North/South differentiation in the distribution of *Cepaea nemoralis* in Spain

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Sixteen enzymatic loci of *Cepaea nemoralis* were studied in an area of 180 km long by 40 km wide in northern Spain. Two new polymorphic loci DIA and GLO, and two monomorphics, ARYL and β -GAL are described.

The analysis of principal components groups the populations in two geographical areas, in the north and south of the sampling area. There is also an intermediate area between them. Heterozygosity in the northern area is significantly higher than in the southern one. Heterozygosity seems to diminish in unfavourable conditions (greater dryness or greater altitude), which is in accordance with the niche width variation hypothesis. There seems to exist an association between alleles of GLO and PGM and altitude.

The geographical areas created by the biochemical polymorphism coincide with those of the visible polymorphism, which in turn correspond to climatic zones. The northern area, according to visible and biochemical polymorphism is located in the so-called "Green Iberia", and the southern area in "Brown Iberia". The intermediate area corresponds to the area of climatic transition between them. These areas could correspond to "geographical races" of *Cepaea nemoralis*.

Climatic selection may be acting upon some loci such as PGM and GLO while in others random drift could be the cause of heterogeneity.

INTRODUCTION

Band pattern and colour polymorphism of the shell have been widely studied in the land snail *Cepaea nemoralis*, and much geographical variation revealed. In some areas, abrupt changes have been discovered in the frequency of morphs. These have been called "area effects" (Cain and Currey, 1963). These could correspond to parapatric or stasipatric speciation in the absence of geographical isolation (White, 1978). Biochemical polymorphism in *Cepaea nemoralis* is less studied (Manwell and Baker, 1968; Levan and Fredga, 1972; Oxford, 1973; Brussard and McCracken, 1974; Johnson, 1976, 1979); it also shows wide geographical variation, as in the case of the Pyrenees (Jones *et al.*, 1980; Cougant *et al.*, 1982; Valdez-Forsans, 1983) and "molecular area effects" have even been detected (Ochman *et al.*, 1983).

Large scale studies of visible (Jones *et al.*, 1977) and biochemical (Ochman *et al.*, 1987) polymorphism have demonstrated the existence of gradients in the frequencies. If the area effects were an early

stage of speciation, a coincidence in variation could be expected between the loci of biochemical polymorphism and the morphological characters of the shells. In some places this association has been found, but not in others (Johnson, 1976; Jones *et al.*, 1980).

In *Cepaea nemoralis* it has been postulated that climatic selection is acting upon visible polymorphism (see Jones *et al.*, 1977). For biochemical polymorphisms, in other species, significant differences have been found in gene frequencies of different enzymatic loci between drier and warmer south-facing slopes and wetter and cooler north-facing slopes, *e.g.*, Nevo *et al.* (1982). The different slope patterns appear to be adaptive, and are presumably maintained by microclimatic diversifying selection. On the other hand, Ochman *et al.* (1983) did not find differences between the north and south of the Pyrenees, but found three regions (East, Centre, West) which were relatively uniform, and would correspond to an allopatric differentiation model with subsequent secondary contacts. Selander and Kaufmann (1975) found

that random drift was the cause of the differences between gene frequencies between populations of *Helix aspersa*.

For visible polymorphism, Mazon *et al.* (1987) found that the *Cepaea nemoralis* populations of northern Spain were grouped in two large geographical areas which coincide with the existence of two climatic zones: "Green Iberia" and "Brown Iberia". This suggested climatic selection as the cause of this division. The aim of this study is to learn whether the morphological differentiation of these two zones also manifests itself in biochemical polymorphism, and thus to be able to learn whether selection can act at a deeper level, such as the molecular level. If this is so, we have an apparent case of White's "geographical races" (White, 1978).

MATERIALS AND METHODS

Thirty-one populations were sampled along the 3°W meridian in an area 200 km long, and 40 km wide (Mazon *et al.*, 1987), fig. 1.

