Genetica 75: 3–9, 1987

Analysis of microdifferentiation in a Spanish cellar population of *Drosophila melanogaster*

M. Aguadé & L. Serra
Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Spain

Received 18.10.1985 Accepted 6.6.1986

Abstract

Variation in *Adh* and *αGpdh-1* gene frequencies has been used to check for microdifferentiation in Spanish samples of *Drosophila melanogaster* inside and outside a wine cellar. Flies were collected after vintage and after overwintering respectively; within each period samples were taken on up to five consecutive days each month. Variation of gene frequencies of *Adh* and *αGpdh-1* can be considered random when samples collected each month are taken into account. When mean monthly frequencies are considered, *αGpdh-1* does not show any significant variation all over the year; yet, variation of the frequency of Adh^S shows a cyclical pattern, its frequency being maximum at the end of the summer and minimum after overwintering. Due to the parallel change of the frequency of the inversion *In(2L)t* and the Adh^S allele, no decision can be made whether the Adh locus itself or the inversion are responsible for the changes.

Introduction

When collecting flies in nature drosophilists have often assumed that flies obtained from a given restricted area are members of a single panmictic unit. There is, however, some experimental evidence which does not support this assumption. In fact, Wallace (1966) detected some microdifferentiation in tropical populations of *Drosophila melanogaster* when studying lethal allelism. Krimbas and Alevizos (1973) also found evidence for spatial microdifferentiation in a Greek population of *Drosophila subobscura* when using inversion polymorphism as a genetic marker and so did Cabrera et al. (1985) in that same species when using the *Adh* locus as a marker. When cellar populations of *Drosophila melanogaster* are considered, the cellar population is often thought to be a single panmictic unit. One of the major features differentiating the cellar and its surroundings is the concentration of ethanol fumes; being ethanol fumes a possible selective agent to maintain *Adh* polymorphism, different authors have compared samples taken inside and outside the cellar and contradictory results have been reported (Briscoe *et al.*, 1975; McKenzie & McKechnie, 1978).

In our previous collections of flies in Spanish cellars (Aguadé & Serra, 1980), a general feature emerged, namely their environmental heterogeneity, which made us think of a possible microdifferentiation inside the cellar. We have therefore tried to detect microdifferentiation both inside and outside the cellar in two periods of the year thought to be important for population structure – after vintage and after overwintering. To trace microdifferentiation we have chosen two enzyme loci – *Adh* and *αGpdh-1* –, both because of their differential relationship to ethanol and their different degree of association with inversion *In(2L)t*. In addition, a polygenic trait – maximum wing length – was studied (data will be published separately).

Material and methods

A cellar located some 70 km northwest of Barcelona – "Cooperativa Vinícola d'Artés" – was chosen for its environmental heterogeneity and the relative high frequency of the slow allele of *Adh* found in its populations (Aguadé & Serra, 1980; Serra & Aguadé, 1982). Samples were taken in two different periods – after vintage and after overwintering –. Within each period samples were taken during up to five consecutive days each month (early October, November and December 1979 and early June and July 1980 for the second). Each day flies were collected at different sites inside and ouside the cellar. Initially flies were taken at a filtering machine (ma), near the grape press (pr), on pomost (remains of pressed grapes) outside the cellar (po) and on pomost kept in a small building close to the cellar itself (pi). Some of these locations had to be changed during the project because no flies could be collected on them. A new sampling location was added in exchange – a leaking barrel (ba). In December due to general scarcity of flies, samples were collected on a single day. Flies were caught directly with a sucking device. No traps were used in order to avoid disturbance of microdifferentiation.

Specimens were classified upon arrival at the laboratory. Whenever possible the number of *Drosophila melanogaster* individuals analyzed per location and date was eighty, although this number could not always be attained. Males were used preferentially and females were only added to the sample whenever necessary to achieve the aimed sample number (homogeneity of gene frequencies between both sexes has been previously proven for both enzyme loci). Wild caught males or females were electrophoresed on starch gel and their genotype for *Adh* and *αGpdh-1* established by differential staining of the gel (Ursprung & Leone, 1965; Grell, 1967).

Results and discussion

Genotypic frequencies

Genotypic frequencies were obtained directly from electrophoretic patterns of *Adh* and *αGpdh-1* (Table 1). Goodness-of-fit to random-mating proportions was tested for each locus independently (chi-squared values given in Table 2). For *Adh,* seven tests out of fifty-four show a significant departure from equilibrium and for *αGpdh-1* five out of fifty-four. Within the significant cases there is no regularity for any particular location or date.

