Variation of allozyme loci in populations of *Drosophila melanogaster* from the former USSR

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Variation of eight allozyme loci, *Acph*, *Adh*, *Est-6*, *Est-C*, α -*Gpdh*, *Idh* (NADP-dependent), *6-Pgd* and *Pgm*, was studied in 20 populations of *Drosophila melanogaster* from the territory of the former USSR, including Eastern Europe, the Caucasus and Central Asia. *Adh* exhibited weak regression on latitude, longitude, and also on the temperature of the hottest calendar month in these localities. For seven other loci, no regular geographical pattern was found. Nei's coefficient of gene differentiation (G_{ST}) ranged from 0.010 (*6-Pgd*, statistically nonsignificant) to 0.178 (*Idh*) and was on average 0.058. The average Nei's genetic distance (D) between the populations was 0.011. It is suggested that this fairly low level of interpopulation differentiation for the examined loci except *Idh* may be explained by extensive gene flow in combination with natural selection.

Keywords: allozymes, *Drosophila melanogaster*, geographical variation, natural populations.

Introduction

In natural populations of *Drosophila melanogaster*, variation of some allozyme loci is well documented for North America, Australia, Western Europe and tropical Africa, East and South Asia (Oakeshott *et al.*, 1981, 1982, 1983a,b and references therein; David, 1982; Triantaphyllidis *et al.*, 1982; Watada *et al.*, 1986; Singh & Rhomberg, 1987a,b; Jiang *et al.*, 1989; Parkash & Shamina, 1994). The pattern of allele frequencies suggests that both selection and genetic drift operate in *Drosophila* populations. At the same time, specific mechanisms that determinate allozyme polymorphism remain obscure. Experimental evaluation of genotype fitness under laboratory conditions is one way towards resolving this problem. Another approach is to elucidate the complete pattern of allozyme differentiation over the world. Combination of these approaches can provide deeper understanding of evolutionary changes at the biochemical level.

In the present work, we report the geographical variation of eight allozyme loci in *D. melanogaster* populations from the former USSR territory. So far, this region remains very poorly investigated with regard to *D. melanogaster* biochemical variation. The

only study dealing with this issue was that of Grossman *et al.* (1970), and it was limited to the alcohol dehydrogenase locus; data on other loci are lacking. We consider variation at the following loci: *Adh* (alcohol dehydrogenase, EC 1.1.1.1), α -*Gpdh* (α -glycerophosphate dehydrogenase, EC 1.1.1.8), *Est-6* (esterase-6, EC 3.1.1.1), *Est-C* (esterase-C, EC 3.1.1.1), *6-Pgd* (6-phosphogluconic dehydrogenase, EC 1.1.1.43), *Pgm* (phosphoglucomutase, EC 5.4.2.2), *Acph* (acid phosphatase, EC 3.1.3.2), and *Idh* (NADP-dependent isocitrate dehydrogenase, EC 1.1.1.42). There is a large body of evidence (e.g. Oakeshott *et al.*, 1981, 1982, 1983a; David *et al.*, 1982; Singh & Rhomberg, 1987b; Parkash & Shamina, 1994) that the first six loci exhibit latitudinal differentiation in some transects, which allows us to look on them as the most probable targets for natural selection.

Materials and methods

Samples of *D. melanogaster* were collected at 20 localities of Eastern Europe, the Caucasus and Central Asia. (Fig. 1, Table 1). The altitude of all localities did not exceed 1000 m above sea level. To eliminate possible seasonal and sampling bias in allele frequencies (see, e.g. Franklin, 1981), we restricted the sampling period to the two hottest

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summer months (from mid-July to early September) when *D. melanogaster* is most abundant in the region surveyed. Collections were made in orchards and vineyards, at fruit-processing factories, and at town fruit markets. In the laboratory, the flies were maintained as separate isofemale lines at 25°C.

