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## Implications of Locus Specific Microevolution Inferred By Genetic Distances Between Two Local Populations of *Drosophila melanogaster* From Turkey

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**Abstract:** The standard genetic distances were calculated with 3 allozyme loci in 2 local populations of *Drosophila melanogaster* from Turkey. One of the distances, i.e. obtained with alcohol dehydrogenase (*Adh*), was completely different from the distances with the other 2 allozyme loci, glycerophosphate dehydrogenase ( $\alpha$ *Gpdh*) and cytosolic malate dehydrogenase (*Mdh1*), leading to a statistically significant inflation in the cumulated distance value obtained over the 3 loci. It is shown that this final significant value was caused by the significant difference in allelic frequencies at the *Adh* loci across local populations. Implications of the different genetic distances in 2 local populations are discussed, especially with respect to putative selection pressures more stressed on the *Adh* than on the other 2 allozyme loci.

**Key Words:** Allozymes, *Drosophila melanogaster*, genetic distance, alcohol dehydrogenase

### Türkiye'den Örneklenen *Drosophila melanogaster*'in İki Lokal Populasyonundaki Genetik Uzaklıkların Gösterdiği Lokusa Özgü Mikroevrim

**Özet:** Türkiye'den örneklenen *Drosophila melanogaster*'in iki lokal populasyonunda üç allozim lokusu açısından genetik uzaklıklar hesaplandı. Hesaplanan uzaklıklardan bir tanesi, alkol dehidrogenaz (*Adh*) ile hesaplanan, diğer iki lokus olan gliserofosfat dehidrogenaz ( $\alpha$ *Gpdh*) ve sitozol malat dehidrogenazı (*Mdh1*) ile hesaplananlardan oldukça farklıydı. Bu durum, üç lokus kullanılarak hesaplanan total uzaklık değerinin istatistiksel olarak anlamlı olacak biçimde artmasına yol açtı. Bu istatistiksel anlamlı değerler lokal populasyonlarda *Adh* alel frekansları arasındaki anlamlı farktan kaynaklandığı anlaşılmaktadır. Lokal populasyonlarda elde edilen farklı genetik uzaklıkların diğer iki lokustakinden daha güçlü olan bir seçilimin *Adh* üzerine etkisinden kaynaklandığı tartışılmaktadır.

**Anahtar Sözcükler:** Allozimler, *Drosophila melanogaster*, genetik uzaklık, alkol dehidrogenaz

### Introduction

Allozymes have received much attention and been much debated since the groundbreaking work of Lewontin and Hubby (1966) for the possibilities they offer in quantifying genetic variation in natural populations and in the association of that variation with various selective mechanisms and population structures (Gillespie, 1991). The use of allozyme variation in the determination and interpretation of hidden genetic variation seems to cause spurious assumptions, especially with loci having a high number of alleles (Barbadilla et al., 1996). However, it is still worthwhile using allozyme data for statistical inferences of populations when the given loci are known to have a few common alleles, especially when the electrophoretic allelic states

correspond closely to the states at the nucleotide level (Barbadilla et al., 1996). In this respect, differences in gene frequencies among populations and between substructured samples could still be considered to track microevolution. One of the favorite areas of research utilizing allozyme data has been to orient natural populations geographically by the genetic distances computed from gene frequency differences. Various genetic distance measures have been developed for different genetic markers with different assumptions of mutation type and rate, but all of them simply interpret the resulting identity and distance profiles on universal microevolutionary events such as the amount of time of separation from a common ancestor, migration rates between populations, reshaping of the genetic

architecture via genetic drift, and different selection regimes in distinct locations (Hedrick, 2000). Obviously, calculating the genetic distances is the first essential step in inferring population history and adaptive evolution.

