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Allozyme polymorphism and linkage disequilibrium of *Adh* and *α-Gpdh* loci in wine cellar and field populations of *Drosophila melanogaster*

A. Alonso-Moraga and A. Muñoz-Serrano

Departamento de Genética, Facultad de Ciencias, E-14071 Córdoba (Spain), and Departamento de Genética, Facultad de Veterinaria, E-14071 Córdoba (Spain), 30 October 1985

Summary. Over three years, the *Adh* and *α-Gpdh* loci have been studied in two cellar populations of *Drosophila melanogaster* and in two field populations which were each near to one of the cellars. Analyses of gene frequencies indicate that the divergence among subpopulaitons is greater in the *Adh* locus than in the *α-Gpdh* locus. Selection for or against Adh^S allele acting on the *In(2L)t* inversion influences of the *α-Gpdh* alleles. This phenomenon may contribute to explain the maintenance of the *Adh* and *α-Gpdh* polymorphism and of the *In(2L)t* inversion.
Key words. Allozyme polymorphism; linkage disequilibrium; wine cellar and field populations; *Drosophila melanogaster*.

One selectionist explanation for the maintenance of the *Adh* locus polymorphism is that of frequency-dependent selection[1], although some authors feel that convincing evidence is lacking[2]. Laboratory experiments in which alcohol was added to the nutrient medium[3] indicate that the Adh^{FF} genotype is better adapted, whereas research in which individuals are submitted to temperature shocks[4] indicates that the Adh^{SS} genotype is better adapted to a higher temperature. The *α-Gpdh* locus codes for an essential enzyme in the energy metabolism of the fly and has been studied to determine the adaptative consequences of the existence of null alleles[5], and whether the maintenance of its polymorphism can be explained by frequency-dependent selection[6]. Linkage disequilibrium has been found between the *Adh* and *α-Gpdh* loci in populations from Japan and Texas[7]; the authors conclude that it is improbable that drift is a cause of these disequilibria; they postulate similar selective environments (ecological and genetic) in the two populations as the cause. On the other hand, the inversion-allozyme linkage is considered as a historical accident maintained by a heterotic effect in the fluctuating environment[8], while the polymorphism of two genes is maintained by random drift in natural populations. Yamaguchi et al.[9] have found a) that the linkage disequilibrium between these two loci is not induced by epistasis but by genetic drift, b) that linkage disequilibria between isozyme genes and polymorphic inversion are destined to disappear after many generations, and c) that linkage disequilibria due to immigration cannot be neglected in continuous populations.

Two wine cellar and two field populations from the southern Iberian Peninsula were analyzed. Samples were taken from each population at the end of the summer over a period of 3 years. 1200 flies were analyzed electrophoretically for *Adh* and *α-Gpdh* loci[10]. The wine cellar populations live in uniform environments where food is abundant in the form of yeast (impregnated by 12–15% alcohol), and where the annual average temperature is between 15 and 20 °C with daily oscillations of ± 1 °C. The field populations live in a variable environment where the annual variation of average temperature is between 10 and 25 °C with daily oscillations of ± 10 °C. For chromosomal analysis, 60 males were crossed with homozygous females of standard arrangement, and 7 larvae of the F_1 progeny were analyzed.

Table 1 shows a substantial deficiency of heterozygotes at the *Adh* locus in the two field populations, whilst the two wine cellar populations are close to equilibrium with a higher frequency of the Adh^F allele. The *α-Gpdh* locus shows a smaller (but still significant) deficiency of heterozygotes in field populations where the $\alpha\text{-}Gpdh^F$ allele is more abundant, and shows equilibrium H-W in wine cellars with the same frequency of the two alleles. The mean frequency of the Adh^F allele for the 1200 individuals is 0.6596 and that of the $\alpha\text{-}Gpdh^F$ allele is 0.6362. The two frequency values are significantly near to equality although they differ significantly for 0.5. Analysis of variation between populations within years (table 2a) shows values significantly different from zero for F, Ø and $f^{[1]}$ for the two loci in the 3 years, except the F values for the *α-Gpdh* locus in the 2nd year. The correlation Ø, which is a measure of the divergence between subpopulations, indicates a greater difference in the *Adh* locus between subpopulations (e.g. field vs wine cellar), with a high homozygosity in the subpopulations which is the cause of the high correlation between genes within individuals (F,f). The *α-Gpdh* locus shows the same annual variation as the *Adh* locus but with significantly lower values for F and f correlations. The Ø correlation (which is Wahlund's variance) can reach values significantly different from zero in two cases: 1) if populations are drifting because of small N_e or 2) if there is an interaction between the selection and the ecological habitat. In the first case, it must be taken into account that migration would reduce the Ø values to 0.2, if there is 1 migrant per subpopulation and per generation ($Ø = 1/1+4Nm$), and at the same time migration will reduce F; so, F would reach value near to zero. In the 2nd case, F and Ø values would be elevated and so would have intermediate values. As can be seen in table 2a, it seems that F, Ø and f values for *α-Gpdh* locus would be consistent with the first assumption, and those for *Adh* locus could be close to the second one. It must be taken into account that population structure influences high values of f, thus, the mixture of subpopulations increases the f value.

