., 1993, The development of *Drosophila melanogaster*, Vol. 2 (Ed., Bate, M., and A. Martinez Arias), Cold Spring Harbor Lab., N.Y. pp. 899-939; Shirk, P.D., P.A. Roberts, and G.H. Harn 1988, Roux's Arch. Dev. Biol. 197: 66-74; Zajonz, M., S.R. Ramesh, and W.-E. Kalisch 1996a, Dros. Inf. Serv. 77: 73-74; Zajonz, M., S.R. Ramesh and W.-E. Kalisch 1996b, Dros. Inf. Serv. 77: 74-76.

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
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).

Figure 1. Comparison of homologous larval secretion fractions in *Drosophila nasuta* using different staining-techniques. (A) CBB [5P]; (B) CBB [5G], (C) Silver [3P], (D) PAS/Alcian [16G], (E) Sudan black [15P]. Number of glands [G] or secretion plugs [P] used for individual lanes are included in parenthesis. 4 - 20% SDS-Polyacrylamide gradient gels covered by 3% stacking gels (not shown). Domain I - V sectioning of secretion fractions according to Kalisch and Ramesh, 1997. For domain 0 fractions and different staining contrast of homologous fractions see text.

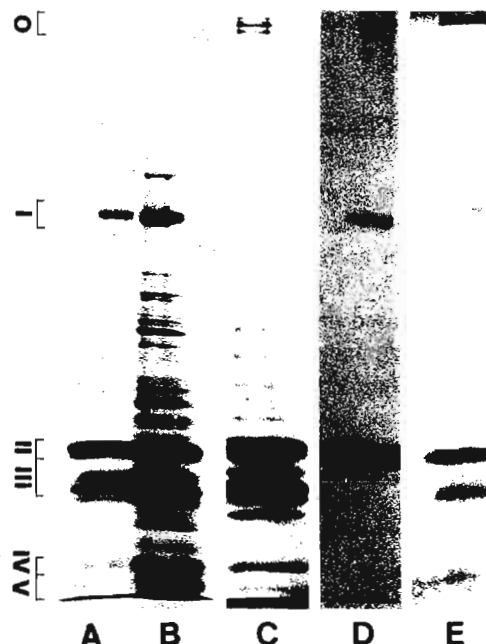
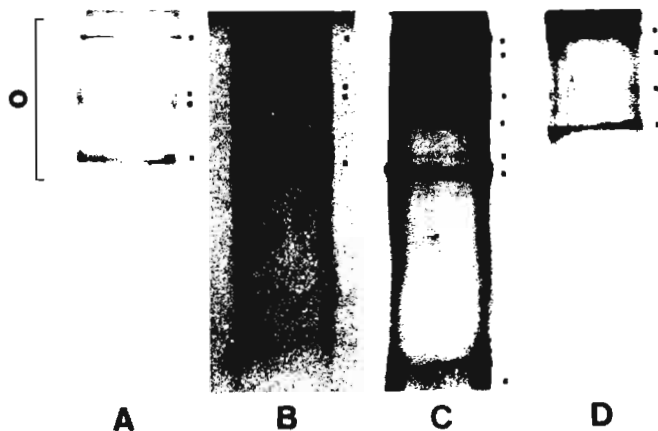


Figure 2. Comparison of domain 0 larval secretion fractions in: (A) *Drosophila nasuta* [1P], (B) *Drosophila repleta* [2P], (C) *Drosophila rubida* [1D], (D) *Drosophila simulans* [2D]. Number of glands [G] or plugs [P] used for individual lanes are included in parenthesis. Silver-staining; 4 - 12% SDS-Polyacrylamide gradient gel covered by a 3% stacking gel (not shown).

By using the more sensitive Silver-staining (Figure 1C), four domain 0 fractions become visible. By the use of phosphorylase b marker proteins (P 8906, SIGMA) we could calculate their Molecular Weight and found a range between 500 - 600kD. Note that we used secretion plugs in (C), but cell fractions attached to the plugs become visible by Silver-staining. However, CBB-staining in (B) and Silver-staining in (C) are not identical, indicating that staining differences in domain 0 fractions are not exceptional for larval secretion fractions.

Biochemical and functional aspects of domain 0 fractions so far are still unknown. The fact that we found quantitatively almost equal amounts of domain 0 fractions in whole salivary glands and in (carefully prepared) glue plugs as well as various patterns in different species (Figure 2) should indicate that: fractions of the cell (housekeeping proteins), nuclear fractions (including fractions of membranes), and/or methodological artifacts (Tasheva and Dessev, 1983) should not be part of the domain 0 fractions.

We used a 4 - 12% SDS-Polyacrylamide gradient gel to spread the domain 0 fractions (Figure 2). We checked *Drosophila nasuta*, *Drosophila repleta*, *Drosophila rubida*, and *Drosophila simulans* in one and the same gel. In several experiments, at least four fractions were found in each species. In *Drosophila rubida* (Figure 2C) we found additional fractions of which it is so far unclear whether or not the smallest one (at the bottom of the lane) belongs to domain 0 or domain I fractions.

