Allozyme variation in populations of *Cepaea nemoralis* from Northern Spain

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Allozyme frequencies at eleven gene loci have been tested electrophoretically in 46 populations of the land snail *Cepaea nemoralis* from the North of Spain. There is some geographic differentiation for molecular polymorphism which coincides to some small extent with the two main climatic regions in the Iberian peninsula, "green" and "brown" Iberia. However, there are no significant correlations between gene frequencies and climatic parameters. At least, part of this geographic change may have emerged through genetic drift, with a counterbalanced action of drift and climatic selection associated with a restricted gene flow, to promote a different genetic structure of *C. nemoralis* populations in each climatic region.

INTRODUCTION

The debate about the maintenance of the shell colour and banding polymorphism of *Cepaea nemoralis* extends to the allelic isozyme variation of this species. Striking geographic differences have been found for several isozyme systems (Johnson, 1976, 1979; Jones *et al.*, 1980; Caugant *et al.*, 1982; Ochman *et al.*, 1987; Mazón *et al.*, 1988) and have been described as "molecular area effects" (Ochman *et al.*, 1983) due to demographic and historic geographic fluctuations of the populations, more than to adaptation to local environment (Selander and Ochman, 1983). Genetic changes for isozymes have hence been thought to reflect mainly the action of genetic drift (Ochman *et al.*, 1987). These conclusions are based on the fact that, in most studies, the geographic changes in allozymes have a distribution pattern unrelated to that of the visible polymorphism (Caugant *et al.*, 1982; Ochman *et al.*, 1983; Jones *et al.*, 1980).

In the north of Spain, Mazón *et al.* (1987) found two large geographic areas in which visible polymorphisms matched the main climatic regions of the Iberian peninsula, the "green" and the "brown" Iberia. We (Vicario *et al.*, 1988) found a similar association in an adjacent zone, which suggested to us some kind of climatic selection pro-

moting the division. More recently, Mazón *et al.* (1988) found the same distribution in a study of polymorphism in 19 isozyme systems, and considered the coincidence as an argument in favour of differentiation into two geographic races due to the effect of climatic selection.

Here we test the generality of the findings of Mazón *et al.*, and whether climatic selection promotes adaptive allozymic and morphological regional differentiation near the limit of the distribution of this species.

MATERIALS AND METHODS

Forty-six populations were sampled in North Spain, in an area 200 km by 60 km (fig. 1) (Vicario *et al.*, 1988), adjacent to the region studied by Mazón *et al.* (1987, 1988). The mean sample size was 54 snails and they were used for the electrophoresis of six enzyme systems with eleven putative loci: Saccharase or invertase (EC 3.2.1.26; SAC, 2 loci), esterases (EC 3.1.1.1: EST, 3 loci), glucose phosphate isomerase (EC 5.3.1.9; GPI, 1 locus), diaphorase (EC 1.6.2.2; DIA, 1 locus), phosphoglucomutase (EC 2.7.5.1; PGM, 3 loci) and superoxide dismutase (EC 1.15.1.1; SOD, 1 locus).

Electrophoresis was based on the techniques of Mazón *et al.* (1988) and staining methods were

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Figure 1 Area and localities sampled.

according to Selander *et al.* (1971) and Harris *et al.* (1976). A modification of the histochemical staining method for disaccharidases (Lojda, 1972) was employed for saccharase. Electrode buffer: TRIS 0.1 M, Boric 0.039 M, citric 0.034 M, LiOH

0.025 M, pH=7.4; gel buffer; electrode buffer diluted 1:9. Stain (for 25 ml. in 1 per cent agar gel): citrate-phosphate buffer 0.1 M pH=6.4, 21.5 ml.; 3,3'-diaminobenzidine (Sigma D-5637), 24 mg.; peroxidase (Sigma P-8735) 0.1 mg.;

Figure 2 Sample coordinates on axes 1 and 2 of the AFC. Numbers correspond to classes 1 to 4 constructed by hierarchical ordering. Squares or circles represent the location of the sample in green or brown Iberia respectively.

glucose oxidase (Sigma G-2133) 10 mg.; sucrose (4 per cent solution) 3.5 ml.; agar 250 mg.

RESULTS

Allele frequencies at the eleven loci studied are shown in table 1.

