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Brief report

Linkage disequilibrium in Hungarian populations of *Drosophila melanogaster*

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In natural populations, nonrandom association of genes, indicated by significant gametic disequilibrium, can be generated when tight linkage is combined with epistatic selection or genetic drift or population subdivision. When gametic disequilibrium is surveyed, however, it is difficult to attribute a significant case to a particular cause (for a review, see HEDRICK et al. 1978). Gametic disequilibria have been extensively investigated in natural and laboratory populations of different species (ALLARD et al. 1972; BAKER 1975; BANTOCK and NOBLE 1973; CLARK et al. 1968; MITTON and KOEHN 1973; MUONA 1982; MUONA et al. 1982; MUONA and SZMIDT 1985). Several studies have demonstrated that significant gametic disequilibria between enzyme loci are seldom found in natural populations of *Drosophila melanogaster* (LANGLEY et al. 1974; LANGLEY 1977). Another finding of some of these surveys is that allozymes are often significantly associated with particular chromosomal arrangements (CHARLESWORTH et al. 1979; LANGLEY and ITO 1976; PRAKASH and LEWONTIN 1968). Studies of DNA sequence polymorphism at the alcohol dehydrogenase (*Adh*) locus of *D. melanogaster*, have revealed strong nonrandom associations among different polymorphic regions (KREITMAN and HUDSON 1991; BEGUN and AQUADRO 1992) and also between the sequence length polymorphism and amino acid replacement (LAURIE et al. 1991; AQUADRO et al. 1986).

The natural food of *D. melanogaster* mostly consists of fermenting fruits. Thus, flies must continuously cope with alcohol in their environments (DAVID 1988). Although the metabolism of ethanol preliminary depends on alcohol dehydrogenase (ADH), there is no consistent evidence which shows that enhanced ethanol tolerance observed in natural populations is the result of ethanol selecting directly on the genetic polymorphism present at the *Adh* locus (e.g., BRISCOE et al. 1975; DAVID et al. 1989; GIBSON et al. 1981; HICKEY and MCLEAN 1980; PECSENYE 1989). An increasing body of the literature shows that adaptation to ethanol is a complex metabolic process which involves several enzymes in addition to ADH (see, for reviews, GEER et al. 1989, 1993). Hence, it can be of interest to search for nonrandom associations among enzyme loci known to participate in the ethanol metabolism in natural populations.

This work reports on gametic disequilibria estimated among five enzyme loci (chromosome II — *αGpdh*, *Mdh*, and *Adh*; chromosome III — *Odh* and *Aldox*) in natural populations of *Drosophila melanogaster* originating from distilleries where ethanol was present at high concentrations, and surrounding areas where ethanol concentrations were low. These enzymes where chosen because in our previous study we showed that environmental ethanol influences their activities (PECSENYE et al. 1994).

Materials and methods

Sample collection and electrophoresis. — Samples of *Drosophila melanogaster* populations originated from Hungary: Hortobágy district and Borsod district. Between 100–120 adults were collected in farmyards and distilleries in four villages of both districts. Five enzyme loci (α-glycerophosphate dehydrogenase — *αGpdh*: 2–20.5; malate dehydrogenase — *Mdh*: 2–37.2; *Adh*: 2–50.1; octanol dehydrogenase — *Odh*: 3–49.1; aldehyde oxidase — *Aldox*: 3–56.6) were studied in each sample using vertical polyacrylamide gel electrophoresis. 80 individuals were investigated at all the five loci in parallel from each sample. Electrophoresis was conducted in a discontinuous buffer system as described by PECSENYE (1989). Then genotype and allele frequencies were estimated.

Estimation of gametic disequilibria. — Gametic disequilibrium between loci A and B (with A, a and B, b alleles) can be estimated by using Burrow's

composite measure (in WEIR 1990, p. 102):

$$\Delta_{AB} = D_{AB} + D_{A/B}$$

where

D_{AB} is the gemetic disequilibrium between A and B loci within individuals;
$D_{A/B}$ is nongametic linkage disequilibrium between individuals (i.e., nonrandom union of gametes).

The composite measure is appropriate to estimate gametic disequilibrium under nonrandom mating as well, when it is defined as:

$$\Delta_{AB} = P_{AB} + P_{A/B} - 2p_A p_B$$

where

$P_{AB} + P_{A/B}$ is the frequency of individuals carrying A and B alleles;
p_A is the frequency of A allele;
p_B is the frequency of B allele.