Allozyme variability was assayed by starch gel electrophoresis using the discontinuous system of Poulik (1957). The staining methods were adapted from Harris & Hopkinson (1976). Fifty males per population were sampled in the F_1 generation for the analysis. For most populations, more than 50 isofemale lines were initiated; in electrophoretic analysis each line was represented by one fly. In three populations (Astrakhan, Gergebil and Makhinjauri), there were fewer than 50 isofemale lines and the samples for electrophoresis were made from 25

lines (2 flies per line). Allele frequency estimates obtained after such sampling procedures are expected to have a minimum bias (Long, 1993).

Expected heterozygosity (H_E) was calculated for each population according to Nei (1978). Genetic diversity analysis (H_T , H_S , D_{ST} , G_{ST}) was performed following the method of Nei & Chesser (1983). Homogeneity of allele frequencies among 20 populations was assessed using the χ^2 procedure of Workman & Niswander (1970).

Unbiased genetic distances between all possible pairs of populations were computed following Nei (1978). UPGMA cluster analysis (Sneath & Sokal, 1973) was performed on this distance matrix and a strict consensus tree was found for the set of tied trees according to Sokal & Rohlf (1981; for review see also Rohlf, 1982).

Table 1 Collection sites and population variability estimates for 20 populations of *Drosophila melanogaster* (size of each sample is 50 individuals)

Population	Geographical co-ordinates		Mean number of alleles per locus	Observed proportion of loci polymorphic*	Mean heterozygosity expected† ± SE
	Latitude °N	Longitude °E			
Eastern Europe					
1 Tartu	58.4	26.7	1.5	0.38	0.126 ± 0.066
2 Ramenskoe	55.6	38.3	1.6	0.38	0.157 ± 0.069
3 Rybnoe	54.7	39.5	1.8	0.63	0.193 ± 0.065
4 Pyany Rog	52.9	33.6	1.6	0.50	0.138 ± 0.059
5 Gomel	52.4	31.0	1.8	0.50	0.202 ± 0.079
6 Khristinovka	48.8	30.0	1.8	0.63	0.167 ± 0.064
7 Kishinev	47.0	28.8	1.8	0.63	0.188 ± 0.061
8 Zabuzan	46.5	48.3	1.6	0.50	0.176 ± 0.074
9 Astrakhan	46.4	48.1	1.9	0.50	0.190 ± 0.070
10 Vilino	44.9	33.6	1.4	0.25	0.115 ± 0.073
Caucasus					
11 Jankhot	44.4	38.2	1.6	0.38	0.136 ± 0.070
12 Sochi	43.6	39.7	1.8	0.50	0.182 ± 0.071
13 Gergebil	42.5	47.1	1.8	0.38	0.162 ± 0.072
14 Makhinjauri	41.7	41.7	1.8	0.38	0.164 ± 0.073
Central Asia					
15 Alma-Ata	43.3	77.0	1.8	0.50	0.212 ± 0.075
16 Akzhar	43.1	71.6	1.6	0.38	0.169 ± 0.079
17 Jambul	42.9	71.4	1.8	0.75	0.244 ± 0.073
18 Kaplanbek	41.5	69.4	1.8	0.38	0.128 ± 0.059
19 Cheptura	38.5	68.4	1.8	0.63	0.239 ± 0.077
20 Bezmein	38.1	58.2	1.8	0.38	0.174 ± 0.073
Average			1.72	0.48	0.173
SE			0.03	0.03	0.008

*A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

†Unbiased estimate according to Nei (1978).