Genetic diversity at the loci analyzed is similar to that in other studies (Ochman *et al.*, 1983; Mazón *et al.*, 1988). Mean heterozygosity is, at $H = 0.284$, greater than that found in many molluscs ($H = 0.148$; Nevo, 1984), but lower than the value found in European populations of *C. nemoralis* ($H = 0.34$; Ochman *et al.*, 1987) using a different set of enzyme loci. *SAC1* and *PGM4* enzyme systems were monomorphic in all populations. *GPI* showed no variation in almost half of the samples.

Saccharase is an enzyme not previously studied in *Cepaea*. We have detected three isozyme systems and for the *SAC3* isozyme Mendelian inheritance and codominance between six alleles has been established (Vicario, 1987).

A Correspondence Factorial Analysis (CFA) (Lebart *et al.*, 1977), based on the χ^2 distance of the more frequent alleles in the populations, shows, in a diagram of the populations' coordinates on the first two axes (fig. 2), an ill-defined grouping of populations by means of their location in green or brown Iberia. However, employing the first six factorial axes, which explain 79.14 per cent of the variance, a hierarchical ordering of the populations may group them in four classes (fig. 3). Classes 1 and 3, consisting of a small number of populations, six and three respectively, are characterised by a high frequency of the *SAC3-B* allele (class 1) and the *EST-C* allele (class 3). Both lack common geographic characteristics and might represent sampling errors. The groups formed by classes 2 and 4 are interesting. In class 4, four populations, Gumiel (43), Ciruelos (39), Espeja (40) and Barbadillo (34), belong to brown Iberia and fifteen to green Iberia. All populations included in class 2 belong to brown Iberia, except La Molina (19) that is located near the boundary between both climatic regions. Class 2 is characterized by high frequencies of the *SOD-B*, *DIA-A*, *DIA-C* and

Table 1 Main allele frequencies for nine polymorphic enzyme loci. *ESTF-C*, *ESTF-D*, *GPI-C*, *DIA-D*, *PGM1-C* and *PGM2-L* alleles have been omitted as their frequencies are zero in most populations

