

Eisses, K.Th., and M. Santos. Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, 08193 Bellaterra (Barcelona), Spain. E-mail: eisses@cc.uab.es Short distance differences in ADH and alphaGPDH allozyme polymorphisms and linkage disequilibrium in *D. melanogaster* in Spanish desert populations.

Understanding why latitudinal clines exist for various characteristics requires studies in areas where these clines are particularly steep. Spain lies in the center of the so-called Mediterranean "instability" with respect to the *Adh* cline (David and Capy, 1988). Several Spanish studies on *Adh* allele frequencies within relatively short distances or short periods of time demonstrate their high variability (Malpica and Vassallo, 1980; Aguadé and Serra, 1980, 1987; Muñoz-

Serrano *et al.*, 1985; Alonso-Moraga and Muñoz-Serrano, 1986).

We captured flies from natural populations in an arid area in southeast Spain without wine cellars nearby. Five locations (Figure 1) near Carboneras (37°00'N; 1°53'W) were sampled during five days with banana traps. Agua Amarga was sampled with decaying *Opuntia ficus-indica* fruits (prickly pears). Flies emerging from *O. ficus-indica* fruits, which were collected in the Rambla location and transferred to the laboratory, were subjected to electrophoresis as well.

The data presented in Tables 1 and 2 reveal rather high frequencies of *Adh*^S compared with *Adh*^S frequencies in France (David *et al.*, 1986), but much lower compared with Central America (Van 't Land *et al.*, 1993). We made a subdivision of the data collected in the location "Rambla", a semi-abandoned *O. ficus-indica* plantation (Table 2). The frequencies of *alpha-Gpdh*^F and *Adh*^S from different locations covaried in similar directions. A linear regression on both allele frequencies gave a correlation coefficient of $R^2 = 0.982$. We found evidence for linkage disequilibrium between the two alleles. Estimates of D were calculated based on the maximum likelihood method as proposed by Hill (1974). Although we did not measure the presence of the *In(2L)t* or other inversions in our populations, Spanish populations contain this inversion in high frequencies in very different regions (Aguadé and Serra, 1980; Sanchez-Refusta *et al.*, 1990; Taberner and González, 1991). In our populations the inversion might be present as well and responsible for covarying frequencies of *alpha-Gpdh*^F and *Adh*^S. The two populations with relatively high *Adh*^S and *alpha-Gpdh*^F frequencies, Sopalmo and Mesa Roldan, were captured in locations above 100 m altitude. The MR site (Figure 1) is an abandoned *O. ficus-indica* plantation in a plain-like environment, whereas the Sopalmo sites were in a small village in a mountain pass with some *O. ficus-indica* and shadow-rich fig trees. Trapped flies in the Rambla location seemed to have systematically slightly higher *Adh*^S and *alpha-Gpdh*^F frequencies than the flies emerging from prickly pears. Whether these differences in allele frequencies are due to sampling errors or to natural selection processes related to microclimatic differences remains to be analyzed. Some additional empirical evidence from another location pointed in the same direction. A small number of *D. melanogaster* emerging from collected prickly pears in Sopalmo revealed a much lower frequency of *Adh*^S than obtained with the trapped flies, 0.269 (n = 26) and 0.568 (n = 37), respectively, whereas the frequency of *alpha-Gpdh*^F was only a little lower, 0.750 and 0.770, respectively. The minimum and maximum temperatures as measured in a cactus tree near site A1 in the *O. ficus-indica* plantation in the Rambla location (Ruiz *et al.*, 1986) were close to the long term average data from the Carboneras weather station shown in Table 3. Nevertheless there is a lack of information on real-temperature profiles over the day at different sites on and above the surface within a variety of locations. In the Rambla location we found a large difference in numbers of trapped *D. melanogaster* between the front rows A and B and the back rows C and D (Table 2), whereas *D. simulans* were present in the back rows in high numbers. The sites C and D were higher up the slope of the plantation and closer to the mountain ridges. A similar difference was found in another plantation at a distance of approximately 1 km (Eisses, Laayouni, Leibowitz, Santos, Galiana and Fontdevilla, unpublished results). It sounds reasonable to assume that different microclimates act differently at the species level. The same may hold for the interactions of microclimate and a set of major gene-protein arrangements. Sunny and shady sites or differences in surface texture of the environment can have a large influence on very local temperatures at short distances (Stoutjesdijk and Barkman, 1992). Seasonal fluctuations in *In(2L)t* frequencies have been reported in a Spanish population caught near Oviedo with the highest frequencies in September-October (Sanchez-Refusta *et al.*, 1990). Experimental populations in a greenhouse and in the laboratory showed similar covarying frequencies of *alpha-Gpdh*^F, *Adh*^S and *In(2L)t* with temperature (Van Delden and Kamping, 1989). Such data point to a temperature effect on a set of genes more or less closely linked to *alpha-Gpdh*^F through the inversion *In(2L)t* and dragging *Adh*^S.