In the present study the results of genetic distances are presented for 2 local populations of *Drosophila melanogaster*, calculated on 3 allozyme loci. The loci chosen are alcohol dehydrogenase (*Adh*), alpha-glycerophosphate dehydrogenase ( $\alpha$ *Gpdh*) and cytoplasmic malate dehydrogenase (*Mdh1*). The alcohol dehydrogenase (*Adh*) and alpha-glycerophosphate dehydrogenase ( $\alpha$ *Gpdh*) loci of *Drosophila melanogaster* are located on the left arm of the 2<sup>nd</sup> chromosome, and both have 2 common allozyme variants (F: Fast and S: Slow, after their electrophoretic mobilities) with substantial frequencies in natural populations (Kamping, 2000). *Adh* frequencies have a distinct geographical distribution pattern; the *Adh*<sup>S</sup> allele frequency decreases with increasing latitude from the tropics to temperate regions, with an almost complete absence in most northern populations (Van Delden and Kamping, 1997). Multicontinental regularity of that pattern strongly suggests the presence of selection on this locus or other loci linked to it physically or functionally (Van Delden and Kamping, 1997 and references therein). *Gpdh* also shows latitudinal frequency changes but not as sharp as those of *Adh* (Kamping, 2000 and references therein). Putative selection agencies for *Adh* could be invoked for its role in alcohol tolerance and utilization when the environment in which females lay eggs has relatively higher alcohol concentrations (Heinstra, 1993). For  $\alpha$ *Gpdh*, various selective scenarios have been proposed ranging from differential efficiencies in lipid metabolism to flight output capacities (Kamping, 2000). It seems there is comparatively less selection pressure on  $\alpha$ *Gpdh* than on *Adh* (Kamping and Van Delden, 1999). *Mdh1* can be classified as a locus with low polymorphism, the most common allele of which has frequencies over 90% (Choudhary et al., 1992, Parkash et al., 1993). No significant selection appears on *Mdh1*, since the homozygotes of a loss-of-function allele *Mdh1*<sup>ml</sup> are viable (Racine et al., 1980). Overall, the 3 loci chosen might be expected to give hints about differential fine scale selection profiles (i.e. particularly with *Adh*) or population structures (i.e. with  $\alpha$ *Gpdh* or *Mdh1*) especially in local population samples, which in fact is the case of the results of this study.

## Materials and Methods

### Populations sampled

Two local populations of *D. melanogaster* were sampled from Ankara province, in the Central Anatolia region in Turkey; one from the Ümitköy district (henceforth called Ankara-1), and the other from the Cebeci district (Ankara-2). A distance of approximately 20 km separates these districts, which might affect the spatial differentiation of populations with low dispersal rates (Endler, 1977), a generality from which *Drosophila* cannot be excluded (Dobzhansky, 1973). Sample collections were performed in August 1999 with fermented food traps. Samples were mixed with individuals of the sibling *D. simulans*, which were discarded after observing the fixed genital pattern difference between *D. melanogaster* and *D. simulans* males (Sturtevant, 1920). Female discrimination in this respect was carried out electrophoretically (e.g., Özsoy, 2002).

### Electrophoresis of the individual flies

Electrophoresis was of the standard PAGE system developed for the combination of the *ADH* and *GPDH* by Van Delden and Kamping (1989). Individual flies were homogenized in demineralized water and 3  $\mu$ l of each homogenate was run on the gel. Running buffer was a mix of 0.0205 M Veronal, 0.003 M EDTA and 0.075 M Tris at pH 8.4. Reaction buffer per gel consisted of 400 mg of Glycero-phosphate, 20 mg of NAD<sup>+</sup>, 20 mg of MTT, and 1 mg of PMS all dissolved in 60 ml of 0.2 M Tris-HCl solution at pH 8.5. After 2.5 h of running the gels were placed in a plastic container containing the reaction buffer and put into an incubator shaker operated at 30 °C for 10 min. After the *GPDH* bands had appeared, 200  $\mu$ l of Isopropanol (propan-2-ol) was added to the total mix in the container and the gel was left for 5 min in the shaker for the appearance of *ADH* bands. A modified version of the *ADH* and *GPDH* method was used for determining *Mdh1* genotypes, L-Malic acid for substrate, and NaCN for the final reaction. When the gels had been clearly stained for the *Adh* and  $\alpha$ *Gpdh*, or *Mdh1* electromorphs, they were photographed for scoring using image analyzer software.