The sampling area was no larger than 400 m². All 1636 specimens were used in the electrophoretic analysis of 10 enzymatic systems with 16 loci:

Phosphogluconate dehydrogenase (PGD), phosphoglucumutase (PGM, 3 loci), β -galactosidase (β -GAL), superoxide dismutase (SOD, 2 loci; anodal and cathodal), glyoxalase I (GLO), isocitrate dehydrogenase (IDH-1), leucine aminopeptidase (LAP, 2 loci), arylsulphatase (ARYL), esterases (EST, 3 loci; EST-1, EST-2, EST-3), NADH diaphorase (DIA).

The samples were prepared from hepatopancreas and ovotestis for all enzymes except GLO, for which the digestive apparatus was used. Organs were homogenized with washed sea-sand and distilled H₂O in the proportion of 1:1 weight/volume, centrifuged at 5000 rpm for 15 min at 5°C and stored at -80°C.

Electrophoresis was in starch gel (Sigma) with the buffers and times shown in table 1. The staining methods were according to Selander *et al.* (1971) and Harris *et al.* (1976). The alleles were named (a-e) from greater to lesser mobility.

To compare the grade of differentiation within and between zones, Wright's (1978) hierarchical *F*-statistics were used. Localities (demes) were considered grouped in eight regions according to geographical and climatic characteristics. The term F_{DR} estimates the variance for demes within the region; F_{RT} , regions within the total area; F_{DT} , demes within the total area.

Figure 1 Area sampled in Spain

RESULTS

Sixteen loci were studied, of which PGM-1, β -GAL and ARYL were monomorphic in all populations. Two loci, IDH-1 and SOD-C, were monomorphic in almost all populations, and others occurred at low frequency, like D and E of PGM-2 and LAP-2, A of SOD and B and E of EST-2 (table 2). For EST-1 and LAP no presence of silent alleles was detected.

The variation between demes within each region (F_{DR}) is relatively high, and furthermore the degree of difference between the demes in each region is not uniform for the sixteen loci. Table 3. The variation between regions (F_{RT}) is in all cases much less than within each region. Therefore, a greater component of diversity exists within the regions.

The most heterogeneous loci, by the measure F_{ST} are EST-3, PGM-3 and SOD. The χ^2 heterogeneity analyses for each locus in each of the eight regions are significant.

Table 1 Electrophoretic conditions and enzymes analysed

Locus	Bridge buffer	Gel buffer	Volt and time
PGD	0.13 M Tris, 0.07 M Boric, 0.003 M EDTA, pH = 8.7	0.045 M Tris, 0.025 M Boric, 0.001 M EDTA, pH = 8.9	8 V/cm 5 h
PGM DIA	0.23 M Tris, 0.05 M Citric, pH = 8.0	1:9 Dilution of the bridge buffer, pH = 8.0	8 V/cm 5 h
β -GAL ARYL	0.22 M Tris, 0.07 M Citric, pH = 6.3	1:10 Dilution of the bridge buffer, pH = 6.3	8 V/cm 5.5 h
GLO SOD	0.21 M Tris, 0.15 M Boric 0.006 EDTA, 0.005 MgCl ₂ , pH = 8.6	1:9 Dilution of the bridge buffer + MgCl ₂ to 0.005 M, pH = 8.6	8 V/cm 5.5 h
IDH LAP	0.41 M Sodium citrate, 0.41 citric, pH = 7.0	0.005 M DL histidine, 0.003 MgCl ₂ , pH = 7.0	10 V/cm 5 h
LAP	0.1 M Tris, 0.1 Maleic, 0.01 EDTA, 0.01 MgCl ₂ , pH = 7.6	1:9 Dilution of the bridge buffer, pH = 7.6	8 V/cm 5 h
EST	0.2 M Boric, 0.03 M Lithium HYDROXIDE, pH = 8.2	0.02 M Boric, 0.004 M Citric 0.03 M Tris, 0.003 M LiOH, pH = 8.2	12 V/cm 6 h

All the polymorphic loci intervene in the analysis of principal components (APC). The first two axes explain 45 per cent of all variance. In fig. 2 it may be observed that the populations can be grouped in two areas, one located in the north of the sampled area, and taking in populations 1–14, and the other in the south, corresponding to populations 15–31. Population 3 (Derio) is the only one which does not fit into its group. Within the southern area, a group of populations located in

the east of the sampling area may be separated (20, 27, 28, 29).