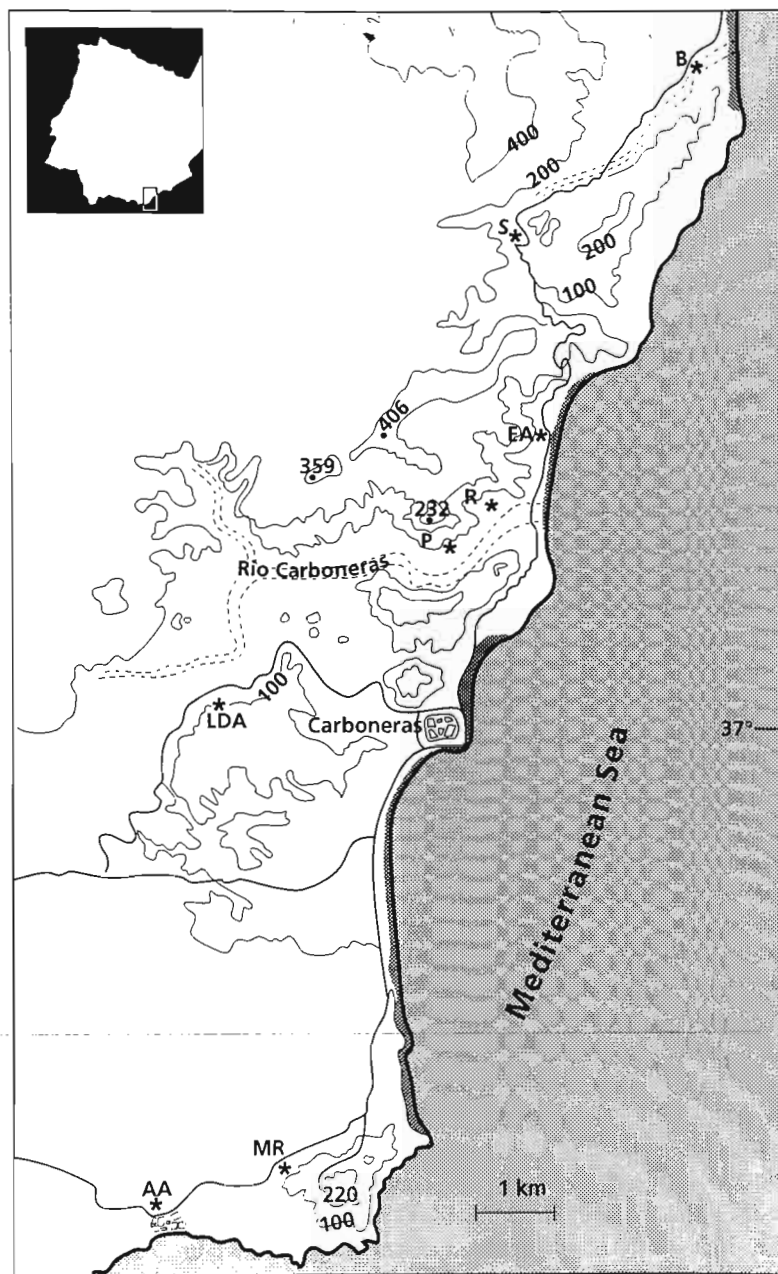


Figure 1. Locations of capture sites in the Carboneras area (37°00'N;1°54'W) is southeast Spain (see insert). B = Blanco, S = Sopalmo, R = Rambla, LDA = Llano Don Antonio, MR = Mesa Roldan, AA = Agua Amarga.