Localities	<i>SAC3-A</i>	<i>SAC3-B</i>	<i>SAC3-C</i>	<i>SAC3-D</i>	<i>SAC3-E</i>	<i>SAC3-F</i>	<i>ESTF-A</i>	<i>ESTF-B</i>	<i>ESTE-A</i>	<i>ESTE-B</i>	<i>ESTC-A</i>
1 Ajo	0.142	0.000	0.302	0.028	0.000	0.528	0.037	0.963	0.213	0.787	0.019
2 Solorzano	0.028	0.093	0.231	0.435	0.009	0.204	0.454	0.546	0.241	0.759	0.130
3 Aranguren	0.056	0.009	0.519	0.093	0.000	0.324	0.222	0.778	0.500	0.500	0.519
4 Valle	0.111	0.176	0.426	0.231	0.000	0.056	0.358	0.642	0.102	0.898	0.028
5 Nava	0.302	0.047	0.340	0.057	0.000	0.255	0.330	0.670	0.113	0.887	0.406
6 Vivanco	0.333	0.094	0.417	0.156	0.000	0.000	0.272	0.728	0.262	0.738	0.357
7 Estacas	0.151	0.047	0.406	0.085	0.066	0.245	0.083	0.917	0.417	0.583	0.657
8 Villabasil	0.256	0.128	0.308	0.192	0.026	0.090	0.272	0.728	0.287	0.713	0.009
9 Castrobarto	0.050	0.030	0.780	0.000	0.010	0.130	0.280	0.720	0.255	0.745	0.500
10 Fresno	0.057	0.019	0.651	0.264	0.000	0.009	0.298	0.702	0.198	0.802	0.373
11 Puntidas	0.113	0.009	0.226	0.377	0.000	0.274	0.321	0.679	0.408	0.592	0.593
12 Rio Losa	0.098	0.049	0.529	0.186	0.000	0.137	0.292	0.708	0.274	0.726	0.594
13 Villamor	0.198	0.000	0.462	0.151	0.113	0.057	0.150	0.850	0.297	0.703	0.444
14 Ciales	0.083	0.231	0.259	0.148	0.000	0.278	0.221	0.779	0.537	0.463	0.361
15 Medina	0.051	0.000	0.755	0.000	0.000	0.194	0.389	0.611	0.359	0.641	0.361
16 Villalain	0.106	0.183	0.212	0.288	0.144	0.067	0.202	0.798	0.028	0.972	0.602
17 Frias	0.128	0.038	0.590	0.154	0.000	0.090	0.232	0.768	0.488	0.512	0.722
18 Barcina	0.083	0.231	0.259	0.213	0.056	0.157	0.147	0.853	0.106	0.894	0.511
19 La Molina	0.102	0.148	0.611	0.093	0.000	0.046	0.250	0.750	0.167	0.833	0.509
20 Mazorra	0.074	0.037	0.315	0.009	0.000	0.565	0.311	0.689	0.352	0.648	0.361
21 Terrazos	0.058	0.106	0.356	0.231	0.038	0.212	0.596	0.404	0.048	0.952	0.425
22 Quintanaurria	0.137	0.118	0.706	0.020	0.000	0.020	0.461	0.539	0.010	0.990	0.304
23 Quintanilla	0.000	0.056	0.917	0.028	0.000	0.000	0.111	0.889	0.271	0.729	0.284
24 Hontomin	0.274	0.104	0.170	0.453	0.000	0.000	0.557	0.443	0.435	0.565	0.444
25 Castil	0.102	0.074	0.796	0.028	0.000	0.000	0.243	0.757	0.093	0.907	0.316
26 Villafranca	0.074	0.546	0.278	0.074	0.019	0.009	0.287	0.713	0.176	0.824	0.389
27 Vivar	0.067	0.288	0.510	0.087	0.029	0.019	0.278	0.722	0.056	0.944	0.575
28 Arlanzon	0.019	0.093	0.593	0.250	0.028	0.019	0.250	0.750	0.265	0.735	0.260
29 Modubar	0.000	0.000	0.861	0.130	0.009	0.000	0.425	0.575	0.260	0.740	0.167
30 Madrigalejo	0.048	0.269	0.644	0.029	0.000	0.010	0.363	0.598	0.397	0.603	0.481
31 Mazueco	0.038	0.000	0.558	0.317	0.000	0.087	0.283	0.717	0.179	0.821	0.806
32 Hoyuelo	0.029	0.125	0.519	0.298	0.029	0.000	0.189	0.811	0.377	0.623	0.057
33 Villangomez	0.176	0.065	0.361	0.389	0.000	0.009	0.292	0.708	0.279	0.721	0.300
34 Barbadillo	0.225	0.000	0.510	0.245	0.000	0.020	0.380	0.620	0.367	0.633	0.290
35 Lerma	0.031	0.260	0.375	0.167	0.000	0.167	0.203	0.797	0.368	0.632	0.407
36 Sto. Domingo	0.028	0.185	0.398	0.287	0.037	0.065	0.264	0.736	0.608	0.392	0.108
37 Rabanera	0.056	0.694	0.083	0.120	0.009	0.037	0.308	0.692	0.255	0.745	0.132
38 La Gallega	0.000	0.520	0.330	0.000	0.000	0.150	0.157	0.843	0.139	0.861	0.139
39 Ciruelos	0.132	0.000	0.302	0.509	0.000	0.057	0.443	0.557	0.213	0.787	0.194
40 Espeja	0.000	0.000	0.565	0.231	0.000	0.204	0.000	1.000	0.235	0.765	0.156
41 Tubilla	0.096	0.250	0.481	0.096	0.000	0.077	0.417	0.583	0.368	0.632	0.500
42 Caleruega	0.000	0.000	0.815	0.102	0.009	0.074	0.389	0.611	0.283	0.717	0.481
43 Gumiel	0.183	0.144	0.269	0.240	0.067	0.096	0.260	0.740	0.330	0.670	0.287
44 Coruna	0.075	0.198	0.283	0.198	0.038	0.208	0.167	0.823	0.139	0.861	0.245
45 Aranda	0.028	0.160	0.443	0.236	0.019	0.113	0.058	0.942	0.056	0.944	0.361
46 Penaranda	0.260	0.048	0.260	0.202	0.010	0.221	0.274	0.726	0.231	0.769	0.260

PGM1-B alleles, and *SOD-A* and *SAC3-A* are frequent in class 4.