When data from different panmictic units are pooled, an excess of homozygotes is expected (Wahlund's effect). Genotypic frequencies from samples obtained each day in different locations of the cellar were pooled and the pooled data tested for a possible departure from Hardy-Weinberg equilibrium. Only four of the eighteen tests performed for *Adh* evidenced a significant excess of homozygotes and only two out of eighteen did so for *αGpdh-1* (Table 3). In all cases the excess of homozygotes can be explained by a significant departure from Hardy-Weinberg equilibrium from at least one of the pooled sets of data. One could therefore talk of a generalized absence of Wahlund's effect, which could be considered as a first indication of the cellar being a single panmictic unit as far as the two loci studied are concerned.

Gene frequencies

More than 4500 flies were assayed for *Adh* and *αGpdh-1* from collections made in 1979 and 1980. Gene frequencies for each collecting day and site are given in Table 1.

Homogeneity of gene frequencies for samples collected each month was tested by means of a non-balanced analysis of variance with the two variable site and data fixed (BMDP, 4V). No significant influence either of site or date was observed (Table 4), which would rule out any microdifferentiation in the population. In December, homogeneity of gene frequencies has been assessed by means of a χ^2 test (for *Adh* $\chi^2_1 = 0.57$, for *αGpdh-1* $\chi^2_1 = 3.22$). When considering *Adh*, this homogeneity of gene frequencies for sites inside and outside the cellar and both for vintage and non-vintage periods would be in agreement with data by McKenzie and McKechnie (1978) and by Oakeshott and Gibson (1981). These authors did not find any difference in gene frequencies for *Adh* between samples taken inside and out-

Table 1. Gene frequency estimates of samples collected in different locations inside and outside the cellar (number of individuals in parentheses). Abbreviations: Ma, filtering machine; Pr, grape press; Pi, pomost in a small building; Po, pomost outside the cellar; Ba, leaking barrel.

Month	Day	Ma	Pr	Pi	Po	Ba
Adh[5]						
October	1	0.279(95)	0.265(97)	0.240(77)	0.266(31)	
	2	0.210(62)	0.150(10)	0.244(88)	0.274(32)	
	3	0.298(67)	0.233(75)	0.134(82)	0.313(32)	
	4	0.186(51)	0.333(81)	0.222(81)	0.159(41)	
	5	0.223(92)	0.150(90)	0.237(78)	0.277(83)	
November	1	0.146(82)	0.173(84)	0.155(90)		0.237(78)
	2	0.227(86)	0.310(100)	0.193(106)		0.190(87)
	3	0.188(88)	0.204(81)	0.189(90)		0.135(89)
	4	0.199(78)	0.293(82)	0.207(87)		0.163(89)
December	1	0.228(46)				0.193(166)
June	1	0.145(100)				0.117(90)
	2	0.165(100)				0.134(138)
	3	0.139(97)				0.149(87)
	4	0.115(126)				0.125(84)
July	1	0.097(98)				0.194(98)
	2	0.170(97)				0.194(97)
	3	0.250(117)				0.188(117)
	4	0.176(88)				0.177(88)
α-GPDH[5]						
October	1	0.552(86)	0.461(51)	0.558(77)	0.518(27)	
	2	0.450(80)	0.600(10)	0.563(79)	0.586(29)	
	3	0.440(67)	0.493(75)	0.507(77)	0.328(30)	
	4	0.560(50)	0.422(81)	0.512(81)	0.634(41)	
	5	0.414(87)	0.500(90)	0.500(78)	0.470(83)	
November	1	0.531(82)	0.542(87)	0.556(90)		0.436(78)
	2	0.448(86)	0.480(100)	0.509(107)		0.477(87)
	3	0.483(88)	0.438(81)	0.533(90)		0.517(89)
	4	0.487(78)	0.421(82)	0.506(87)		0.449(89)
December	1	0.348(46)				0.508(63)
June	1	0.475(100)				0.450(90)
	2	0.535(100)				0.522(138)
	3	0.531(97)				0.500(87)
	4	0.512(126)				0.547(84)
July	1	0.541(98)				0.449(98)
	2	0.474(97)				0.500(90)
	3	0.521(117)				0.547(85)
	4	0.517(88)				0.459(110)

Table 2. X^2 values (with one degree of freedom) for goodness-of-fit to random-mating proportions. See Table 1 for abbreviations.