Maximum likelihood estimates of D_{AB} were obtained in all populations for all pairs of linked loci when both of them were polymorphic. We tested whether estimates of D_{AB} differed significantly from zero, as described by WEIR (1990).

Since we had four samples of the two habitats in both districts, we could perform an overall test for gametic disequilibria in each habitat by using the standardised statistics described by LANGLEY et al. (in MANLY 1985, p. 325):

$$R^2_{AB} = \frac{1/2\Delta^2_{AB}}{p_A(1-p_A)p_B(1-p_B)}.$$

R_{AB} behaves like a correlation coefficient.

Test I for K samples:

$$\chi^2_k = 4N_k R_k$$

where

N_k is the k-th sample size;
R_k is the estimated correlation coefficient for the k-th sample.

The sum of the χ^2_k values for K independent samples has a chi-square distribution with K degrees of freedom. In this test, however, $\Sigma\chi^2_k$ can be significantly large when (i) R has a constant non-zero value in the samples, or (ii) there is a variation in the R values for the different samples. LANGLEY et al. have developed weighted analyses of variance on the R_k values to tell the difference between these two situations (in MANLY 1985, p. 325).

Test II for K samples. — First we estimated the minimum variance weighted mean of the R_k values for the two habitats in both districts:

$$\bar{R} = \sum_{k=1}^{K} N_k R_k \Big/ \sum_{k=1}^{K} N_k$$

$$\chi^2 = \sum_{k=1}^{K} 4N_k \bar{R}^2$$

χ^2 has a chi-square distribution with one degree of freedom.

Using the minimum variance weighted mean of the R_k values we calculated the mean values of gametic disequilibria for the two habitats in both districts. We also estimated the mean values of the normalised gametic disequilibria (D′):

$$D' = D/D_{max}$$

where D_{max} was min ($p_A p_B$ or $p_a p_b$) since $D < 0$ held in all of the significant cases. p_A, p_B, p_a, and p_b values were average allele frequencies in one particular habitat of a district.

Results and discussion

Allele frequency values are presented in Tables 1 and 2. The *Mdh* locus excepted, all populations were polymorphic (frequency of alternative allele > 0.01) at the investigated loci. Most of the populations had the Hardy-Weinberg genotypic proportions at all five loci, with the exception of the *Aldox* locus in the Hortobágy district, where half of the samples had significantly large chi-square values due to a deficiency of heterozygotes.

Tables 3A and 3B show the estimated values of gametic disequilibria in all samples. There were 8 cases (4 for *Mdh-Adh*; 1 for *αGpdh-Mdh*; 3 for *Odh-Aldox*) where the chi-square values indicated significant gametic disequilibria. In four further cases (2 for *αGpdh-Adh* and 2 for *Odh-Aldox*), the chi-square values were close to the significant level. We found $D < 0$ in all significant cases.

Since the *Mdh* locus was monomorphic in 9 samples, the mean values of the correlation coefficients (R) and gametic disequilibria (D) were calculated only for the two districts in combinations with this locus (*Mdh-Adh* and *αGpdh-Mdh*). The mean correlation coefficients showed strong evidence for nonrandom association of alleles at the *Adh* and *Mdh* loci (Table 4). It is also evident from Tables 4 and 5 that the magnitude of the R, D, and D′ values is rather similar for this pair of loci in the two districts. Our result is interesting,

Table 1. F allele frequencies at the five investigated loci in the Hortobágy district. The chi-square values indicating the departures from the Hardy-Weinberg equilibria, are shown in brackets, * significant at 0.05 level, ** significant at 0.01 level, *** significant at 0.001 level. FY — farmyards, DI — distilleries

