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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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 (Liberman and Feldman, 1986), although the cost of fidelity may counterbalance this trend leading to some intermediate equilibrium value of *mtr* (Ishii *et al.*, 1989; Kondrashov, 1995). With frequently changing environment, selection may favour higher *mtr*s (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

Drosophila from the Canyon were obtained on isofemale lines maintained in standard laboratory conditions for about 1-2 years.

Here we report the results on spontaneous mutation rate in males of *D. melanogaster* from the Canyon. The rate of sex-linked recessive lethal mutations was evaluated using the standard *Basc* test (Ashburner, 1989). The stock *Basc*, $sc^{8}sc^{Sl}w^{a}B^{l}$ was obtained from the European Drosophila Stock Center, Umea, Sweden. *D. melanogaster* males were taken from two opposite slopes of the Canyon during June-August 1996.

Freshly caught males were mated *en masse* to homozygous *Basc* females, P1 males and females were kept together for 3 days at 22-25°C, then discarded and the cultures incubated at 22-25°C. F1 offspring were collected for matings between 10th-to-17th days and their F1 daughters then mated individually to 3-4 *Basc* males. Each F2 culture carrying a putative lethal was tested by taking three F2 females and crossing them individually with their F2 brothers. It is noteworthy that the genetic background of the compared males in our test is of natural origin, in contrast to other known studies of mutation rate that are based on the accumulation technique.

Table 1. Frequency of newly arisen six-linked recessive lethals and sterile cultures derived from freshly caught males of *D. melanogaster* from the opposite slopes of Lower Nahal Oren Canyon (Mount Carmel, Israel)

Slope	Chromosomes			Mutation rate[a]		
	lethal	sterile	total	per X	per genome[b]	per locus[c]
NFS	1	0	1410	7.1×10^{-4}	3.0×10^{-3}	8.9×10^{-7}
SFS	6	2	1291	4.6×10^{-3}	2.0×10^{-2}	5.8×10^{-6}

[a] calculated for recessive lethality only; [b] assuming that the proportion of loci that can mutate to recessive lethals is the same in X chromosome and autosomes, and applying the estimate that X chromosome comprises about 23.6% of *D. melanogaster* genome (Ashburner, 1989); [c] applying the estimate that mutation at about 800 loci in the X chromosome can result in recessive lethality (Abrahamson *et al.*, 1980).

The results we obtained are shown in Table 1. Fisher's exact text for 2×2 contingency tables gives a significant ($P = 0.04$ $9< 0.05$) difference between NFS and SFS in the frequency of lethals and no difference ($P = 0.227 > 0.05$) for the frequency of sterility. The same test gives $P = 0.014$ when lethal and sterile cases are combined. Thus, we can conclude that higher mutation rate is characteristic of SFS, although further tests are desirable.

Recently, singificant intra-population difference in the rate of recessive sex-linked lethals was described when the scoring was conducted separately on large- and small-sized males (Ivannikov and Zakharov, 1996). The authors interpret the established dependence of *mtr* on body size as an indicator of stress conditions that might affect the organism's development. This explanation corroborates the known tendency of increased mutation when the organism is subjected to adverse ecological conditions (reviewed in Woodruff *et al.*, 1983; Ashburner, 1989). The question we tried to address is whether population adaptation to adverse conditions brings about a genetically determined increase in *mtr*. Our current data together with indirect evidence based on increased male recombination (Derzhavets *et al.*, 1996) allow us to assume that this is indeed the case. Similar results were obtained on mutation rate in the fungus *Sordaria* from the Canyon (Lamb *et al.*, 1996).

One additional point deserves mentioning here. Experimental tests showed that genetic material selected for increased resistance to a specific ecological stress may manifest, as a correlated response, a reduced level of spontaneous mutations and resistances to other stresses including mutagenic ones (Hoffmann and Parsons, 1989; Meerson *et al.*, 1993). If the interslope differences in *mtr* are a manifestation of such a correlated response, one should expect a lower mutation rate in the stress-resistant material, *i.e.*, that from SFS. Exactly the opposite was found in our test (see Table 1), which fits the theoretical explanation assuming that in a population adapting to stressful and variable conditions an increased mutation rate is evolutionarily profitable and is selected for (Gillespie, 1981; Ishii *et al.*, 1989).

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