Table 2 Allele frequencies of eight allozyme loci in 20 populations of *Drosophila melanogaster* (sample size is 50 flies per population)

Locus	Allele	Eastern Europe										Caucasus										Central Asia									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20										
<i>Acph</i>	S	—	—	—	—	—	—	—	—	0.07	—	—	0.01	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—	—	—	—	—
	F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.93	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.96	—	—	—	—	—	—	—	—	—	—
	1.10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.04	—	—	—	—	—	—	—	—	—	—
<i>Adh</i>	S	0.06	0.04	0.10	0.03	0.06	0.02	—	—	0.20	—	0.07	0.06	0.04	0.16	0.15	0.04	0.11	0.08	0.23	0.19	—	—	—	—	—	—	—	—	—	—
	F	0.94	0.96	0.90	0.97	0.94	0.98	1.00	0.93	0.80	1.00	0.93	0.94	0.96	0.84	0.85	0.96	0.89	0.89	0.77	0.81	—	—	—	—	—	—	—	—	—	—
	UF	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.03	—	—	—	—	—	—	—	—	—	—	—	—
<i>Est-6</i>	S	0.64	0.61	0.71	0.62	0.66	0.59	0.74	0.60	0.63	0.69	0.50	0.61	0.63	0.54	0.64	0.49	0.68	0.66	0.50	0.59	—	—	—	—	—	—	—	—	—	—
	F	0.36	0.39	0.29	0.38	0.34	0.41	0.26	0.40	0.37	0.31	0.50	0.39	0.37	0.46	0.36	0.51	0.32	0.34	0.50	0.41	—	—	—	—	—	—	—	—	—	—
<i>Est-C</i>	VS	—	—	—	—	—	—	0.03	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	—	—	0.02	—	0.02	0.06	0.15	—	0.01	—	0.01	0.04	0.02	0.01	—	—	0.05	—	0.09	0.03	—	—	—	—	—	—	—	—	—	—
	F	1.00	1.00	0.98	1.00	0.98	0.94	0.82	1.00	0.99	1.00	0.99	0.96	0.98	0.99	1.00	1.00	0.95	1.00	0.91	0.97	—	—	—	—	—	—	—	—	—	—
α - <i>Gpdh</i>	S	0.24	0.30	0.28	0.12	0.36	0.24	0.15	0.33	0.35	0.36	0.26	0.20	0.38	0.30	0.27	0.42	0.39	0.16	0.43	0.32	—	—	—	—	—	—	—	—	—	—
	F	0.76	0.70	0.72	0.88	0.64	0.76	0.85	0.67	0.65	0.64	0.74	0.80	0.62	0.70	0.73	0.58	0.61	0.84	0.57	0.68	—	—	—	—	—	—	—	—	—	—
<i>Idh</i>	S	1.00	0.89	0.74	0.86	0.60	0.87	0.73	0.99	0.97	1.00	0.98	0.68	0.88	0.99	0.61	1.00	0.57	0.9	0.76	1.00	—	—	—	—	—	—	—	—	—	—
	F	—	0.11	0.26	0.14	0.40	0.13	0.27	0.01	0.03	—	0.02	0.32	0.12	0.01	0.39	—	0.43	0.02	0.24	—	—	—	—	—	—	—	—	—	—	—
<i>6-Pgd</i>	S	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	F	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	—	—	—	—	—	—	—	—	—	—
<i>Pgm</i>	0.70	0.03	0.04	0.06	0.06	0.03	0.05	0.08	0.20	0.03	0.01	—	—	0.01	0.04	0.04	0.15	0.12	0.03	—	0.01	—	—	—	—	—	—	—	—	—	—
	1.00	0.97	0.96	0.94	0.94	0.97	0.95	0.92	0.80	0.97	0.99	1.00	1.00	0.99	0.96	0.96	0.84	0.88	0.97	1.00	0.99	—	—	—	—	—	—	—	—	—	—
	1.20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.01	—	—	—	—	—	—	—	—	—	—	—	—	—	—

For explanation, see Table 1.

The qualitative estimates of the level of gene flow among populations were obtained with Slatkin's method based on private allele frequencies (Slatkin, 1985) and Crow's method based on G_{ST} of populations at equilibrium (Crow, 1986; for theoretical background see Wright, 1969). The Ewens-Watterson test of neutrality for the loci was performed following Manly (1985).

Statistical computations were performed using the BIOSYS-1 (Swofford & Selander, 1989) and NTSYS-pc (Rohlf, 1988) computer programs.