Month	Day	Adh					α-Gpdh-1				
		Ma	Pr	Pi	Po	Ba	Ma	Pr	Pi	Po	Ba
October	1	8.17**	1.28	0.12	0.19		4.32*	1.17	0.84	1.80	
	2	0.95	3.69	0.19	1.44		2.21	0.28	1.97	0.68	
	3	0.36	3.54	5.77*	0.52		0.25	0.01	1.05	0.20	
	4	0.51	0.25	0.41	0.00		0.15	3.53	0.32	5.59*	
	5	2.40	6.71*	1.02	7.42**		0.16	1.90	0.05	0.08	
November	1	8.23**	3.64	0.43		0.75	1.68	0.27	0.57		0.29
	2	2.52	0.25	0.00		0.62	1.46	0.26	1.57		0.01
	3	0.40	0.06	2.32		0.12	0.42	0.42	0.35		0.01
	4	0.00	0.27	3.17		1.13	0.05	1.27	0.35		4.63*
December	1	0.11				0.17	0.08				1.92
June	1	1.40				5.65*	1.90				0.01
	2	0.41				5.03*	0.06				0.69
	3	0.34				0.28	0.91				0.01
	4	0.04				0.07	1.99				1.53
July	1	0.29				0.03	0.90				0.01
	2	1.61				0.02	1.68				1.11
	3	2.77				1.21	11.61***				5.93*
	4	0.96				1.77	1.16				0.70

* $0.01 < P < 0.05$
** $0.001 < P < 0.01$
*** $P < 0.001$

side the cellar, although they observed a significant difference in ethanol tolerance. In contradiction, Briscoe *et al.* (1975) found that the frequency of the slow allele of *Adh* is higher in a neighbouring population than in the cellar.

This very homogeneity allowed us to pool the data and estimate mean gene frequencies for each month (Table 5). Variation in *αGpdh-1* throughout the year can be considered random, ($\chi^2_4 = 3.948$). For *Adh*, however, there are significant differences not only when a general test yearround is performed ($\chi^2_4 = 72.428$) but also when any two consecutive sampling months are compared – except November vs. December – (Table 6).

If we consider for *Adh* three additional samples collected in the same cellar early October 1978, late May 1979 (Aguadé & Serra, 1980) and early October 1980 (unpublished data), a cyclical change in gene frequencies becomes apparent (Fig. 1) – the frequency of Adh^S is minimum just after winter and maximum at the end of the summer during vintage. This seasonal change would be in agreement with already published data on latitudinal (Pipkin *et al.*, 1973; Johnson & Schaffer, 1973; Vigue & Johnson, 1973; Voelker *et al.*, 1978; Oakeshott *et al.*, 1982) and altitudinal clines (Malpica & Vassallo, 1980), as well as with the kinetic properties of both alleles with regard to temperature (Johnson & Powell, 1974). The common conclusion of all these studies is that the slow allele of *Adh* seems to have a selective advantage at higher temperatures. When shifting from a spatial to a temporal dimension, one would expect an increase – if any – in the frequency of the slow allele in the warmer months and a decrease at the end of the summer. Both Johnson and Burrows (1976) and Langley *et al.* (1977) found such an increase in the frequency of Adh^S from spring to winter when surveying repeatedly the same population. This is in agreement with the before cited expectations and with our results. Yet, Cavener and Clegg (1981) found

Table 3. χ^2 values (with one degree of freedom) for goodness-of-fit to random-mating proportions when all samples are pooled (number of individuals in parentheses).

Month	Day	Adh		α-Gpdh-1	
October	1	2.806	(254)	6.713**	(241)
	2	0.437	(191)	0.303	(178)
	3	9.364**	(256)	1.243	(251)
	4	0.653	(254)	2.691	(253)
	5	1.021	(343)	0.087	(338)
November	1	4.404*	(334)	0.553	(334)
	2	1.056	(379)	0.040	(379)
	3	0.147	(348)	0.182	(348)
	4	0.619	(336)	3.159	(336)
December	1	0.001	(109)	2.253	(109)
June	1	10.265**	(198)	0.603	(198)
	2	6.281	(238)	0.225	(238)
	3	1.705	(184)	0.366	(184)
	4	3.084	(210)	3.553	(210)
July	1	0.007	(196)	0.739	(196)
	2	1.919	(187)	0.044	(187)
	3	2.010	(197)	17.473***	(202)
	4	2.767	(198)	1.673	(198)

* $0.01 < P < 0.05$
** $0.001 < P < 0.01$
*** $P < 0.001$

homogeneity of gene frequencies for *Adh* and *αGpdh-1* when surveying a North American population for a period of two years.