To augment the explained variance, a dendrogram is constructed on the basis of the six principal axes (which explain 83.3 per cent of the total variance) (fig. 3). It may be observed that in the first ramification, the northern populations are separated from the southern ones, except populations 3, 12, 14 and 15. According to this analysis, the northern area is positively characterized by

Figure 2 Distribution of populations on Factor I and II scores of analysis of principal component based on allele frequencies of 16 polymorphic enzymatic loci. Right of figure, northern populations; middle, intermediate populations; left, southern populations. (Numbers refer to localities in table 2).

Table 2 Allelic frequencies of 16 enzymatic loci in 31 populations of Spain

Locality	PGD-A	PGD-B	PGD-C	PGM2-A	PGM2-B	PGM2-C	PGM2-D	PGM2-E	PGM3-A
1 Arminza	0.000	0.659	0.341	0.025	0.762	0.213	0.000	0.000	0.000
2 Munguia	0.000	0.541	0.459	0.115	0.623	0.262	0.000	0.000	0.098
3 Derio	0.019	0.423	0.558	0.000	0.173	0.827	0.000	0.000	0.033
4 Gardeliz	0.041	0.568	0.391	0.071	0.737	0.192	0.000	0.000	0.071
5 Llodio-Amu	0.098	0.621	0.280	0.016	0.778	0.198	0.000	0.008	0.052
6 Saracho	0.000	0.591	0.409	0.061	0.598	0.341	0.000	0.000	0.088
7 Orduña 1	0.000	0.722	0.278	0.053	0.553	0.395	0.000	0.000	0.146
8 Orduña-2	0.000	0.545	0.453	0.000	0.186	0.786	0.000	0.029	0.042
9 Orduña-3	0.000	0.857	0.143	0.022	0.789	0.178	0.000	0.011	0.114
10 Guinea	0.000	0.594	0.406	0.016	0.688	0.297	0.000	0.000	0.145
11 Espejo	0.179	0.515	0.306	0.000	0.393	0.600	0.000	0.007	0.064
12 Fontecha	0.160	0.373	0.467	0.120	0.278	0.602	0.000	0.000	0.042
13 M. Pastor	0.000	0.278	0.722	0.000	0.359	0.598	0.033	0.011	0.000
14 Altable	0.185	0.457	0.359	0.439	0.265	0.296	0.000	0.000	0.009
15 Tirgo	0.474	0.474	0.051	0.204	0.505	0.292	0.000	0.000	0.005
16 Herramallu	0.378	0.244	0.378	0.391	0.383	0.223	0.000	0.000	0.075
17 Ezcaray-Po	0.000	0.125	0.875	0.000	0.473	0.527	0.000	0.000	0.000
18 El Rio	0.000	0.245	0.755	0.000	0.163	0.837	0.000	0.000	0.000
19 Zaldierna	0.000	0.595	0.405	0.000	0.286	0.714	0.000	0.000	0.045
20 Anguiano	0.221	0.297	0.483	0.080	0.233	0.687	0.000	0.000	0.602
21 Altuzarra	0.000	0.267	0.733	0.000	0.548	0.452	0.000	0.000	0.078
22 Fresneda S.	0.000	0.083	0.917	0.000	0.250	0.750	0.000	0.000	0.036
23 Valvanera	0.048	0.073	0.879	0.000	0.464	0.536	0.000	0.000	0.018
24 Pineda S.	0.000	0.625	0.375	0.000	0.344	0.656	0.000	0.000	0.000
25 Riocabado	0.152	0.572	0.275	0.149	0.216	0.634	0.000	0.000	0.000
26 Barbadillos	0.310	0.280	0.410	0.038	0.462	0.500	0.000	0.000	0.012
27 Ribaflecha	0.000	0.100	0.900	0.000	0.614	0.386	0.000	0.000	0.394
28 Pradillo	0.000	0.487	0.513	0.000	0.425	0.575	0.000	0.000	0.207
29 Pedroso	0.000	0.579	0.421	0.000	0.528	0.472	0.000	0.000	0.033
30 Castroviejo	0.034	0.560	0.405	0.000	0.367	0.633	0.000	0.000	0.000
31 Cardenas-10	0.000	0.267	0.733	0.000	0.179	0.821	0.000	0.000	0.000