The fact that the electrophoretic mobility of most of the domain 0 fractions is similar and can only be spread sufficiently in a 4 - 12% gradient gel obviously does not reflect biochemical similarity. We could show by Alcian-blue-staining (together with PAS-staining in Figure 1C) that only one of the four domain 0 fractions is Alcian-positive. From

the experiments with 4 - 12% gradient gels (data not shown), we know that the third fraction of domain 0 fraction (from top) is the Alcian-positive one.

We used Alcian blue in a salt solution (Scott and Dorling, 1965). By this, our data could be interpreted that the domain 0 fraction contains acid glycosaminoglycans (mucopolysaccharides). Staining of the same domain 0 fraction and the domain I - III fractions by Sudan black (Figure 1D) could be a hint that the Alcian-positive domain 0 fraction is a protein. But lipoproteins are also stained by the Sudan black technique we used.

Contrast of Silver-stained domain I fraction in Figure 1C is very low by the original light-yellow color of this fraction (compare Kalisch and Ramesh, 1997). Domain III-V fractions in Figure 1D are PAS-negative by the staining combination used (domain III) as well as by the completely missing (domain IV and V) glycosylation (Ramesh and Kalisch, 1989).

Data so far collected indicate that domain 0 fractions quantitatively are a minor group of biochemically different larval secretion fractions which are found not only in the *Drosophila nasuta* subgroup, but in others too.

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References: Andrews, A.T., 1986, Electrophoresis. Clarendon Press, Oxford; Ansorge, W., 1985, J. Biochem. Biophys. Methods 11: 13-20; Diezel, W., G. Kopperschlager, and E. Hofmann 1972, Anal. Biochem. 48: 617-620; Jay, G.D., D.J. Culp, and M.R. Jahnke 1990, Anal. Biochem. 185: 324-330; Kalisch, W.-E., and S.R. Ramesh 1997, Dros. Inf. Serv., this issue; Krueger, R.C., and N.B. Schwarz 1987, Anal. Biochem. 167: 295-300; Ramesh, S.R., and W.-E. Kalisch 1988, Biochem. Genetics Vol. 26, Nos. 7/8: 527-541; Scott, J.E., and J. Dorling 1965, Histochemie 5: 221 - 233; Tasheva, B., and G. Dessev 1983, Analyt. Biochem. 129: 98-102.

Derzhavets, Elena, A. Korol, T. Pavlicek, and E. Nevo. Institute of Evolution of Haifa, Mount Carmel, Haifa 31905, Israel, E-mail: Korol@esti.haifa.ac.il. Adaptation to stressful environment and mutation rate: a case study in *Drosophila melanogaster*.

Mutation rate is one of the most fundamental characteristics of the genetic system. Theoretical models predict that under normal constant environmental conditions mutation rate (*mtr*) should evolve toward the lowest possible level (Lieberman and Feldman, 1986), although the cost of fidelity may counterbalance this trend leading to some intermediate equilibrium value of *mtr* (Ishii *et al.*

et al., 1989; Kondrashov, 1995). With frequently changing environment, selection may favour higher *mtrs* (Gillespie, 1981; Ishii *et al.*, 1989; Haraguchi and Sasaki, 1996). An appreciable genetic variation in spontaneous mutation rate has been documented for *Drosophila* (see Ashburner, 1989). Likewise, it is well known that an individual subjected to stressful ecological conditions reacts by an increase in *mtr* (reviewed in Woodruff *et al.*, 1983; Ashburner, 1989). However, next to nothing is known whether microevolutionary adaptation to stressfully fluctuating conditions in natural populations indeed results in increased level of mutations.

We conducted an experimental test of this prediction employing freshly collected material from the "Evolution Canyon" at Mount Carmel, Israel (Nevo, 1995). The opposite slopes of the Canyon differ in the level of solar radiation up to three-fold. Consequently, the abiotic conditions on the south-facing slope (SFS) are much more stressful and variable as compared to those on the north-facing slope (NFS). Earlier, we have shown that isofemale lines of *D. melanogaster* and *D. simulans* established from flies of the opposite slopes demonstrate significant differences for a number of adaptively important traits (Derzhavets *et al.*, 1996; Nevo *et al.*, 1997): lines derived from the SFS appeared to manifest higher resistance to heat and drought stress. Likewise, our recent fluctuating asymmetry test of wing measurements on *D. melanogaster* showed a significantly higher between-individual variation on SFS as compared to NFS (see Derzhavets *et al.*, this issue). These data can be considered as an indication of population differentiation on a microscale level, in spite of a rather small distance of 200 m between the stations 2 and 6 on the opposite slopes.

According to the foregoing theoretical models, one could expect a higher mutation rate in the SFS subpopulation as compared to the NFS. This expectation was confirmed in experiments with the fungus *Sordaria fimicola* collected from the Canyon (Lamb *et al.*, 1997). Likewise, we have compared isofemale lines of *D. melanogaster* from the opposite slopes of the Canyon for the rate of male recombination (Derzhavets *et al.*, 1996, and unpublished results). It appeared that SFS-flies manifest significantly higher recombination rate. Although increased male recombination is a component of the hybrid dysgenesis syndrome, the latter cannot be considered as an explanation of our results because the foregoing interslope difference was observed for both directions of crosses between the wild type flies and multiple marker stocks (see also Scobie and Schaffer, 1982). The reviewed data on