DISCUSSION

The large number of alleles of the *SAC3* locus is characteristic of enzymes acting on an external substrate and their geographic variation may perhaps reflect their susceptibility to microgeographic

variation in the nature of their substrate in the snail's diet (Gillespie and Kojima, 1968). However, this variation does not seem to fit a geographic pattern.

A dendrogram based on the genetic distances, not presented here, would construct some geographic groups, but differing on the clear division in two zones for the visible and enzyme polymorphism described by Mazón *et al.* (1987, 1988). They are more similar to the geographic structuring

ESTC-B	ESTC-C	GPI-A	GPI-B	DIA-A	DIA-B	DIA-C	PGM1-A	PGM1-B	PGM2-A	PGM2-B	PGM2-C	SOD-A	SOD-B
0.824	0.157	1.000	0.000	0.100	0.700	0.200	0.958	0.042	0.677	0.226	0.097	0.130	0.870
0.769	0.102	0.657	0.000	0.056	0.750	0.139	0.940	0.060	0.725	0.275	0.000	0.377	0.623
0.481	0.000	1.000	0.000	0.038	0.764	0.198	0.944	0.056	0.362	0.638	0.000	0.685	0.315
0.943	0.028	0.778	0.000	0.269	0.546	0.185	1.000	0.000	0.274	0.453	0.274	0.769	0.231
0.594	0.000	1.000	0.000	0.278	0.422	0.300	0.948	0.052	0.476	0.524	0.000	0.648	0.352
0.552	0.091	0.385	0.500	0.239	0.620	0.141	0.894	0.096	0.385	0.563	0.052	0.553	0.447
0.315	0.028	1.000	0.000	0.019	0.898	0.083	1.000	0.000	0.578	0.422	0.000	0.394	0.606
0.755	0.236	1.000	0.000	0.410	0.360	0.230	0.925	0.075	0.567	0.433	0.000	0.694	0.306
0.500	0.000	1.000	0.000	0.272	0.522	0.207	0.910	0.070	0.610	0.390	0.000	0.770	0.230
0.569	0.059	1.000	0.000	0.284	0.569	0.147	1.000	0.000	0.594	0.406	0.000	0.792	0.208
0.407	0.000	1.000	0.000	0.082	0.786	0.133	0.902	0.098	0.510	0.490	0.000	0.528	0.472
0.406	0.000	1.000	0.000	0.212	0.567	0.221	0.857	0.143	0.338	0.662	0.000	0.833	0.167
0.556	0.000	1.000	0.000	0.148	0.639	0.213	0.930	0.070	0.566	0.434	0.000	0.694	0.306
0.347	0.292	1.000	0.000	0.132	0.623	0.189	0.954	0.046	0.489	0.227	0.284	0.906	0.094
0.306	0.333	0.944	0.000	0.500	0.258	0.242	0.958	0.042	0.541	0.388	0.071	0.296	0.704
0.398	0.000	1.000	0.000	0.148	0.833	0.019	0.858	0.142	0.763	0.286	0.041	0.815	0.185
0.264	0.014	1.000	0.000	0.172	0.531	0.297	0.988	0.012	0.553	0.447	0.000	0.598	0.402
0.489	0.000	1.000	0.000	0.216	0.568	0.216	0.811	0.189	0.587	0.413	0.000	0.074	0.926
0.491	0.000	0.981	0.000	0.284	0.402	0.314	0.981	0.019	0.667	0.333	0.000	0.370	0.630
0.639	0.000	1.000	0.000	0.292	0.519	0.189	0.944	0.056	0.283	0.425	0.292	0.325	0.675
0.575	0.000	1.000	0.000	0.490	0.176	0.333	0.972	0.028	0.683	0.173	0.144	0.585	0.415
0.696	0.000	1.000	0.000	0.306	0.612	0.082	0.694	0.306	0.530	0.470	0.000	0.441	0.559
0.373	0.343	0.991	0.009	0.148	0.741	0.111	1.000	0.000	0.463	0.361	0.176	0.306	0.694
0.417	0.139	1.000	0.000	0.392	0.490	0.118	0.954	0.046	0.741	0.259	0.000	0.102	0.898
0.633	0.051	1.000	0.000	0.192	0.558	0.250	0.944	0.056	0.840	0.160	0.000	0.407	0.593
0.611	0.000	0.877	0.123	0.151	0.594	0.255	0.972	0.028	0.479	0.436	0.085	0.509	0.491
0.425	0.000	0.991	0.009	0.250	0.558	0.192	0.889	0.111	0.756	0.220	0.024	0.269	0.731
0.350	0.390	0.991	0.009	0.067	0.644	0.288	0.991	0.009	0.696	0.283	0.022	0.694	0.306
0.510	0.324	1.000	0.000	0.074	0.657	0.269	1.000	0.000	0.683	0.288	0.029	0.538	0.462
0.519	0.000	0.898	0.102	0.259	0.630	0.111	0.991	0.009	0.357	0.296	0.347	0.120	0.880
0.167	0.028	1.000	0.000	0.444	0.324	0.231	0.954	0.046	0.745	0.255	0.000	0.130	0.870
0.877	0.066	1.000	0.000	0.481	0.148	0.370	0.676	0.324	0.402	0.598	0.000	0.324	0.676
0.700	0.000	1.000	0.000	0.123	0.594	0.283	0.972	0.028	0.520	0.388	0.092	0.102	0.898
0.490	0.220	0.981	0.019	0.104	0.833	0.063	0.991	0.009	0.408	0.592	0.000	0.427	0.573
0.593	0.000	0.778	0.222	0.241	0.519	0.241	0.824	0.176	0.648	0.352	0.000	0.170	0.830
0.735	0.157	1.000	0.000	0.194	0.676	0.130	0.963	0.037	0.394	0.462	0.144	0.093	0.907
0.868	0.000	1.000	0.000	0.000	0.509	0.491	0.925	0.075	0.557	0.245	0.179	0.489	0.511
0.861	0.000	0.824	0.176	0.000	0.981	0.019	1.000	0.000	0.567	0.433	0.000	0.602	0.398
0.583	0.222	0.509	0.491	0.000	0.813	0.187	1.000	0.000	0.731	0.269	0.000	0.917	0.083
0.615	0.229	0.596	0.404	0.111	0.759	0.130	1.000	0.000	0.519	0.317	0.163	0.548	0.452
0.500	0.000	0.935	0.065	0.250	0.241	0.509	0.827	0.173	0.433	0.308	0.260	0.690	0.310
0.509	0.009	0.833	0.167	0.046	0.546	0.407	0.657	0.333	0.417	0.583	0.000	0.852	0.148
0.617	0.096	0.760	0.240	0.173	0.442	0.346	0.991	0.009	0.557	0.443	0.000	0.657	0.343
0.623	0.132	0.663	0.337	0.130	0.426	0.444	0.889	0.111	0.304	0.554	0.141	0.676	0.324
0.639	0.000	0.704	0.296	0.179	0.377	0.443	0.849	0.151	0.538	0.462	0.000	0.620	0.380
0.310	0.430	0.657	0.343	0.287	0.250	0.463	0.944	0.056	0.471	0.363	0.167	0.611	0.389