Locality	PGM3-B	PGM3-C	SOD-A	SOD-B	SOD-C	SODC-A	SODC-B	GLO-A	GLO-B
1 Arminza	1.000	0.000	0.000	0.366	0.634	0.025	0.975	0.487	0.329
2 Munguia	0.902	0.000	0.000	0.467	0.533	0.000	1.000	0.482	0.321
3 Derio	0.950	0.017	0.000	0.232	0.768	0.000	1.000	0.067	0.817
4 Gardeliz	0.904	0.026	0.000	0.205	0.795	0.000	1.000	0.486	0.392
5 Llodio-Amu	0.843	0.104	0.000	0.254	0.746	0.000	1.000	0.420	0.442
6 Saracho	0.912	0.000	0.000	0.098	0.902	0.000	1.000	0.418	0.436
7 Orduña 1	0.854	0.000	0.000	0.119	0.881	0.000	1.000	0.341	0.549
8 Orduña-2	0.958	0.000	0.000	0.205	0.795	0.029	0.971	0.361	0.347
9 Orduña-3	0.886	0.000	0.000	0.219	0.781	0.000	1.000	0.438	0.354
10 Guinea	0.855	0.000	0.000	0.188	0.813	0.000	1.000	0.516	0.250
11 Espejo	0.936	0.000	0.014	0.150	0.836	0.021	0.979	0.457	0.257
12 Fontecha	0.953	0.005	0.000	0.668	0.332	0.019	0.981	0.392	0.387
13 M. Pastor	1.000	0.000	0.000	0.478	0.522	0.011	0.989	0.446	0.391
14 Altable	0.945	0.045	0.000	0.455	0.545	0.000	1.000	0.412	0.475
15 Tirgo	0.954	0.041	0.000	0.473	0.527	0.000	1.000	0.368	0.473
16 Herramallu	0.915	0.009	0.000	0.755	0.245	0.000	1.000	0.337	0.471
17 Ezcaray-Po	1.000	0.000	0.000	0.563	0.438	0.000	1.000	0.329	0.553
18 El Rio	0.961	0.039	0.000	0.541	0.459	0.000	1.000	0.225	0.637
19 Zaldierna	0.932	0.023	0.000	0.625	0.375	0.000	1.000	0.159	0.795
20 Anguiano	0.398	0.000	0.000	0.506	0.494	0.000	1.000	0.397	0.486
21 Altuzarra	0.922	0.000	0.000	0.938	0.063	0.000	1.000	0.281	0.478
22 Fresneda S.	0.904	0.000	0.000	0.639	0.361	0.000	1.000	0.264	0.694
23 Valvanera	0.936	0.045	0.000	0.442	0.398	0.000	1.000	0.343	0.583
24 Pineda S.	1.000	0.000	0.000	0.122	0.878	0.000	1.000	0.000	0.744
25 Riocabado	0.986	0.014	0.000	0.420	0.580	0.000	1.000	0.436	0.514
26 Barbadillos	0.988	0.000	0.000	0.413	0.587	0.000	1.000	0.397	0.474
27 Ribaflecha	0.530	0.076	0.000	0.375	0.625	0.000	1.000	0.091	0.455
28 Pradillo	0.780	0.012	0.000	0.575	0.425	0.000	1.000	0.128	0.526
29 Pedroso	0.867	0.100	0.000	0.472	0.528	0.000	1.000	0.200	0.675
30 Castroviejo	0.948	0.052	0.000	0.078	0.922	0.000	1.000	0.230	0.672
31 Cardenas-10	0.967	0.033	0.000	0.423	0.577	0.000	1.000	0.267	0.700

