Fitness related traits in *Drosophila melanogaster* subpopulations from the opposite slopes of "Evolution Canyon", Lower Nahal Oren, Mount Carmel, Israel.

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"Evolution Canyon" on Mount Carmel in Israel has become a natural laboratory where local adaptation of a number of species, including *Drosophila,* is being studied within the framework of a large interdisciplinary program initiated by the Institute of Evolution, Haifa University (reviewed in Nevo, 1995, 1997; Nevo *et al.,* 1998). The opposite slopes of the canyon show strong biotic contrasts, which have considerable consequences for species composition and population genetic structure in a diversity of animals and plants. The subpopulations on the slopes, separated by 100 and 400 m at bottom and top respectively, have to cope with markedly different environments (Nevo, 1995, 1997; Harry *et al.,* 1999). Our previous studies showed a strong interslope differentiation for a complex of adaptive traits related to adaptation (resistance to high temperature and drought stress) in *Drosophila.* These included induced viability and longevity scores under short-term and life span temperature treatments and changes in fly weight due to desiccation treatment. We also found behavioral differentiation, manifested in habitat choice (Nevo *et al.,* 1998), and positive assortative mating (Rashkovetsky *et al.,* 1997; Korol *et al.,* 2000).

Here we provide the results of some further examination of a few fitness-related traits: fecundity, fertility, viability, developmental time, and survival in two temperature regimes (24°C and 29°C). Wild-type flies were sampled in 1997 in the middle parts of the opposite canyon slopes. Each female inseminated in nature was used to establish a separate isofemale line. Experimental synthetic populations were constructed from 25 isofemale lines derived from each of the canyon slopes (SFS – south facing slope; NFS – north facing slope) and maintained in standard laboratory conditions for two years before the experiments. To estimate the indicated components of fitness, 60 virgin females and 80 males from each variant (SFS and NFS) were introduced in half-pint bottles with fresh medium and transferred to new ones for three days in order to reduce the effects of overcrowding. After this procedure flies were introduced into bottles with separate bottoms covered by 4% agar spreading by fresh yeast. ***Fecundity*** was scored as the number of eggs laid per female for 24 hours. For each variant, 250 eggs were sampled and transferred to Petri dishes with agar and yeast. The number of non-hatched larvae was recorded 30 hours later. ***Fertility*** was calculated as proportion of eggs laid that hatched. Hatched larvae (first instar stage) were transferred into vials with fresh medium (25 larvae per vial) and maintained under the two temperature regimes (24°C and 29°C) till eclosion. ***Viability*** was estimated as proportion of eclosed imago. To characterize the time of development, 10 females and 15 males were introduced in Petri dishes for 2 hours for oviposition. The larvae were sampled 24-26 hours later and transferred into standard glass vials with fresh medium. Half of the larvae were placed in an incubator (29°C) and maintained till eclosion, the remainder were cultivated in normal temperature (24°C). The ***time of development*** was characterized by measuring the number of newly eclosed imago in equal periods of time (twelve hours). Each of the foregoing tests was conducted in seven replicates.

The obtained scores of fecundity, fertility, and viability for the synthetic populations derived from flies from the opposite slopes are presented in Table 1. The results showed a significantly lower fecundity of flies from the north-facing slope (Table 1). However, no difference in fertility was found. The significant interslope difference in viability of larvae when the development was maintained at normal temperature (24°C) disappears at elevated (29°C) temperature. This pattern is caused by sensitivity of NFS larvae to increased temperature and stability of the corresponding score for SFS ones. This suggests a relatively more secure survival (thermotolerance) of the SFS subpopulation in changing temperature conditions as compared with the NFS subpopulations. Our previous fluctuating asymmetry test of wing measurements in the same *D. melanogaster* subpopulations showed a significantly higher between-individual variation on SFS than on NFS (Derzhavets *et al.*, 1997a). Thus, increased fecundity and thermotolerance of flies derived from the south-facing slope, which is more climatically stressful and variable, seem to be compensatory mechanisms that together with other adaptation components (Nevo *et al.,* 1998) ensure the survival.

Table 1. Fitness components characterizing the synthetic populations of *D. melanogaster* derived from flies from the opposite slopes of the "Evolution canyon".