found by Ochman *et al.* (1983) in the Pyrenees, though in our study, some of the groups do accord to some extent with zones of visible polymorphism (Vicario *et al.*, 1988). The FCA, on the removal of rare alleles and redundancy for diallelic loci, clarifies the spatial variation of these more frequent genes which might differentiate the populations in green and brown Iberia. Any geographic differentiation in molecular polymorphism, which coincides with the two climatic regions, might suggest an

adaptive response of the *Cepaea* populations to climatic differences. However there is no apparent relation between gene frequencies and climatic parameters for most of the loci analyzed. Part of the observed variability might hence have emerged as an effect of genetic drift. A selective effect acting on visible polymorphism from both zones, might produce a "balance point" (Falconer, 1964) where genetic drift and selection trends counteract each other. Geographic isolation produced by the Ebro

Figure 3 Hierarchical ordering of the populations based on the first six factorial axes (reciprocal neighbour method).

river and the mountains that divide green and brown Iberia, prevent genetic flow between the zones, as geographically neighbouring populations do have smaller genetic distances than those more distant.

Jones *et al.* (1980) suggest that demographic history explains the pattern of variation of enzyme polymorphisms in *C. nemoralis* in the Pyrenees. In our case, genetic change due to drift, and conditioned by the action of climatic selection on the visible polymorphism, appears to have created two

zones. Morphological variation is reflected as different genetic structure for enzyme loci of *C. nemoralis* populations in each zone.

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