Original article

A comparison of the level of enzyme polymorphism in cosmopolitan *Drosophila* species between populations collected in distilleries and in their surroundings in Hungary

K. Pecsenye

Kossuth Lajos University, Department of Zoology and Anthropology, Debrecen 10, PO Box 3, H-4010 Hungary

Summary — The level of enzyme polymorphism was studied in populations of *Drosophila melano-gaster* and *D. hydei* from three different regions of Hungary. Collections were made in distilleries or by outside baits. Allozyme variability was investigated for five loci (*Adh; Odh; Mdh; α–Gpdh; α–Amy*) by means of polyacrylamide gel electrophoresis. Two different rare alleles were detected for the *Adh* locus in *D. hydei* in Hungary. The number of species was lower in distilleries than outside. The heterozygosity level in samples from distilleries was generally lower than in samples from outside. This result gives support to the hypothesis that the more diverse the environment the higher the level of polymorphism maintained.

enzyme polymorphism – distillery – *Drosophila hydei*

Résumé — Comparaison du polymorphisme enzymatique chez des espèces cosmopolites de drosophiles, entre des populations Hongroises échantillonnées dans des distilleries et dans les environs. *Le polymorphisme enzymatique a été étudié dans des populations de* Drosophila melanogaster *et de* D. hydei *issues de trois régions de Hongrie. Les récoltes ont été réalisées dans des distilleries et à l'extérieur par piégeage. La variabilité des allozymes a été étudiée en cinq locus (Adh; Odh; Mdh; α-Gpdh; α-Amy) par électrophorèse sur gel de polyacrilamide. Deux allèles rares ont été détectées au locus Adh dans les populations hongroises de* D. hydei. *Le nombre d'espèces est plus petit dans les distilleries qu'à l'extérieur. Le niveau d'hétérozygotie est en général plus bas dans les échantillons prélevés dans les distilleries qu'à l'extérieur. Ce résultat renforce l'hypothèse que le polymorphisme est maintenu à un niveau d'autant plus élevé que le milieu est plus variable.*

polymorphisme enzymatique – distillerie** – Drosophila melanogaster – **Drosophila hydei

Introduction

Genetic differentiation within a species is a common response to environmental heterogeneity. Some of the existing field studies indicate association between the level of polymorphism at several enzyme loci and the geographical variation of different environmen-

tal factors (Nevo, 1978; Triantaphyllidis *et al.*, 1980; Oakeshott *et al.*, 1982; Singh *et al.*, 1982; Van Delden, 1982; Oakeshott *et al.*, 1983; Nevo *et al.*, 1984).

Many authors have studied microdifferentiation of *Drosophila* populations living in wine cellars and in the surroundings (McKenzie and Parsons, 1974; Briscoe *et al.*, 1975; McKenzie and McKenzie, 1978; Parsons, 1980; McKenzie and McKenzie, 1983). Their main interest was the gene frequency distribution at the *Adh* locus in populations from the 2 types of micro-habitats. It would also be interesting, however, to study the difference in the genetic diversity of the 2 kinds of populations. In the case of laboratory populations, several observations have revealed differences in the average frequency of heterozygotes when Drosophilids were kept in homogeneous and heterogeneous environments (Powell, 1971; McDonald and Ayala, 1974; Hale and Birley, 1983).

This study provides data for a comparison of the level of polymorphism at 4 enzyme loci among village populations of *Drosophila melanogaster* and *D. hydei*, and those living in distilleries. We have found that the average frequency of heterozygotes is higher in the village populations at the investigated loci.