Analysis of variation between genes within subpopulations (table 2b) shows that F and f are greater in field than in wine cellar (as Comstock's test indicates) and also F and f are greater for *Adh* than for the *α-Gpdh* locus, (as can be seen from the results of Fisher's *z*-method). The Ø value is homogeneous for the four populations and it reaches the same value for the *Adh* locus as for the *α-Gpdh* locus; this is a reasonable result taking into account that the analysis has been performed for the 3 years within a same population. This homogeneity indicates that the changes produced in the subpopulations between years are not significant for both loci and for both environments (wine cellar, field). The high values of F in field are due to the existence for a

Table 1. Genotypes (FF, FS, SS), allelic frequencies (p), sample size and X^2 values in Hardy-Weinberg expectations

Year	Population	Sample size	*Adh* FF	FS	SS	(p) F	X^2	*α-Gpdh* FF	FS	SS	(p) F	X^2
1	Cellar 1	100	56	37	7	0.745	0.069	36	48	16	0.600	0.000
	Cellar 2	100	71	19	10	0.805	15.587***	29	40	31	0.490	3.987*
	Field 1	100	45	30	25	0.600	14.063***	46	35	19	0.635	6.000*
	Field 2	100	52	6	42	0.550	77.227***	59	27	14	0.725	10.425**
2	Cellar 1	100	39	43	18	0.605	1.007	46	47	7	0.695	1.180
	Cellar 2	100	72	23	5	0.835	2.733	33	46	21	0.560	0.443
	Field 1	100	35	30	35	0.500	16.000***	55	36	9	0.730	0.753
	Field 2	100	18	17	65	0.265	31.764***	82	15	3	0.895	4.077*
3	Cellar 1	100	71	28	1	0.850	0.961	33	47	20	0.565	0.192
	Cellar 2	100	84	11	5	0.895	17.201***	19	45	36	0.415	0.536
	Field 1	100	73	19	8	0.825	11.670***	37	39	24	0.565	4.268*
	Field 2	100	36	16	48	0.440	45.606***	66	20	14	0.760	20.411***

*p < 0.05; **p < 0.01; ***p < 0.001. All the X^2 have one degree of freedom.

Table 2. Values of correlation coefficients for the *Adh* and α-*Gpdh* loci

a) Year		F *Adh*		*α-Gpdh*	Ø *Adh*		*α-Gpdh*	f *Adh*		*α-Gpdh*
1		0.4842***	≠	0.2178***	0.0575***	=	0.0336***	0.4527***	≠	0.1906***
2		0.4596***	≠	0.1277*	0.2090***	=	0.0872***	0.3168***	≠	0.0443 ns
3		0.5312***	≠	0.2425***	0.2188***	≠	0.0748***	0.3999***	≠	0.1813***
	X^2	1.832 ns		3.115 ns	6.739*		0.572 ns	5.153 ns		5.522 ns
b) Population										
Cellar 1		0.1094 ns	=	0.0021 ns	0.0695***	=	0.0144*	0.0429 ns	=	0.0000 ns
Field 1		0.4495***	≠	0.2089**	0.1097***	=	0.0238***	0.3816***	≠	0.1896***
Cellar 2		0.3301***	≠	0.1327*	0.0099*	=	0.0155*	0.3234***	≠	0.1191*
Field 2		0.7402***	≠	0.3799***	0.0746***	=	0.0419**	0.7193***	≠	0.3528***
	X^2	108.185***		24.677***	1.548 ns		0.150 ns	116.285***		33.494***

F: correlation between genes within individuals ($F = \sigma_a^2 + \sigma_b^2/\sigma^2$); Ø: correlation between genes of different individuals ($Ø = \sigma_a^2/\sigma^2$); f: correlation between genes within individuals within subpopulations ($f = \sigma_b^2/\sigma_w^2 + \sigma_b^2$); σ_a^2 = variance among subpopulations; σ_b^2 = variance between individuals; σ_w^2 = variance within individuals; σ^2 = total variance; Fisher's *z*-method was applied for testing the homogeneity of two correlation coefficients; χ^2 was applied for testing the homogeneity of several correlation coefficients. *p < 0.05; **p < 0.01; ***p < 0.001.