the ADH-Fast allozyme due to high alcohol concentrations within a wine cellar, the temperatures inside a cellar are supposedly much more constant and lower than in the field, especially in the summer. In a wine cellar population near Montemayor at high altitude, the Adh^F frequency stayed above 0.8 all year, whereas in a field population nearby large but covarying fluctuations in Adh^S (0.3-0.9) and $\alpha\text{-Gpdh}^F$ (0.55-0.95) frequencies occurred (Muñoz-Serrano *et al.*, 1985).

Acknowledgments: Drs. Jan van 't Land and Albert Kamping from the Department of Genetics, Rijksuniversiteit Groningen are thanked for providing the values of D by means of their LINKDIS program. These data were collected during a project funded by Contract No. CHRX-CT92-0041 from the Commission of the European Communities.

Some attention has been paid to the temperature constraint on body size of ectotherms as a consequence of the temperature dependency of biochemical reactions related to development and growth (Van der Have and De Jong, 1996). High temperature regimes result in small body sizes, short wings and high wingbeat frequencies, whereas low temperature regimes result in large body sizes, long wings and low wingbeat frequencies. As $\alpha\text{-GPDH}$ activity is directly coupled to flight muscle activity, it could well be that the presence of $\alpha\text{-GPDH}^F$ favours the mobility of small flies, because $\alpha\text{-GPDH}^{F/F}$ activity was higher than $\alpha\text{-GPDH}^{F/S}$ or $\alpha\text{-GPDH}^{S/S}$ activity, especially in an $ADH^{S/S}$ background (Oudman *et al.*, 1991). Selection for short wings gave a quick response in the increase of the $\alpha\text{-Gpdh}^F$ and Adh^S allele frequencies, in spite of the absence of the $In(2L)t$ inversion, whereas selection for long wings resulted in the increase of the $\alpha\text{-Gpdh}^S$ and Adh^F allele frequencies (Serra and Oller, 1984). Their results were discussed in the light of selection on a set of genes acting as a "supergene" surrounding the marker genes. However, these marker genes themselves might play a role as well. Comparison of wing lengths and $\alpha\text{-Gpdh}^F$, Adh^S and $In(2L)t$ frequencies of flies from an after-vintage population of *D. melanogaster* with an overwintering population showed longer wing lengths in the overwintering population in addition to considerably lower frequencies of these alleles and the $In(2L)t$ inversion (Aguadé and Serra, 1980). Apart from the selection pressure favoring flies or larvae with

Table 1. Alpha-GPDH and ADH allozyme combinations in trapped and emerged flies from samples taken in the Carboneras area (Almería, Spain) in September 1995. The first two characters point to alpha-GPDH dimers, and the other two to ADH dimers. D is an approximation of the linkage disequilibrium, based on gamete frequencies (Hill, 1974).

Location	Number	FF_FF	FF_FS	FF_SS	FS_FF	FS_FS	FS_SS	SS_FF	SS_FS	SS_SS	Adh ^s	Gpdh ^f	D
Trapped Flies													
Blanco	154	8	42	20	20	28	8	16	8	4	0.461	0.636	-0.07011*
Sopalmó	37	3	12	8	1	8	2	2	0	1	0.568	0.770	-0.05111
Rambla	259	23	50	35	30	69	22	11	15	4	0.494	0.651	-0.04438*
LlanoDonAntonio	82	5	7	6	21	22	6	7	8	0	0.372	0.518	-0.05879
Mesa Roldán	97	16	17	18	6	19	9	3	7	2	0.521	0.701	-0.00673
Agua Amarga	78	5	18	9	8	20	5	5	7	1	0.481	0.622	-0.05812
Total Trapped	707	60	146	96	86	166	52	44	45	12			
alpha-GPDH			302			304			101			0.642	-0.05311*
ADH		190	357	160							0.479		
Emerged Flies from <i>O. ficus-indica</i> fruits in Rambla location													
Total Emerged	1094	107	189	125	145	284	89	74	63	18			
alpha-GPDH			421			518			155			0.622	-0.04804*
ADH		326	536	232							0.457		