Results

The variability estimates for 20 populations of *D. melanogaster* from Eastern Europe, the Caucasus and Central Asia are presented in Table 1. An average population was polymorphic for 48 per cent of its loci according to the 95 per cent criterion and had on average 1.72 alleles per locus. The average heterozygosity per locus was 0.173. The mean number of alleles per locus was virtually constant from one population to another. The proportion of polymorphic loci and heterozygosity estimates were more variable but no obvious geographical trends were detected.

In Tables 2 and 3, the allele frequencies of the eight loci surveyed and their variability estimates over 20 populations are presented. All the loci were polymorphic according to the 99 per cent criterion and seven loci were polymorphic according to the 95 per cent criterion (the exception was *6-Pgd*). The mean number of alleles per population ranged from

1.05 (*6-Pgd*) to 2.00 (*Est-6*, α -*Gpdh*) and was, on average, 1.63. For six loci, most populations had two alleles. One allele predominated in the populations for *Acph* and *6-Pgd*. There were only three cases in which three alleles of one locus were present in a population simultaneously (see Table 2).

Only the *Adh* locus exhibited a statistically significant relationship with the geographical position of populations and temperature of the hottest calendar month (July). Regression coefficients of the *Adh-F* allele frequency on latitude, longitude and temperature were 5.69×10^{-3} ($P < 0.05$), -2.35×10^{-3} ($P < 0.01$) and -11.57×10^{-3} ($P < 0.01$), respectively.

Gene diversity analysis indicated that only 5.81 per cent of the total genetic variation resided among populations (Table 3). However, seven of the eight loci (exception *6-Pgd*) were found to be heterogeneous among populations according to the Workman-Niswander χ^2 test, with *Idh* displaying considerable interpopulation differentiation.

Nei's genetic distance estimates were fairly low amounting on average to 0.011 ± 0.001 . The regression of genetic distance on geographical distance was very slight (regression coefficient of 6.45×10^{-7} , $P < 0.05$) and accounted for only 2.74 per cent of the total genetic distance variation. It is not surprising then that cluster analysis could not reveal clear geographical population groups (Fig. 2). As no single solution in the construction of the dendrogram was available, we represented on Fig. 2 a strict consensus tree with a CI_C consensus index of 0.39.

Both Slatkin's and Crow's methods gave high estimates of gene flow among the populations studied.

Table 3 Variation of eight allozyme loci over 20 populations of *Drosophila melanogaster*

Locus	Proportion of populations polymorphic†	Mean number of alleles per population	Gene diversity statistics‡				Heterogeneity test§, χ^2
			H_T	H_S	D_{ST}	G_{ST}	
<i>Acph</i>	0.05	1.20	0.014	0.013	0.001	0.047	***
<i>Adh</i>	0.65	1.95	0.161	0.152	0.009	0.055	***
<i>Est-6</i>	1.00	2.00	0.478	0.468	0.009	0.019	**
<i>Est-C</i>	0.20	1.65	0.053	0.050	0.003	0.061	***
α - <i>Gpdh</i>	1.00	2.00	0.418	0.403	0.015	0.036	***
<i>Idh</i>	0.55	1.80	0.251	0.206	0.045	0.178	***
<i>6-Pgd</i>	0.00	1.05	0.001	0.001	0.000	0.010	ns
<i>Pgm</i>	0.35	1.90	0.098	0.092	0.006	0.059	***
Average	0.48	1.69	0.184	0.173	0.011	0.058	***
SE	0.14	0.13	0.065	0.062	0.005	0.018	—

†A population is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

‡According to Nei & Chesser (1983).

§According to Workman & Niswander (1970).

*** $P < 0.01$, ** $P < 0.001$; NS, nonsignificant.

Fig. 1 Geographical locations of the 20 sampled populations of *Drosophila melanogaster*.