One should point out the existence of a strong association of alleles Adh^S and $\alpha Gpdh\text{-}1^F$ with inversion *In(2L)t* and also some relevant differences between North American and Spanish cellar

Table 5. Monthly mean frequencies.

Month, year	Adh^S	$\alpha\text{-}Gpdh^S$
October 1979	0.235 ± 0.008	0.501 ± 0.010
November 1979	0.201 ± 0.007	0.490 ± 0.009
December 1979	0.201 ± 0.019	0.450 ± 0.066
June 1980	0.136 ± 0.008	0.510 ± 0.012
July 1980	0.167 ± 0.009	0.500 ± 0.013

Table 6. Homogeneity χ^2 values (with one degree of freedom) for *Adh* frequencies when two consecutive sampling months are compared.

October *vs.* November	9.247**
November *vs.* December	1.69×10^{-4}
December *vs.* June	11.212***
June *vs.* July	6.345**
July *vs.* October	27.009***

* $0.01 < P < 0.05$
** $0.001 < P < 0.01$
*** $P < 0.001$

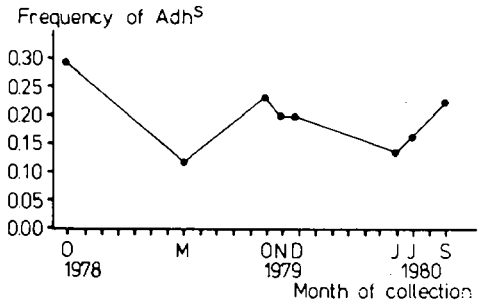

Fig. 1. Monthly gene frequencies.

Table 4. Analysis of variance of gene frequencies (using angular transformants).

Month	*Adh*						*α-Gpdh-1*					
	Day			Place			Day			Place		
	F	d.f.	Prob	F	d.f.	Prob	F	d.f.	Prob	F	d.f.	Prob
October	0.20	(4,12)	0.934	0.25	(3,12)	0.860	1.63	(4,12)	0.231	0.37	(3,12)	0.774
November	1.64	(3,9)	0.249	1.97	(3,9)	0.189	1.40	(3,9)	0.306	2.08	(3,9)	0.173
June	1.37	(3,3)	0.402	0.70	(1,3)	0.464	6.11	(3,3)	0.086	0.63	(1,3)	0.487
July	0.67	(3,3)	0.624	3.57	(1,3)	0.155	0.56	(3,3)	0.676	0.68	(1,3)	0.471

populations. Voelker *et al.* (1978) concluded that only 0.23 of the cline of Adh^S present on the east coast is due to association with *In(2L)t*, the remainder due to a cline in the frequency of Adh^S in the standard chromosome. Oakeshott *et al.* (1982) and Knibb (1983), on the other hand, stated that in Australia even a lower percentage of the Adh^S cline can be accounted for by the inversion. Samples collected in Artés in October 1978 and May 1979 – as well as samples collected in other Spanish cellars (Aguadé & Serra, 1980) – show a slightly different pattern. In fact, Adh^S shows the general strong association with *In*(2L)t, its conditional frequency within the inversion being close to 1.0, but in contrast with the American populations the frequency of Adh^S within the standard gene arrangement is very low, 0.08 and 0.0 respectively for the above mentioned collections in Artés. Even without direct information about inversions, there is no reason to believe that the situation has changed and one would expect a parallel change in the frequency of *In(2L)t* and Adh^S.

The assumed association between *In(2L)t* and Adh^S renders us unable to attribute the observed seasonal changes in the frequency of *Adh* either to the locus itself or to the inversion. The absence of a similar cyclical change in the frequency of $\alpha Gpdh\text{-}1^F$, which also shows association with the inversion and which is located within the inversion, does not help at all. The reason is first that the association with the inversion is weaker and second that the frequency of $\alpha Gpdh\text{-}1^F$ is higher. Both these circumstances make that the frequency of $\alpha Gpdh\text{-}1^F$ does not follow the frequency of inversion *In(2L)t*.

Acknowledgements

Thanks are given to "Cooperativa Vinícola d'Artés" for allowing us into its facilities. Thanks are given to professor D. Sperlich for critically reading the manuscript.