*significantly different from D = 0

Table 2. Alpha-GPDH and ADH allozyme combinations in samples of trapped and emerged flies from the Rambla location near Carboneras (Almería, Spain) in September 1995. The Rambla location has been subdivided in smaller areas (Ruiz *et al.*, 1986). Between brackets the number(s) of the sites are given.

Location	Number	FF_FF	FF_FS	FF_SS	FS_FF	FS_FS	FS_SS	SS_FF	SS_FS	SS_SS	Adh ^s	Gpdh ^f	D
Trapped Flies													
Subdivisions													
Rambla A (1-2)	129	6	25	21	14	36	12	6	8	1	0.531	0.643	-0.07601*
Rambla B (1-2)	113	13	22	11	16	29	7	5	7	3	0.442	0.637	-0.01802
Rambla C-D (1-2)	17	4	3	3	0	4	3	0	0	0	0.559	0.794	+0.09083
Total Rambla	259	23	50	35	30	69	22	11	15	4	0.494	0.651	-0.04438*
Emerged Flies													
Subdivisions													
Rambla A (13)	506	39	90	60	55	143	53	27	31	8	0.500	0.622	
Rambla A (E1)	30	5	1	6	2	7	0	3	3	3	0.483	0.550	
Rambla A (E2)	174	11	34	26	24	37	18	11	11	2	0.500	0.635	
Rambla A (E3)	38	2	2	5	8	10	2	1	4	4	0.500	0.500	
Total A (13-E3)	748	57	127	97	89	197	73	42	49	17	0.499	0.616	-0.04704*
Rambla B (14)	182	36	28	15	32	39	6	21	5	0	0.315	0.646	
Rambla C (15)	14	0	3	0	3	6	2	0	0	0	0.464	0.607	
Rambla C (E4)	41	7	3	7	5	11	4	2	2	0	0.463	0.670	
Rambla D (16)	109	7	28	6	16	31	4	9	7	1	0.404	0.610	
Total B-D (14-16)	346	50	62	28	56	87	16	32	14	1	0.366	0.634	-0.05731*

* significantly different from D = 0

References: Aguadé, M., and L. Serra 1980, *Genetika* (Beograd) 12:111-120; Aguadé, M., and L. Serra 1987, *Genetica* 75:3-9; Alonso-Moraga, A., and A. Muñoz-Serrano 1986, *Experientia* 42:1048-1050; David, J.R., and P. Capy 1988, *TIG* 4:106-111; David, J.R., H. Merçot, P. Capy, S.F. McEvey, and J. Van Herrewege 1986, *Génét. Sé. Evol.* 18:405-416; Hill, W.G., 1974, *Heredity* 33:229-239; Malpica, J.-M., and J.M. Vassallo 1980, *Nature* 286:407-408; Muñoz-Serrano, A., A. Alonso-Moraga, and A. Rodero 1985, *Genetica* 67:121-129; Oudman, L., W. van Delden, A. Kamping, and R. Bijlsma 1991, *Heredity* 67:103-115; Ruiz, A., A. Fontdevila, M. Santos, and E. Torroja 1986, *Evolution* 40:740-755; Sanchez-Refusta, F., E. Santiago, and J. Rubio 1990, *Genet. Sel. Evol.* 22:47-56; Serra, L., and J.M. Oller 1984, *Genetica* 63:39-47; Stoutjesdijk, Ph., and J. J. Barkman 1992, *Microclimate, vegetation and fauna*. Opulus Press; Taberner, A., and A. González 1991, *Heredity* 67:307-316; Van Delden, W., and A. Kamping 1989, *Evolution* 43:775-793; Van der Have, T. M., and G. de Jong 1996, *J. Theor. Biol.* 183:329-340; Van 't Land, J. Van Delden, and A. Kamping 1993, *Dros. Inf. Serv.* 72:102-104.