Materials and Methods

Drosophilids were collected in 3 large regions of Hungary: the Central Tisza region (region I), the Bereg plain (region II) and the Sajo and Hernad valley (region III). Signs on the map (Fig. 1) show the distilleries where collection took place. Enzyme polymorphism
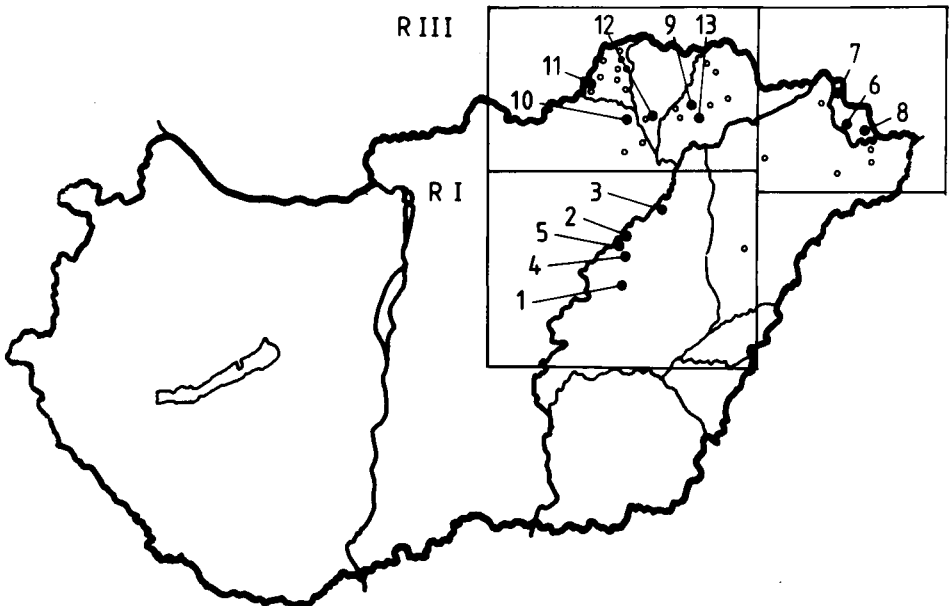
Fig. 1. Map showing the collection sites in the northern and eastern parts of Hungary. R I: Kunhegyes (1), Tissafured (2), Tiszacsegezs (3), Tiszaszentimre (4), Tiszaszolos (5); R II: Jand (6), Lonya (7), Tarpa (8); R III: Abaujszanto (9), Sajoszentpeter (10), Serenyfalva (11), Szikszo (12), Tallya (13). Open circles: collection in distilleries; full circles: collection both in distilleries and on baits.

was determined from 13 samples with high individual counts of both *D. melanogaster* and *D. hydei* (full circles). In order to obtain field populations we used normal maize-sucrose media as baits in the farmyards of the villages close to these distilleries. Similarly to the fermenting mash in the distilleries, this bait attracted the flies so we were able to collect them easily in the surroundings. A glass suction tube was used for the collection in both micro-habitats.

Four or 5 loci – alcohol dehydrogenase (*Adh*), octanol dehydrogenase (*Odh*), malate dehydrogenase (*Mdh*), α–glycerophosphate dehydrogenase (*α–Gpdh*) and α–amylase (*α–Amy*) – were examined in each sample. Electrophoresis was conducted on vertical polyacrylamide slabs using a discontinuous buffer system (O'Brien, 1973; Doane *et al.*, 1975; Clark, 1983; Winberg *et al.*, 1983; Batterham *et al.*, 1984). Genotype and allele frequencies were then calculated.

Statistical procedures

Standard errors of heterozygosity were calculated on a Commodore 64 computer by means of the Number Cruncher 1 programme.

As the proportion of heterozygotes was close to zero for most of the investigated loci, we used the angular transformation of frequency data when the *t*-tests were calculated. A paired *t*-test was performed on a Commodore 64 computer using the Number Cruncher 1 programme.

Results

The common species in distilleries were *D. melanogaster* and *D. hydei*. Some individuals of other species also appeared, such as *D. immigrans, D. funebris* and *D. busckii*. The bait in the villages, however, attracted more species: besides the 2 common ones, we collected quite large samples of *D. immigrans* in each location and some samples of *D. funebris* and *D. busckii* in region I. Other species such as *D. repleta., D. obscura* and *D. subobscura* were scarce (Table I).

Table I. *Drosophila* species distribution in the 2 micro-habitats.