Localities		Chromosome II			Chromosome III	
		Adh	*Mdh*	*αGpdh*	*Odh*	*Aldox*
Kunhegyes	FY	0.981	0.013	0.885	0.974	0.289
		(0)	(0)	(0.5)	(0.1)	(3.8*)
	DI	0.950	0	0.852	0.963	0.181
		(0.1)	(−)	(0.5)	(0.1)	(6.5*)
Tiszacsege	FY	0.988	0.013	0.713	0.900	0.244
		(0)	(0)	(0)	(1.0)	(1.9)
	DI	0.983	0	0.767	0.975	0.308
		(0)	(−)	(0.3)	(0)	(6.8**)
Tiszafüred	FY	0.975	0	0.781	0.899	0.304
		(0)	(−)	(0.3)	(1.0)	(3.9*)
	DI	0.994	0.013	0.772	0.943	0.241
		(0)	(0)	(1.5)	(0.3)	(0.1)
Tiszaszentimre	FY	0.981	0.013	0.750	0.963	0.219
		(34.9***)	(0)	(0)	(0.2)	(0)
	DI	0.970	0	0.753	0.952	0.176
		(0.1)	(−)	(1.1)	(0.3)	(0)

Table 2. F allele frequencies at the five investigated loci in the Borsod district. (For further explanation, see the legend of Table 1)

Localities		Chromosome II			Chromosome III	
		Adh	*Mdh*	*αGpdh*	*Odh*	*Aldox*
Abaujszántó	FY	0.975	0.013	0.744	0.988	0.269
		(0)	(0)	(0)	(0)	(0.2)
	DI	0.963	0.019	0.700	0.988	0.463
		(0.1)	(0)	(1.4)	(0)	(0.7)
Sajószentpéter	FY	0.956	0	0.719	0.944	0.275
		(0.2)	(−)	(0.5)	(0)	(0.3)
	DI	0.956	0	0.713	0.988	0.163
		(5.1*)	(−)	(0.6)	(0)	(0.8)
Szikszó	FY	0.981	0	0.775	0.981	0.188
		(0)	(−)	(0.5)	(0)	(0)
	DI	0.956	0	0.717	0.994	0.128
		(0.2)	(−)	(0.9)	(0)	(0.2)
Tállya	FY	0.994	0	0.718	0.987	0.234
		(0)	(−)	(0.2)	(0)	(0)
	DI	0.963	0.006	0.725	0.987	0.184
		(0.1)	(0)	(2.7)	(0)	(0.3)

since in spite of the relatively tight linkage (map distance 9.9 cM) LANGLEY and ITO (1977) could not detect significant gametic disequilibrium in a North Carolina population.

It is interesting that significant disequilibria were seldom found between the *Odh* and *Aldox* loci in individual populations (Tables 3A and 3B), and yet, the average correlation coefficients indicate a highly significant nonrandom association between these two loci in both habitats in the Hortobágy district and in the farmyards in the Borsod district (Table 2). The detailed analysis of Tables 3A and 3B, however, can give an explanation of the contradiction in these results. In the Hortobágy district (average R value was significant in both habitats), we found one single sample with $D > 0$; in the

Table 3A. Gametic disequilibria estimated in the populations of the Hortobágy district. FY — populations originating from farmyards; DI — populations originating from distilleries; N — number of adults sampled; D — gametic disequilibrium (when one of the loci was monomorphic, D was not calculated); * significant at 0.05 level

Localities			Chromosome II			Chromosome III
			MdhF AdhF	*αGpdhF MdhF*	*αGpdhF AdhF*	*OdhF AldoxF*
Kunhegyes	FY	N	78	78	78	78
		D	+0.0005	−0.0128	+0.0164	−0.0045
		χ^2	0.008	5.68*	3.61	0.25
	DI	N				80
		D	–	–	–	−0.0179
		χ^2				3.77
Tiszacsege	FY	N	80	80	80	80
		D	+0.0003	−0.0054	−0.0009	+0.0114
		χ^2	0.05	0.88	0.03	0.60
	DI	N			60	60
		D	–	–	−0.0079	−0.0013
		χ^2			1.18	0.01
Tiszafüred	FY	N			80	79
		D	–	–	−0.0047	−0.0402
		χ^2			0.46	5.98*
	DI	N	79	79	79	79
		D	−0.0062	+0.0058	+0.0035	−0.0107
		χ^2	25.66*	1.07	0.75	1.00
Tiszaszentimre	FY	N	79	79	80	
		D	−0.0244	0	+0.0032	–
		χ^2	41.83*	–	0.14	
	DI	N			99	80
		D	–	–	+0.0002	−0.0153
		χ^2			0	3.40

Table 3B. Gametic disequilibria estimated in the populations of Borsod district. (For further explanation, see the legend of Table 3A)