### Statistics and genetic distances

Single and 3 locus genetic distances were calculated as Nei's standard distance coefficient (Nei, 1972), in which the distance is  $D = -\ln(I)$ , where  $I$  is the identity between 2 samples calculated for the given loci (Nei, 1972). This distance measure assumes the infinity of allelic states by mutation and neutrality between alternative alleles, and is a good indicator of population differentiation, for it increases linearly with time when these assumptions hold (Nei, 1972; Hedrick, 2000). Understanding identity levels quantitatively is directly achieved by testing the populations as to whether the allelic differences at chosen loci are significantly different. In this respect a goodness of fit test ( $\chi^2$ ) was performed with individual loci to obtain a cumulative test value for the allelic differences. The  $\chi^2$  test used the weighted variance and weighted average of allele frequencies over the populations compared (Workman and Niswander 1970; Hedrick, 2000 p.83).

### Results

#### *Adh* and $\alpha$ *Gpdh* polymorphisms

Table 1 shows the allele frequencies at *Adh* and  $\alpha$ *Gpdh*. The first thing to note is the considerable difference at the *Adh* locus between the 2 local populations. *Adh<sup>S</sup>* frequency, for example, is almost halved in Ankara-1 with respect to that in Ankara-2. The frequencies of the F allele are considerably greater than the S frequencies in both populations. Finally, the 2 alleles (Fast: F and Slow: S) of *Adh* detected in the study are almost the only variants segregating in natural

populations worldwide (Van Delden and Kamping, 1997). As for  $\alpha$ *Gpdh*, there seems to be little difference between the allelic classes (Fast and Slow types) across the 2 local populations (Table 1). The 2 allelic types of  $\alpha$ *Gpdh* detected in the present study are also the most common worldwide (Van Delden and Kamping, 1997).

#### *Mdh1* polymorphism

Allelic states and the level of polymorphism at the *Mdh1* locus in both samples on the gel allowed the detection of only 2 alleles that were decided as the slower rare allele, *Mdh1<sup>2</sup>*, and the faster common allele *Mdh1<sup>4</sup>* (O'Brien and O'Brien, 1969; Alahiotis, 1979). The allele frequency difference between the populations is insignificant, and smaller than that of *Adh* (Table 1).

#### Genetic distances

Nei's distances were calculated per individual locus and for the 3 loci taken together. The distances obtained from  $\alpha$ *Gpdh* and *Mdh1* frequencies are quite small, revealing the near identity of the local populations with respect to these loci (Table 2). In contrast, the distance obtained with *Adh* was considerably larger than the distances based on  $\alpha$ *Gpdh* and *Mdh1* (Table 2). When the cumulative distance over the 3 loci was calculated, it was found that the differentiation between the local populations was inflated (Table 2, boldfaced distance figure). This must be exclusively due to the considerable *Adh* frequency difference between the populations. Indeed, this enlargement of the multilocus distance value can easily be analyzed into component parts by testing the

Table 1. Allele frequencies at 3 loci in the study. Alternative alleles (*Adh<sup>F</sup>*,  $\alpha$ *Gpdh<sup>S</sup>* and *Mdh1<sup>2</sup>*) are shown in parentheses.

Population	Size	<i>Adh<sup>S</sup></i>	$\alpha$ <i>Gpdh<sup>F</sup></i>	<i>Mdh1<sup>4</sup></i>
Ankara-1	52	0.156 (0.844)	0.466 (0.534)	0.942 (0.058)
Ankara-2	53	0.380 (0.620)	0.500 (0.500)	0.858 (0.142)

Table 2. Genetic distances of individual loci. Identities are given in parentheses. Boldfaced figure in the upper right is the distance calculated over 3 loci.