Table 3. Linkage disequilibrium (D and R values) and inversion frequencies

Population		Year of sample 1	2	3	In (2L) t frequency
Cellar 1	R	−0.5139***	−0.5534***	−0.3466***	0.191
	D	−0.1586	−0.1515	−0.0831	
Cellar 2	R	−0.3678***	−0.5683***	−0.4852***	0.243
	D	−0.1288	−0.1054	−0.1604	
Field 1	R	−0.4623***	−0.4801***	−0.2088**	0.067
	D	−0.1000	−0.1070	−0.0359	
Field 2	R	−0.4491***	−0.2960**	−0.3682***	0.143
	D	−0.1151	−0.0608	−0.0686	

$D = D_w + 2D_b = N/N-1[2N_{11} + N_{12} + (N_{22}/2)]/N-2pq$.
$R = D/\sqrt{[p(1-p)(N_1/N-p^2)][q1-q)(N_1/N-q^2)]}$.
$X^2 = ND^2/p(1-p)q(1-q)$, d.f. = 1.
N_{11}: frequency of Adh^{FF}/α-$Gpdh^{FF}$ homozygote.
N_{22}: frequency of Ahd^{FS}/α-$Gpdh^{FS}$ heterozygote.
p: Adh^F allele frequency.
q: α-$Gpdh^F$ allele frequency.
*p < 0.05; **p < 0.01; ***p < 0.001.

larger component 'between individuals' since Ø values are uniform and lower than 0.11; this may indicate the existence of a structure of subpopulations in the field for *Adh* locus which affects in the α-*Gpdh* locus (which has significantly lower F and f values). At the same time, all the wine cellar populations are more uniform than the field populations perhaps because there is uniform exological environment. Both F and f values are homogeneous in the course of the 3 years. The Ø value is homogeneous for the α-*Gpdh* locus but not for the *Adh* locus since this locus presents very low Ø values in the first year. Perhaps this may be due to sampling error, or because the eclosion had begun when this sample was taken (the eclosion is maximum during the vintage and it renders the populations uniform).

Linkage disequilibrium and inversion frequency. The observation of the maintenance of significant linkage disequilibrium in different populations and at different times is a very sensitive indicator of natural selection[12]. We have found continuous significant linkage disequilibria in the repulsion phase in all four populations as shown in table 3. The inversion *In(2L)t* is present in our populations at a frequency of 0.161 (0.217 in wine cellar and 0.105 in field). The most abundant gametes in wine cellars are Adh^F/α-$Gpdh^F$ and Adh^F/α-$Gpdh^S$ (perhaps because Adh^F allele is favored in environments with alcohol), whereas in the field, Adh^S/α-$Gpdh^F$ and Adh^F/α-*GpdhF* are the more frequent gametes. That is to say, the Adh^F/α-$Gpdh^S$ gamete (which is associated with the standard arrangement) is abundant in wine cellars and (although to a lesser extent) it is abundant in the field too. The Adh^S/α-$Gpdh^F$ gamete (which is associated with the *In(2L)t* inversion) is abundant in the field. The origin of the linkage disequilibrium between these two loci and between each of these loci and the *In(2L)t* may be due to the founder effect[13]. It is reasonable to think that the fact that the alleles Adh^S and α-$Gpdh^F$ are associated with the inversion is a principal product of a stochastic process. However, the persistence of this disequilibrium may be due to the opposite selective influences which are exerted on the *Adh* locus in different environments. In wine cellars and in environments which contain alcohol, the Adh^S allele is negatively selected and therefore both the α-$Gpdh^F$ allele and the inversion are indirectly selected, whereas the Adh^S allele is positively selected in some field subpopulations in consequence of the high temperatures reached in the summer, and both the inversion and the α-$Gpdh^F$ allele are favored indirectly; in other field subpopulations another variable correlated with the temperature, such as the concentration of alcohol in the nutrient medium, may result in a phenomenon similar to that observed in the wine cellars.

The results reported here suggest subdivision of the field populations but not subdivision of the cellar populations, and that selection for or against the Adh^S allele acting on the entire content of the inversion will influence the α-*Gpdh* alleles. This phenomenon may be a factor helping to explain the maintenance of polymorphism in the *Adh* and αGpdh loci and also the maintenance of the *In(2L)t* inversion.