Species	Distillery	Villages
D. melanogaster	2	2
D. hydei	2	2
D. immigrans	1	2
D. funebris	1	2
D. busckii	1	2
D. repleta	0	1
D. obscura	0	1
D. suboscura	0	1

2 = species with large population size; 1 = scarce species; 0 = species not found

Tableau IIa. Allele frequencies at 5 investigated loci in *D. melanogaster* populations collected in distilleries.

Regions	RI					RII			RIII				
Populations	1	2	3	4	5	6	7	8	9	10	11	12	13
Adh sample size	84	84	81	87	82	82	83	84	84	84	84	83	83
Alleles													
S	0.030	0.030	0.010	0.020	0.035		0.010	0.010		0.020	0.005	0.010	
F	0.970	0.970	0.990	0.980	0.965	1.000	0.990	0.990	1.000	0.980	0.995	0.990	1.000
χ^2	0.087	0.001	0	0	0.003		0	0		0	0.001	0	
Odh sample size	84	84	81	87	82	82	83	84	84	82	84	83	83
Alleles													
S	0.010	0.005		0.005						0.010			
F	0.990	0.995	1.000	0.995	1.000	1.000	1.000	1.000	1.000	0.990	1.000	1.000	1.000
χ^2	0	0.001		0.001						0			
Mdh sample size	84	84	76	84	82	75			84	56	70	82	84
Alleles													
A	0.970	0.980	0.980	1.000	1.000	0.980			0.985	0.980	0.970	1.000	0.990
B	0.030	0.020	0.020			0.020			0.015	0.020	0.030		0.010
χ^2	0.019	0	0			0.087			0.032	0	0.002		0
α–Gdph sample size	76	56	83	84	84		84	70	68	84	84	82	80
Alleles													
S	0.290	0.352	0.238	0.236	0.393		0.333	0.286	0.264	0.292	0.173	0.287	0.344
F	0.710	0.648	0.762	0.764	0.607		0.667	0.714	0.736	0.708	0.827	0.713	0.656
χ^2	0.002	0.002	0.001	0.116	0.021		0.004	0.007	0.044	0	0.017	0.005	0
α–Amy sample size	84	84	84	84	84		84	84	112	84	84	54	84
Phenotypes													
1–3				0.012	0.012	0.012	0.012	0.012					0.012
1–2					0.012			0.012				0.012	
1–1	1.000	0.982	0.964	0.976	0.952		0.988	0.964	0.976	0.952	1.000	0.976	0.988
1–1*		0.018	0.024	0.012	0.024			0.012	0.024	0.048		0.012	

χ^2 values indicate the deviation from the expectation on the basis of the Hardy-Weinberg equilibrium.

The distribution of allele frequencies at the investigated loci in *D. melanogaster* populations collected in distilleries and in villages using baits is shown in Table IIa and IIb, respectively. At the *Adh* locus, almost all the populations were polymorphic; however, the frequency of the slow allele was rather low. This is in good agreement with the European frequency gradient (Oakeshott *et al.*, 1982). The populations investigated were less polymorphic at the *Odh* than at the *Adh* locus. For the *Mdh* and *α-Amy* loci, we found that the frequencies of alternative alleles were also rather low. As the α-amylase enzyme is enco-

Table IIb. Allele frequency distribution at 5 investigated loci of *D. melanogaster* populations collected in villages.