Fitness score	SFS	NFS	P (for t-test)
Fecundity	11.76 ± 0.42	7.92 ± 0.51	0.0004 ***
Fertility (%)	97.00 ± 0.47	97.80 ± 0.41	NS
Viability, % (1)	73.14 ± 3.67	90.29 ± 2.87	0.003 **
Viability, % (2)	74.86 ± 4.59	78.29 ± 2.87	NS

P- significance level (based on t-test); NS - non-significant; (1) and (2) - viability estimates obtained for larvae development at 24°C and 29°C, respectively.

Table 2. Estimates of developmental time for the synthetic populations of *D. melanogaster* derived from flies from the opposite slopes of the "Evolution canyon".

Populations	Temperature (°C)	Developmental time (hours)	Variance
SFS	24	265.6 ± 1.7	208.1
SFS	29	243.6 ± 1.9	291.8
NFS	24	258.5 ± 0.9	78.8
NFS	29	225.5 ± 1.2	81.7

Analysis of developmental time (Table 2) clearly showed that flies from the SFS develop significantly slower at both normal and elevated temperature. Moreover, the difference considerably increases in the case of development at elevated temperature. Two-way ANOVA showed highly significant effects of *slope* ($F_{1,48}$ = 73.2, $P < 10^{-6}$), *temperature* ($F_{1,48}$ = 346.7, $P < 10^{-6}$) and interaction '*slope × temperature*' ($F_{1,48}$ = 13.6, $P < 0.0005$). Likewise, significant differences were found between the slopes with respect to the within-variant variance in developmental time (see Table 2). Namely, at both temperatures, variation between individuals of the SFS synthetic population is significantly much higher than that of NFS ($P < 10^{-6}$). The prolongation of the developmental period and its inter-individual variation can be interpreted as an adaptive strategy reducing the chance that the entire progeny of a female will be eliminated due to a severe stress at a critical period of development (larvae), *e.g.* by longer pupation.

The series of experiments with flies maintained in different photoperiod regimes show that the regimen experienced by parents exerts strong effects on developmental time of their progeny (Giesel, 1986, 1988). Progeny of flies reared in a short-day environment had significantly shorter development times than progeny of flies reared in a long-day environment. Although the "Evolution Canyon" has a typically Mediterranean climate and ecosystem, the opposing slopes show contrasting ecology due to the higher (up to 600%) solar radiation on the SFS than on the NFS (Pavlicek *et al.*, 2000). It is natural to suppose that the foregoing results are partially connected to different lighting regime at the canyon slopes as well as to contrasted temperature, humidity etc. Short-term indirect selection for heat-sensitivity and heat resistance (Kilias and Alahiotis, 1985) resulted in diverse correlated responses in behavioral, biochemical, and fitness components. Selective regimes, even of a short duration (ten generations only) can induce significant adaptive and evolutinary changes. Taking into consideration the dramatic biotic contrasts in the Nahal Oren canyon, we can conclude that our data corroborate these cited results, but further direct tests are needed.

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Tumor inducing *Drosophila*: resistance to hydroxyurea and methane sulfonic acid methyl ester.

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Abstract: Tumor induction lies in proliferative gene defects in *Drosophila* and humans. Mutant proliferative genes allow replication. Recent results obtained in human leukaemic cell lines point towards an involvement of the repair system: DNA damage response is inadequate in tumors. Hydroxyurea inhibits semiconservative replication but allows repair replication. Methane sulfonic acid methyl ester damages DNA and induces apoptosis. Proliferative gene mutants of *Drosophila* show intrinsic resistance to hydroxyurea and methane sulfonic acid methyl ester. This raises evidence, that repair replication is constitutively active and circumvents semiconservative replication, response to DNA damage is inadequate.

Introduction

Tumor formation lies in gene defects that alter the normal program of cell proliferation. *Drosophila* stock Malignant Brain Tumor (MBT) has been genetically analyzed. Out of six mutant genes, those defects have been identified that are responsible for loss of control over cell proliferation. Tumor growth in flies is dependent on mutation of a proliferative gene. Proliferative genes are defined as the class of genes, that, when mutant, allow cell divisions in cell cycle competent cells. Replication allowance has been identified in cell cycle restricted cells, which aberrently polytenize DNA. Thus, the mutation of a proliferative gene could shortcut the cell cycle by onset of replication (Riede, 1996, 1997, 1998). Due to the phenotypic expression of proliferative gene defects in *Drosophila* – somatic pairing defect in combination with replication allowance – the involvement of the repair system has been anticipated. Recombination repair is dependent on homologous pairing of chromosomes and is able to initiate replication. Recent studies with human