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High level of genetic heterozygosity in the hyperparasitic wasp, *Mesochorus nigripes*[1]

A. C. F. Hung*, R. C. Hedlund**[2] and W. H. Day***

*Agricultural Research Service, USDA, *Beneficial Insects Laboratory, Beltsville (Maryland 20705, USA), **European Parasite Research, Béhoust (France), and ***Beneficial Insects Research Laboratory, Newark (Delaware 19713, USA), 13 November 1985*

Summary. The hyperparasitic ichneumonid wasp, *Mesochorus nigripes,* from Southern Sweden was found to have an over-all heterozygosity (H_a) of 0.187 based on allele frequencies of 12 loci in 10 enzyme systems. This high level of genetic heterozygosity has not been reported in any hymenopteran species.
Key words. Mesochorus; Hymenoptera; Ichneumonidae; electrophoresis; heterozygosity.

Over the last 20 years it has become clear that natural populations of animals possess a wealth of genetic polymorphism. However, very low levels of electrophoretic variation have been found in hymenopteran species. Some species even lack variation altogether[3,4]. Several hypotheses concerning these low levels of intraspecific genetic variability in Hymenoptera have been proposed[5–10]. We report here a case of genetic heterozygosity of a much higher level than previously reported for any hymenopteran species.

The ichneumonid wasp, *Mesochorus nigripes,* is a well-known hyperparasite of two other ichneumonid wasps, *Bathyplectes curculionis* and *B. stenostigma.* These two *Bathyplectes* wasps are themselves larval parasites of the alfalfa weevil *Hypera postica.* Three trips were made to Skurup in Southern Sweden to collect larvae of the alfalfa weevil from which the *Bathyplectes* parasites and *M. nigripes* hyperparasites would emerge. Rearing experiments indicated that this population was arrhenotokous[11]. A total of 92 females and 14 males of *M. nigripes* were obtained and stored at −65 °C for use in isozyme analysis. Starch gel electrophoretic techniques and staining methods are described elsewhere[11]. The following enzyme systems were examined: aldehyde oxidase (AO), esterase (EST), alpha-glycerophosphate dehydrogenase (GPDH), hexokinase (HK), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and superoxide dismutase (SOD). Two buffer systems of Siciliano and Shaw[12] were used: the tris-citrate buffer system for HK, LAP, LDH, MDH, ME and SOD; and the tris-versene-borate buffer system for AO, EST, GPDH, IDH, PGI and PGM.

Although we originally planned to survey 12 enzyme systems, no usable gels were obtained for EST and LAP. Variations were observed in AO-1, EST and GPDH-1 gels, but they could not be clearly scored, and were thus excluded from the analysis. With the exception of HK-2 locus, the genetic basis of observed isozyme variation was confirmed by examining the electromorphs of 11 haploid males (hemizygote pattern). The gels for HK-2 locus in males could not be scored.

Allele frequencies for the 12 loci that could be scored are listed in the table with the results partitioned into the P, H_p, and H_a components of Pamilo and Crozier[8]. As shown in the table, the level of electrophoretic variation in *M. nigripes* is much higher than any hymenopteran species reported in the literature[5,6]. The highest level of heterozygosity in Hymenoptera other than Ichneumonidae was found in the ant, *Conomyrma bicolor,* with $H_a = 0.100$ based on 9 loci[5]. The greatest degree of genetic polymorphism reported among insects is that of the tobacco budworm, *Heliothis virescens,* having an average H value of 0.389[13,14].

So far, only 7 species of ichneumonid wasps have been studied electrophoretically. The proportion of polymorphic loci (P) in

Allele frequencies for 12 enzyme loci in *Mesochorus nigripes* (based on female wasps)

AO-2	GPDH-2	HK-1	HK-2	IDH-1	IDH-2
N = 69 1.000	N = 72 1.000	N = 52 0.337	N = 56 0.411	N = 67 1.000	N = 67 1.000
$h = 0$	$h = 0$	0.634	0.553	$h = 0$	$h = 0$
		0.029	0.036		
		$h = 0.483$	$h = 0.524$		

LDH	MDH	ME	PGI	PGM	SOD
N = 83 0.813	N = 91 0.368	N = 84 1.000	N = 57 0.079	N = 87 0.017	N = 77 0.130
0.187	0.632	$h = 0$	0.921	0.948	0.870
$h = 0.304$	$h = 0.466$		$h = 0.146$	0.035	$h = 0.226$
				$h = 0.100$	

Proportion of polymorphic loci, P = 0.583. Gene diversity at polymorphic loci, $H_p = 0.321 \pm 0.065$ SE. Gene diversity over all loci, $H_a = 0.187 \pm 0.060$ SE.