Regions	RI					RII			RIII				
Populations	1	2	3	4	5	6	7	8	9	10	11	12	13
Adh sample size	56	83	84	84	84	84	56	84	83	84	84	78	84
Alleles													
S	0.025	0.030	0.025	0.025	0.030		0.005	0.010	0.025	0.020	0.010	0.025	0.010
F	0.975	0.970	0.975	0.975	0.970	1.000	0.995	0.990	0.975	0.980	0.990	0.975	0.990
χ^2	0.002	0.001	0.002	0.002	0.001		0.002	0.001	0.002	0	0.001	0.002	0.001
Odh sample size	56	83	84	84	84	84	56	84	83	84	84	78	84
Alleles													
S	0.060	0.005		0.010	0.030		0.005		0.010		0.010		0.010
F	0.940	0.995	1.000	0.990	0.970	1.000	0.995	1.000	0.990	1.000	0.990	1.000	0.990
χ^2	0.005	0.001		0	0.001		0.001		0		0		0
Mdh sample size	56	84	84	84	84	84	84	84	82	71	84	82	89
Alleles													
A	0.980	0.990	0.980	0.990	0.990	0.960	0.970	0.970	0.960	0.970	0.970	0.990	0.960
B	0.020	0.010	0.020	0.010	0.010	0.040	0.030	0.030	0.040	0.030	0.030	0.010	0.040
χ^2	0.001	0.001	0.001	0.001	0.001	0.002	0.002	0.086	0.084	0.002	0.001	0	0.001
α–*Gdph* sample size	83	82		84	84	84	81	56	56		56	82	80
Alleles													
S	0.241	0.305		0.386	0.280	0.251	0.302	0.285	0.197		0.170	0.247	0.253
F	0.759	0.695		0.614	0.720	0.749	0.698	0.715	0.803		0.830	0.753	0.747
χ^2	0.006	0.007		0.036	0.043	0.020	0.007	0.001	0.018		0.006	0.005	0
α–*Amy* sample size	82	56		84	112	56	70	84	112	84		84	84
Phenotypes													
1–3							0.048		0.012				0.024
1–2								0.012					
1–1	0.952	0.988		0.952	0.976	0.982	0.952	0.964	0.964	0.964		0.964	0.952
1–1*	0.048	0.012		0.048	0.024	0.018		0.024	0.024	0.036		0.036	0.024

χ^2 values indicate the deviation from the expectation on the basis of the Hardy-Weinberg equilibrium.

ded by a duplicated locus we did not calculate allele frequencies, thus only the phenotype frequencies are presented in the tables (Doane *et al.*, 1975; Singh *et al.*, 1982). At the α-Gpdh locus the average frequencies of the slow allele were 0.291 for the populations originating from distilleries and 0.265 for those collected in villages. On the basis of the results of a χ^2 test we concluded that all the populations at all the investigated loci were in Hardy-Weinberg equilibrium.

Drosophila hydei was the other cosmopolitan species in our study. As opposed to D . melanogaster, this species did not occur in large masses either in distilleries or on bait.

The allele frequency values at all the investigated loci in D. hydei populations collected in distilleries and in villages by baits are presented in Table IIIa and IIIb, respectively. The Adh locus is known to be monomorphic in populations of D. hydei in the United States (Batterham et al., 1984). In some of the collecting sites, however, we found 2 different rare alleles at this locus. Figure 2 shows the new genotypes. The F allele was the most common, and the rare alleles showed either faster or slower migration. These rare alleles appeared only in a few populations, mostly in region I. At the Mdh locus 3 alleles, i.e. 6 genotypes, appeared in Hungarian populations. Allele S* was found only in populations collected on baits, and the frequency of allele F was slightly higher in these popula-

Table IIIa. Distribution of allele frequencies at 4 investigated loci in D. hydei populations collected in distilleries.

Regions	RI					RII			RIII				
Populations	1	2	3	4	5	6	7	8	9	10	11	12	13
Adh sample size	56	78	67	84	56	78	56	80	83	79	82	57	56
Alleles													
S		0.010											
F	1.000	0.990	0.980	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
F*			0.020										
χ^2		0.006	0.002										
Mdh sample size	56	82		168		83	82	84	56	84	121		
Alleles													
S*													
S	0.720	0.950		0.990		0.990	0.950	0.880	0.840	0.980	0.980		
F	0.280	0.050		0.010		0.010	0.050	0.120	0.160	0.020	0.020		
χ^2	0.044	0.004		0.018		0	0.005	0.001	0.001	0.001	0.001		
α–Gdph sample size	84	75			56		70	84	84	84	84	70	84
Alleles													
S	1.000	1.000			1.000		0.994	0.994	1.000	1.000	1.000	1.000	1.000
F							0.006	0.006					
χ^2							0	0					
α–Amy sample size	74	84			56		56	84	84	84	83	84	84
Alleles													
8*												0.006	
8								0.018	0.006	0.006	0.018	0.012	
7	1.000	1.000			1.00		1.000	0.982	0.994	0.982	0.994	0.982	1.000
χ^2				0		0	0	0	0				

χ^2 values indicate the deviation from the expectation on the basis of the Hardy-Weinberg equilibrium.