The average frequency of the private alleles (alleles that were found in only one population), $p(1)$ was 0.024 ± 0.006 and according to Slatkin's method Nm (the average number of migrants exchanged between populations) was 6.43. Nm calculated according to Crow's method using the average G_{ST} was 3.66.

Table 4 represents the results of the Ewens–Watterson test of neutrality for seven loci that exhibited statistically significant among-population heterogeneity (see Table 3). Because the migration rate was found to be quite high, we considered the situation when the samples were treated as belonging to one population. Except *Acph*, all approximate percentage points for sample homozygosity were between 0.05 and 0.95 and thus the hypothesis of neutrality could not be rejected for these loci.

Discussion

In the present study, we deal with variation of allozyme loci most of which exhibit a considerable differentiation among the *D. melanogaster* populations throughout the world (Oakeshott *et al.*, 1981, 1982, 1983a; David, 1982; Triantaphyllidis *et al.*, 1982; Lamooza *et al.*, 1985; Watada *et al.*, 1986; Singh & Rhomberg, 1987a,b; David *et al.*, 1989; Jiang *et al.*, 1989; Parkash & Shamina, 1994). Though the populations that we surveyed were geographically distant and occupied both temperate and subtropical habitats, the range of allele frequencies and level of interpopulation differentiation were shown to be rather low.

There are two main factors which result in low

Fig. 2 UPGMA cluster analysis of Nei's genetic distances among 20 populations of *Drosophila melanogaster*: a strict consensus tree ($CI_C = 0.39$) for the set of tied dendrograms.

variation of polymorphic loci over the range of a species. One is extensive gene flow which equalizes allele frequencies of neutral loci. Another factor is strong selection associated with some quite constant climatic parameter(s). There can also be some interaction of these factors.

The qualitative evaluation of gene flow among the populations of Eastern Europe, the Caucasus and Central Asia indicates that it is quite large ($Nm > 1$). This is in good agreement with the results obtained previously for *D. melanogaster* (Coyne *et al.*, 1982, 1987; Singh & Rhomberg, 1987a; Hale & Singh,

Table 4 Ewens–Watterson test of neutrality with an assumption of high migration between the populations of *Drosophila melanogaster**

Locus†	Allele number, <i>k</i>	Sample homozygosity, $\hat{F}$	Approx. corresp. percentage points, <i>P</i>
<i>Acph</i>	3	0.987	0.95
<i>Adh</i>	3	0.841	0.55
<i>Est-6</i>	2	0.527	0.08
<i>Est-C</i>	3	0.947	0.78
α - <i>Gpdh</i>	2	0.586	0.12
<i>Idh</i>	2	0.752	0.25
<i>Pgm</i>	3	0.903	0.68

*According to Manly (1985).

†6-*Pgd* was excluded from consideration because of its statistically nonsignificant differentiation among the populations.

1991) and appears to be reasonable for the region surveyed. Traditional extensive transportation of fruits and vegetables from the south to north, existing in this territory, can promote a high level of passive migration of *D. melanogaster*.

A comparison of the allele frequencies scored in the present study with data of other authors for Eurasian populations (see references in the first paragraph) indicates that selection can also maintain the low allozyme variation found in Eastern Europe, the Caucasus and Central Asia. The allele frequencies in this region resemble those obtained previously for temperate and subtropical Eurasian populations, with *Acph*, α -*Gpdh*, *Est-C*, 6-*Pgd* and *Pgm* exhibiting low or very low interpopulation differentiation. At the same time, a parallel latitudinal variation between temperate/subtropical and tropical habitats in different continents was documented for *Adh*, α -*Gpdh*, *Est-6*, *Est-C*, 6-*Pgd* and *Pgm*. Such a geographical pattern suggests some selection pressure on these loci. The most probable type of selection appears to be a balancing diversifying one, with coefficients corresponding to climatic gradients between high latitudes and the equator (see Singh & Rhomberg, 1987b for discussion). At the *Acph* locus, one allele predominates in both tropical and temperate populations; this may result from strong purifying selection against rare alleles.