References

Aguadé, M. & Serra, L., 1980. Spanish cellar populations of Drosophila melanogaster. I. Study of variability at three different levels: quantitative, chromosomal and molecular. Genetika (Yugoslavia) 12(2): 111–120.

Briscoe, D. A., Robertson, A. & Malpica, J. M., 1975. Dominance at Adh locus in response of adult Drosophila melanogaster to environmental ethanol. Nature 255: 148–149.

Cabrera, V. M., González, A. M., Hernández, M., Larruga, J. & Martell, M., 1985. Microgeographical and temporal genetic differentiation in natural populations of Drosophila subobscura. Genetics 110: 247–256.

Cavener, D. R. & Clegg, M. T., 1981. Temporal stability of allozyme frequencies in a natural population of Drosophila melanogaster. Genetics 98: 613–623.

Grell, E. H., 1967. Electrophoretic variants of α-glycerophosphate dehydrogenase in Drosophila melanogaster. Science 158: 1319.

Johnson, F. M. & Powell, A., 1974. The alcoholdehydrogenase Drosophila melanogaster: frequency changes associated with heat and cold shock. Proc. natn. Acad. Sci. U.S.A. 71: 1783–1784.

Johnson, F. M. & Burrows, P. M., 1976. Isozyme variability in species of the genus Drosophila. VIII. The alcohol dehydrogenase polymorphism in North Carolina populations of Drosophila melanogaster. Biochem. Genet. 14: 47–58.

Johnson, F. M. & Schaffer, H. E., 1973. Isozyme variability in species of the genus Drosophila. VII. Genotype-environment relationships in populations of Drosophila melanogaster from the eastern United States. Biochem. Genet. 10: 149–163.

Knibb, W. R., Chromosome inversion polymorphism in Drosophila melanogaster. III. Gametic disequilibrium and the contributions of inversion clines to the Adh and Gpdh-1 allozyme clines in Australasia. Genetica 61: 139–146.

Krimbas, C. B. & Alevizos, V., 1973. The genetics of Drosophila subobscura populations. IV. Further data on inversion polymorphism in Greece – evidence of microdifferentiation. Egyp. J. Genet. Cyt. 2: 121–132.

Langley, C. H., Ito, K. & Voelker, R. A., 1977. Linkage disequilibrium in natural populations of Drosophila melanogaster. Seasonal variation. Genetics 86: 447–454.

Malpica, J. M. & Vassallo, J. M., 1980. A test for the selective origin of environmentally correlated allozyme patterns. Nature 286: 407–408.

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

Oakeshott, J. G. & Gibson, J. B., 1981. Is there selection by environmental ethanol in the alcohol dehydrogenase locus in Drosophila melanogaster ?. In: Genetic Studies of Drosophila populations, ed. J. B. Gibson & J. G. Oakeshott, pp. 103–120. Australian University Press.

Oakeshott, J. G., Gibson, J. B., Anderson, P. R., Knibb, W. R., Anderson, D. G. & Chambers, G. K., 1982. Alcohol de-

hydrogenase and glycerol-3-phosphate dehydrogenase clines in Drosophila melanogaster in different continents. Evolution 36: 86–96.

Pipkin, S. B., Rhodes, C. & Williams, N., 1973. Influence of temperature on Drosophila alcohol dehydrogenase polymorphism. J. Hered. 64: 181–185.

Serra, L. & Aguadé, M., 1982. Heterozygosity and variation for a quantitative trait. Advances in Genetics, Development and Evolution of Drosophila, pp. 309–317. Plenum Press.

Ursprung, H. & Leone, J., 1965. Alcohol dehydrogenase: a polymorphism in Drosophila melanogaster. J. exp. Zool. 160: 147.

Vigue, C. L. & Johnson, F. M., 1973. Isozyme variability in species of the genus Drosophila. VIII. Frequency–Property–Environment. Relationships of allelic alcoholdehydrogenases in Drosophila melanogaster. Biochem. Genet. 9: 213–227.

Voelker, R. A., Cockerham, C. C., Johnson, F. M., Schaeffer, H. E., Mukai, T. & Mettler, L. E., 1978. Inversions fail to account for allozyme clines. Genetics 88: 515–527.

Wallace, B., 1966. Distance and the allelism of lethals in a tropical population of Drosophila melanogaster. Am. Nat. 180: 565–578.