Table IIIb. Allele frequency values at 4 investigated loci of *D hydei* populations collected in villages.

Regions	RI					RII			RIII				
Populations	1	2	3	4	5	6	7	8	9	10	11	12	13
Adh sample size	82	58	82	84	62	83	84	80	84	82			83
Alleles													
S		0.040			0.020								
F	1.000	0.890	0.990	1.000	0.980	0.990	1.000	1.000	1.000	1.000			1.000
F*		0.070	0.010			0.010							
χ^2		0.032	0.005		0.007	0.005`							
Mdh sample size	84	65	84	84	68	83	83	84	66	56			69
Alleles													
S*		0.040	0.010	0.010			0.010	0.010	0.030	0.010			0.050
S	0.890	0.890	0.950	0.930	0.860	0.960	0.960	0.960	0.810	0.920			0.810
F	0.110	0.070	0.040	0.060	0.140	0.040	0.030	0.030	0.160	0.070			0.140
χ^2	0.016	0.219	0.053	0.005	0.005	0.005	0.006	0.031	0.019	0.016			0.033
α–Gdph sample size	79	84	84		84	84	80	84	56	84	`51		84
Alleles													
S	1.000	0.994	1.000		1.000	0.988	0.988	0.923	0.957	1.000	1.000		0.988
F		0.006				0.012`	0.012	0.077	0.043				0.012
χ^2		0				0	0	0.007	0.004				0
α–Amy sample size	80	84	84	56	84	82	83	84	79	84	55		84
Alleles													
8*						0.006				0.006			
8	0.030	0.045				0.018			0.065	0.006	0.006		0.030
7	0.970	0.955	1.000	1.000	1.000	0.982	0.994	1.000	0.935	0.988	0.994		0.970
χ^2	0	0				0	0		0	0	0		0

χ^2 values indicate the deviation from the expectation on the basis of the Hardy-Weinberg equilibrium.

tions. The *α–Gpdh* locus was actually monomorphic with rare alleles appearing mainly in region II. Similarly to the *Adh*, the *α–Amy* locus had 2 rare alleles (Doane *et al.*, 1975) that were mainly found in populations of region III.

Discussion

We compared the level of polymorphism in populations originating from distilleries to those collected in villages in the case of both species. Some important data – as a basis of comparison – are presented in Table IV for *D. melanogaster* populations. All 3 of the parameters – proportion of polymorphic populations (frequency of rare alleles > 0.01),
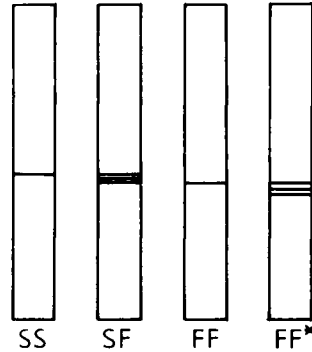
SS   SF   FF   FF*

Fig. 2. New genotypes found at the *Adh* locus in *D. hydei* populations.

Table IV. The level of polymorphism in *D. melanogaster* populations originating from both micro-habitats.