	Ank-2 ( <i>Adh</i> )	Ank-2 ( $\alpha$ <i>Gpdh</i> )	Ank-2 ( <i>Mdh-1</i> )
Ank-1 ( <i>Adh</i> )	0.166 (0.844)		<b>0.237(0.763)</b>
Ank-1 ( $\alpha$ <i>Gpdh</i> )		0.002 (0.998)	
Ank-1 ( <i>Mdh-1</i> )			0.005 (0.995)

magnitude of variance in allele frequency over the populations. The results of such a test, a  $\chi^2$ , are given in Table 3. It is clearly shown that the final highly significant  $\chi^2$  value obtained by pooling the 3 individual values (each from a locus) is greatly enlarged by the *Adh* contribution, which is also highly significant (Table 3). In the case here, *Adh* frequency seems to disturb the otherwise substantially similar genetic profile between the local populations studied.

### Discussion

The results of the present study indicate that different genetic distances from different loci can reveal the differential nature of evolutionary paths even in populations separated by a small distance. Especially with *Adh*, a locus on which considerable selection pressure seems to occur worldwide (Kreitman, 1983; Berry and Kreitman, 1993; Van Delden and Kamping, 1997), the allele frequencies vary dramatically between the 2 local populations (Tables 1 and 3). This change is also reflected by the rather dissimilar *Adh* distance value compared to those obtained with the other 2 loci,  $\alpha$ *Gpdh* and *Mdh1*, in the study (Table 2). Alcohol dehydrogenase enzyme (*ADH*) is essential in detoxifying and utilizing the alcohol in larval environments (Freriksen et al., 1994; Van Delden and Kamping, 1997), and there may be great variations in alcohol levels in the adjacent habitats in which females lay eggs (McKenzie and Parsons, 1972; McKechnie and Morgan, 1982). Therefore, the most likely explanation for the greater genetic distance obtained with *Adh* is that the microspatial habitat differentiation between the 2 populations (i.e. Ankara-1 and Ankara-2) might provide an alcohol related selection regime on *Adh*. However, there seem to be no effectively disturbing forces on  $\alpha$ *Gpdh* and *Mdh1* loci in that the frequency differences gave almost negligible distances between local populations (Tables 1 and 2). For  $\alpha$ *Gpdh*,

the genetic distance value is very small and indicates insignificantly different allele trajectories in the 2 populations (Table 3). This picture is concordant with the long time-coursed observation that less selection operates on  $\alpha$ *Gpdh* compared with *Adh* (Kamping and Van Delden, 1999). As for *Mdh1*, the distance value is also small, although the allele frequencies significantly differ between the populations (Table 3). However, this significant figure could largely be due to a moderate sampling error, or drift, because the level of polymorphism at *Mdh1* is very small in natural populations (Choudhary et al., 1992; Parkash et al., 1993). Otherwise a selection effect could have been invoked, which would be nonrealistic in the context that a loss-of-function allele of *Mdh1*, *Mdh1<sup>nl</sup>*, is viable (Racine et al., 1980).

In conclusion, the genetic distances calculated in this study with the loci *Adh*,  $\alpha$ *Gpdh* and *Mdh1* clearly point to different evolutionary processes that may be ongoing even in populations separated by a small distance. This inference is based on the statistical description of the genetic variation profiles of the 3 marker loci that may lead to different frequency trajectories in different environments; further elaboration of the evolution of these 3 loci awaits for studies determining the relevant structure of the habitats from which the local populations were sampled.

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Table 3. Differences in allele frequencies between the 2 local populations. Numbers are the sample  $\chi^2$  values per loci and for the 3 loci pooled (boldfaced figure in the upper right).

	Ank-2 ( <i>Adh</i> )	Ank-2 ( $\alpha$ <i>Gpdh</i> )	Ank-2 ( <i>Mdh-1</i> )
Ank-1 ( <i>Adh</i> )	11.082***		15.449***
Ank-1 ( $\alpha$ <i>Gpdh</i> )		0.344	
Ank-1 ( <i>Mdh-1</i> )			4.023*

\* P<0.05  
 \*\*\* P<0.001

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