Localities			Chromosome II			Chromosome III
			MdhF AdhF	*αGpdhF MdhF*	*αGpdhF AdhF*	*OdhF AldoxF*
Abaujszántó	FY	N	80	80	80	80
		D	−0.0057	+0.0065	−0.0003	−0.0122
		χ^2	8.75*	1.44	0	5.09*
	DI	N	80	80	80	80
		D	−0.0049	−0.0076	−0.0038	+0.0054
		χ^2	3.0	1.37	0.18	0.68
Sajószentpéter	FY	N			80	80
		D	–	–	+0.0004	−0.0028
		χ^2			0	0.49
	DI	N			80	80
		D	–	–	−0.0028	−0.0022
		χ^2			0.08	0.26
Szikszó	FY	N			80	80
		D	–	–	+0.0104	−0.0119
		χ^2			2.92	3.94*
	DI	N			90	90
		D	–	–	+0.0026	+0.0014
		χ^2			0.07	0.31
Tállya	FY	N			78	77
		D	–	–	−0.0037	−0.0070
		χ^2			0.76	1.65
	DI	N	80	80	80	79
		D	−0.0059	−0.0028	−0.0019	+0.0047
		χ^2	12.49*	0.43	0.03	0.98

Table 4. Results of over all analyses on gametic disequilibria estimated in the populations of both districts. N — average number of samples; R — the minimum variance weighted mean of the correlation coefficients (see text); χ_1 and χ_2 summed chi-square values for all the populations in the two districts estimated in two different ways (see text): *significant at 0.05 level, ** significant at 0.01 level, ***significant at 0.001 level

Chromosome	Loci		Hortobágy		Borsod	
			Farmyards	Distilleries	Farmyards	Distilleries
II	*Mdh-Adh*	N	79		80	
	(9.9 cM)	R	−0.3088		−0.3064	
		χ_1^2	268.39***		96.92***	
		χ_2^2	120.52***		90.12***	
	αGpdh-Mdh	N	79		80	
	(22.3 cM)	R	−0.0856		−0.0235	
		χ_1^2	30.51**		12.95*	
		χ_2^2	6.92**		0.48	
	αGpdh-Adh	N	79.5	79.5	79.5	82.5
	(32.2 cM)	R	+0.0542	−0.0016	+0.0240	−0.0173
		χ_1^2	20.45**	8.51	14.74**	1.44
		χ_2^2	3.71	0.01	0.18	0.10
III	*Odh-Aldox*	N	79.3	78.3	79.3	82.3
	(7.5 cM)	R	−0.1088	−0.1201	−0.1748	+0.0516
		χ_1^2	38.88***	32.74***	44.64***	8.90
		χ_2^2	14.96***	18.05***	38.69***	3.51

Table 5. Estimated mean values of gametic disequilibria (D) and normalized gametic disequilibria (D′) in those cases where the mean correlation coefficients (R) over all the districts and habitats were significant (see Table 4)

Chromosome	Loci		Hortobágy		Borsod	
			Farmyards	Distilleries	Farmyards	Distilleries
II	*Mdh-Adh*	D	−0.0041		−0.0062	
	(9.9 cM)	D_{max}	−0.0128		−0.0126	
		D′	0.320		0.494	
	αGpdh-Mdh	D	−0.0040		–	
	(22.3 cM)	D_{max}	−0.0104		–	
		D′	0.396		–	
III	*Odh-Aldox*	D	−0.0119	−0.0101	−0.0117	–
	(7.5 cM)	D_{max}	−0.0486	−0.0325	−0.0190	–
		D′	0.245	0.311	0.615	–

other 7 samples, D varied between −0.0045 and −0.0402. In the farmyard samples of the Borsod district (average R value was again highly significant), all D values were negative and varied between −0.0028 and −0.0122. In contrast, we observed positive D values in 3 of the 4 samples that originated from the distilleries of Borsod district (the average R value was not significant). It thus appears that the average R values were significant in those cases when the variation in the D values of the individual samples was relatively little. When the nonrandom association between the *Odh* and *Aldox* loci was significant, the average values of R, D, and D′ were all similar in their size and direction (Tables 4 and 5). This also indicates that there is a significant gametic disequilibrium between the alleles at the *Odh* and *Aldox* loci in the Hungarian *D. melanogaster* populations. Our finding is in agreement with the results of LANGLEY et al. (1974) and LANGLEY (1977), who also found significant gametic disequilibrium between the *Odh* and *Aldox* loci in a Japanese population.

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