Localities	Distilleries					Villages				
Loci	Adh	Odh	Mdh	α–Gpdh	α–Amy	Adh	Odh	Mdh	α–Gpdh	α–Amy
No. of populations	13	13	11	12	12	13	13	13	11	11
Proportion of polymorphic populations*	0.69	0.15	0.73	1.00	0.38	0.85	0.46	1.00	1.00	0.75
Average no. of alleles	1.77	1.31	1.73	2.00	2.44	1.85	1.62	2.00	2.00	2.55
Average frequency of heterozygotes	0.026	0.005	0.025	0.428	0.020	0.037	0.020	0.045	0.416	0.037
Standard error of heterozygosity	0.022	0.008	0.021	0.063	0.015	0.020	0.033	0.020	0.082	0.011

*Rare allele frequency is > 0.01.

average number of alleles (each investigated allele taken into account) and average heterozygosity – indicate a higher level of polymorphism in the field as compared with the distillery populations at 4 of the investigated loci. In *D. melanogaster* the highly polymorphic α–*Gpdh* locus was, however, an exception.

In the case of *D. hydei* populations, Table V shows the most basic data for comparison. The 3 examined parameters show the level of polymorphism to be higher in village populations for 3 of the investigated loci. The only exception was the highly polymorphic *Mdh* locus.

As the average frequencies of heterozygotes have rather high standard errors, we tested the statistical significance of differences between populations originating from the 2 habitats, villages *versus* distilleries. Results of the *t*-test are shown in Table VI. The dif-

Table V. The level of polymorphism in *D. hydei* populations originating from both micro-habitats.

Localities	Distilleries				Villages			
Loci	Adh	Mdh	α–Gpdh	α–Amy	Adh	Mdh	α–Gpdh	α–Amy
No. of populations	13	9	10	9	13	13	12	12
Proportion of polymorphic populations	0.15	1.00	0	0.33	0.23	1.00	0.45	0.42
Average no. of alleles	1.15	2.00	1.22	1.56	1.42	2.73	1.33	1.78
Average frequency of heterozygotes	0.003	0.167	0.003	0.011	0.028	0.158	0.032	0.043
Standard error of heterozygosity	0.009	0.135	0.005	0.015	0.077	0.101	0.054	0.045

ferences approached significance or were significant at all the investigated loci except α–Gpdh in *D. melanogaster* and *Mdh* in *D. hydei*; *i.e.*, genic diversity appears higher in the villages as compared with the distilleries.

It can be concluded that field populations had a higher level of enzyme polymorphism in comparison with those living in distilleries. This tendency clearly appears at those enzyme loci with a low heterozygosity level. A possible explanation for the situation is that both species develop in villages in more diverse resources, in fermenting windfalls, in rotting vegetables, in rubbish, etc. In distilleries, however, Drosophilids grow in a more uniform environment, on mash with rather high alcohol concentrations. It is interesting, however, that the highly polymorphic loci (*D. melanogaster*: α–Gpdh, *D. hydei*: *Mdh*) do not show such a difference.

Environments in nature are usually heterogeneous in time and space – the environment of the population has a grain structure. A fine grain would make polymorphism less

Table VI. Results of *t*-tests for the comparison of the arcsin transformation of the average heterozygosity between the 2 micro-habitats in both species.

Species		Adh	Odh	Mdh	α–Gpdh	α–Amy
D. melanogaster	t	1.86	1.91	1.78	1.07	1.88
	P	0.09	0.08	0.10	0.32	0.07
D. hydei	t	3.73	–	0.63	2.31	1.89
	P	0.02	–	0.50	0.06	0.09

t: *t*-values; *P* : level of significance.

likely to be achieved, or would reduce the stability of polymorphism already attained (Levins and Macartur, 1966). With coarseness of grain, however, the population may maintain some choice of genotypes over the types of conditions available (Levins and Macartur, 1966; Gillespie and Langley, 1974; Taylor, 1975). Our results support the hypothesis that the more diverse the environment, the higher the level of polymorphism that can be maintained (Powell, 1971; McDonald and Ayala, 1974; Nevo et al., 1984).