As to *Idh*, no data on geographical variation in other transects are available for this locus. In our study, it shows the highest and random interpopulation differentiation, i.e. it more closely resembles a neutral locus.

The results of the Ewens–Watterson test of neutrality may appear at first sight to conflict with the conclusions based on the worldwide pattern of

geographical variation. According to the test, only the *Acph* locus is under selection. Of course, we should view this conclusion with some scepticism. Rigorous results can be obtained only for low homozygosity values, provided that there is an equilibrium distribution of allele numbers and all alleles are identified (for details see Manly, 1985). Not all these requirements can be met in our study. However, a consideration of the test results in relation to data on the loci variation over the world allows this situation to be regarded as quite realistic for the region studied.

The locus *Acph* is monomorphic or almost monomorphic in *D. melanogaster* populations all over the world. It has high sample homozygosity that according to the Ewens–Watterson test will give a statistically significant deviation from neutrality for any part of the species range. The loci *Adh*, α -*Gpdh*, *Est-6*, *Est-C*, 6-*Pgd* and *Pgm* exhibit considerable differentiation between temperate/subtropical and tropical habitats. However, these loci normally show low or moderate variation within temperate/subtropical climates. Their sample homozygosity values are intermediate there; consequently, they cannot give statistically significant deviation from neutrality. If we combine temperate/subtropical populations with tropical ones, which have another ratio for the main allele frequencies, we might obtain a decrease in sample homozygosity. In this case, the Ewens–Watterson test is likely to provide evidence for balancing selection rather than neutrality.

It is usually suggested that the most apparent selective agent to maintain the geographical variation between temperate/subtropical and tropical habitats is temperature in *Drosophila* breeding sites. Especially for *Adh*, there is a large body of data on

this issue, derived from both field observations and laboratory experiments (for review, see e.g. Chambers, 1988). Even with the low interpopulation differentiation, shown in the present study, the *Adh* allele frequencies exhibited a statistically significant regression on latitude and the temperature of the hottest month. Clinal geographical and altitudinal *Adh* variation in this region has also been previously demonstrated by Grossman *et al.* (1970). For the loci α -*Gpdh*, *Est-6*, *Est-C*, *6-Pgd* and *Pgm*, the temperature gradient along the transect was likely to be insufficient to result in such regular geographical variation. The pattern of their allele frequency distribution could be determined by interaction between gene flow and some specific factors within the limits of macroclimatic temperature-dependent selection.

Subtle mechanisms of selection are, however, uncertain. *In vitro* analysis of thermostabilities and kinetic properties of allozymes under different temperatures yielded results which were frequently in contrast with what could be expected from the existent geographical and seasonal variation (see e.g. Zera *et al.*, 1984 and Chambers, 1988 for reviews; Oakeshott *et al.*, 1983a and White *et al.*, 1988 for discussions). Therefore, it was hypothesized that temperature had also various indirect effects on allozyme genotypes. For instance, selection might maintain the *Adh* clines through a higher developmental rate of the *Adh-FF* genotypes as a result of higher enzyme activity of ADH-F (Oudman *et al.*, 1991). Rapid development (*r*-strategy) is held to be an advantage in extreme climates far from the equator (Parsons, 1983).

Another possibility is hitch-hiking with cosmopolitan inversions. These inversions display clear latitudinal differentiation in *D. melanogaster* (see Lemeunier *et al.*, 1986; Sperlich & Pfriem, 1986 for reviews) which can reflect adaptation to environmental stress (Dobzhansky, 1970). In laboratory experiments, van Delden & Kamping (1989) demonstrated that the *Adh-s* and α -*Gpdh-F* alleles may be hitch-hiking with In(2L)t. However, some authors (e.g. Voelker *et al.*, 1978; Oakeshott *et al.*, 1982; Singh & Rhomberg, 1987b) argue that the inversions fail to account fully for the allozyme geographical variation in *D. melanogaster*. Obviously, more data are required for elucidation of selective mechanisms in natural populations.

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