Table 3. Temperatures in °C at the Carboneras weather station "Central Termica" as measured from 1986 - 1995. Data from January are given, because it is the coldest month on the average.

Month	Mean temp.	Mean Max.	Mean Min.	Absol. Max.	Absol. Min.
June	22.3	25.6	18.9	34.5	9.5
July	25.5	28.5	22.4	41.6	12.5
August	26.5	29.6	23.3	39.3	16.3
September	23.8	26.9	20.7	33.6	9.4
October	19.8	23.1	16.5	32.5	9.4
November	16.6	19.8	13.3	28.6	5.3
December	14.0	17.1	10.8	23.9	5.0
January	12.4	15.8	8.9	24.4	-4.5

The mean year temperature over ten years was 18.7 °C.

Eisses, K.Th., and M. Santos. Universitat Autònoma de Barcelona, Departament de Genètica i de Microbiologia, 08193 Bellaterra (Barcelona), Spain. Hybrids between *Drosophila melanogaster* and *D. simulans* in a Spanish natural population.

The sibling species *Drosophila melanogaster* and *D. simulans* coexist in natural conditions in various abundances. The siblings are so closely related that it took a while until the two species were distinguished as such (Sturtevant, 1919). Interspecific matings can easily be provoked in laboratory conditions and used as a tool for studying processes of speciation (e.g., Davis *et al.*,

1996). We screened field populations for these species because of ecological and genetical reasons and used gel electrophoresis as a tool, which method revealed an approximation of the frequency of interspecific hybrids between *D. melanogaster* and *D. simulans*. Data about frequencies of such hybrids in natural populations are very scarce.

Flies were captured with mashed banana traps during five days in Carboneras (Almería, Spain; 37°00'N; 1°53'W) and other locations up to 10 km north and south (Eisses and Santos, 1997). *Opuntia ficus-indica* fruits (prickly pears) were put in an experimental design for almost seven days in a semi-abandoned *O. ficus-indica* plantation near Carboneras. After recollection of the fruits emerging flies were aspirated. Captured and emerged flies were checked for *D. melanogaster* morphology and frozen at -29°C until gel electrophoresis and staining for alcohol dehydrogenase (ADH; EC 1.1.1.1.), which is a diagnostic enzyme between *D. melanogaster* and *D. simulans* (Eisses, Van Dijk and Van Delden, 1979). Gel buffer and electrophoresis buffer were according to the system of Poulik (1957).

Figure 1 shows the dimeric hybrid enzyme band between the parental ADH-S band of *D. melanogaster* and the ADH-simulans band in the lane labelled with *. However, the hybrids resulting from a cross between *D. melanogaster* ADH-Fast and *D. simulans* did not show a dimeric hybrid enzyme band. In these cases only the parental ADH-bands were visible on the gels (not shown on photograph). We do not know whether these hybrid dimers do not exist or are inactive. Four female hybrids were found, three of them were ADH-Fast/ADH-simulans. One of the hybrids was found among 137 captured *D. melanogaster* flies in Llano de Don Antonio. One natural hybrid on a total of 425 captured females in the wider Carboneras area means a frequency of 2.4×10^{-3} . Three interspecific hybrid females among 1096 females, emerging from prickly pears in the Carboneras *O. ficus-indica* plantation in September 1995, means a frequency of 2.7×10^{-3} . *D. simulans* was approximately three times more abundant than *D. melanogaster* in this area of Spain in this time of the year (Eisses, Laayouni, Leibowitz, Santos and Fontdevila, unpublished results).

The frequency of hybrids in the Carboneras population with 2.7×10^{-3} was about 2.5 times lower than the frequency reported in a Barcelona population (Mensua and Pérez, 1977). Much higher frequencies have been reported for natural populations and ranged from 1 to 40% (Casares and Carracedo, 1985; Inoue *et al.*, 1990; Sperlich, 1962).