Acknowledgments

References

Batterham P., Chambers G.K., Starmer W.T. & Sullivan D.T. (1984) Origin and expression of an alcohol dehydrogenase gene duplication in the genus Drosophila. Evolution 38, 644-657

Briscoe D.A., Robertson A. & Malpica J. (1975) Dominance at Adh locus is response of adult Drosophila melanogaster to environmental alcohol. Nature 255, 148-149

Clark B.A. (1983) Cytogenetic localization by variation in electrophoretic allozyme phenotype: Drosophila Odh. Biochem. Genet. 21, 375-390

Doane W.W., Abraham I., Kolar M.M., Martenson R.E. & Deibler G.E. (1975) Purified Drosophila α–amylase isozymes: genetical, biochemical, and molecular characterization. In: Isozymes. IV. Genetics and Evolution (C.L. Markert, ed), Academic Press, New York, pp. 585-607

Gillespie J.H. & Langley C.H. (1974) A general model to account for enzyme variation in natural populations. Genetics 76, 837-848

Hale C.S. & Birley A.J. (1983) The genetical response to natural selection by varied environments. II. Observations on replicate populations in spatially varied laboratory environments. Heredity 51, 581-606

Levins R. & Macartur R. (1966) Maintenance of genetic polymorphism in a heterogeneous environment: variations on a theme by Howard Levene. Am. Nat. 100, 585-590

McDonald J.F. & Ayala F.J. (1974) Genetic response to environmental heterogenity. Nature 250, 572-574

McKechnie S.W. & McKenzie J.A. (1983) Polymorphism of alcohol dehydrogenase (ADH) in a winery cellar population of Drosophila melanogaster : gene frequency association with temperature and genotypic differences in progeny production Evolution 37, 850-853

McKenzie J.A. & McKechnie S.W. (1978) Ethanol tolerance and the Adh polymorphism in a natural population of Drosophila melanogaster. Nature 272, 75-76

McKenzie J.A. & Parsons P.A. (1974) Microdifferenciation in a natural population of Drosophila melanogaster to alcohol in the environment. Genetics 77, 385-394

Nevo E. (1978) Genetic variation in natural populations: patterns and theory. Theor. Pop. Biol. 13, 121-177

Nevo E., Beiles A. & Ben-Shlomo R. (1984) The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. In: Evolutionary Dynamics of Genetic Diversity. (G.S. Mani, ed.), Lecture Notes in Biomathematics 53, Springer-Verlag, Berlin, pp. 13-213

Oakeshott J.C., Gibson J.B., Anderson P.R. & Knibb W.R. (1982) Alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase clines in Drosophila melanogaster on different continents. Evolution 36, 86-96

Oakeshott J.G., Gibson, J.B. & Willcocks D.A. (1983) Latitudinal variation in octanol dehydrogenase and acid phosphatase allele frequencies in *Drosophila melanogaster. Theor. Appl. Genet.* 65, 191-196

O'Brien S.J. (1973) Comparative analysis of malate dehydrogenase of *Drosophila melanogaster. Biochem. Genet.* 10, 191-205

Parsons P.A. (1980) Responses of *Drosophila* to environmental ethanol from ecologically optimal and extreme habitats. *Experientia* 36, 1070-1071

Powell J.R. (1971) Genetic polymorphism in varied environments. *Science* 174, 1035-1036

Singh R.S., Hickey D.A. & David J. (1982) Genetic differentiation between geographically distant populations of *Drosophila melanogaster. Genetics* 101, 235-256

Taylor C.E. (1975) Genetic loads in heterogeneous environments. *Genetics* 80, 621-635

Triantaphyllidis C.D., Panourgias J.N., Scouras Z.G. & Ioanuidis G.C. (1980) Comparison of gene-enzyme variation between *Drosophila melanogaster* and *Drosophila simulans. Genetica* 51, 227-231

Van Delden W. (1982) The alcohol dehydrogenase polymorphism in *Drosophila melanogaster. Evol. Biol.* 15, 187-222

Winberg J., Thatcher D.R. & McKinley-McKee J.S. (1983) *Drosophila melanogaster* alcohol dehydrogenase: an electrophoretic study of the *AdhS, AdhF* and *AdhUF* alloenzymes. *Biochem. Genet.* 21, 63-80