Table 2 continued

Locality	GLO-C	IDH1-A	IDH1-B	IDH1-C	LAP1-A	LAP1-B	LAP2-A	LAP2-B	LAP2-C
1 Arminza	0.184	1.000	0.000	0.000	0.910	0.090	0.000	0.155	0.845
2 Munguia	0.196	1.000	0.000	0.000	0.941	0.059	0.131	0.025	0.844
3 Derio	0.117	1.000	0.000	0.000	1.000	0.000	0.000	0.017	0.983
4 Gardeliz	0.122	1.000	0.000	0.000	1.000	0.000	0.000	0.263	0.737
5 Llodio-Amu	0.138	0.855	0.065	0.080	0.971	0.029	0.007	0.184	0.809
6 Saracho	0.145	1.000	0.000	0.000	0.864	0.136	0.000	0.129	0.871
7 Orduña 1	0.110	1.000	0.000	0.000	0.878	0.122	0.000	9.100	0.900
8 Orduña-2	0.292	1.000	0.000	0.000	0.847	0.153	0.000	0.114	0.871
9 Orduña-3	0.208	1.000	0.000	0.000	0.927	0.073	0.020	0.092	0.888
10 Guinea	0.234	1.000	0.000	0.000	0.563	0.438	0.000	0.000	1.000
11 Espejo	0.286	1.000	0.000	0.000	0.943	0.057	0.000	0.058	0.942
12 Fontecha	0.221	1.000	0.000	0.000	0.833	0.167	0.165	0.109	0.826
13 M. Pastor	0.163	1.000	0.000	0.000	1.000	0.000	0.000	0.054	0.946
14 Altable	0.112	1.000	0.000	0.000	0.882	0.118	0.000	0.144	0.844
15 Tirgo	0.159	1.000	0.000	0.000	1.000	0.000	0.000	0.087	0.784
16 Herramallu	0.192	1.000	0.000	0.000	1.000	0.000	0.000	0.147	0.637
17 Ezcaray-Po	0.118	1.000	0.000	0.000	1.000	0.000	0.287	0.188	0.525
18 El Rio	0.137	1.000	0.000	0.000	1.000	0.000	0.000	0.490	0.510
19 Zaldierna	0.045	1.000	0.000	0.000	1.000	0.000	0.000	0.184	0.816
20 Anguiano	0.116	0.885	0.115	0.000	1.000	0.000	0.000	0.114	0.886
21 Altuzarra	0.141	1.000	0.000	0.000	1.000	0.000	0.000	0.113	0.806
22 Fresneda S.	0.042	1.000	0.000	0.000	1.000	0.000	0.028	0.056	0.917
23 Valvanera	0.074	1.000	0.000	0.000	1.000	0.000	0.118	0.333	0.549
24 Pineda S.	0.256	1.000	0.000	0.000	1.000	0.000	0.000	0.045	0.955
25 Riocabado	0.050	1.000	0.000	0.000	0.950	0.050	0.000	0.008	0.992
26 Barbadillos	0.128	1.000	0.000	0.000	1.000	0.000	0.000	0.010	0.990
27 Ribaflecha	0.455	0.800	0.186	0.014	0.971	0.029	0.157	0.186	0.657
28 Pradillo	0.346	1.000	0.000	0.000	0.975	0.025	0.000	0.183	0.817
29 Pedroso	0.125	1.000	0.000	0.000	1.000	0.000	0.000	0.306	0.694
30 Castroviejo	0.098	1.000	0.000	0.000	0.983	0.017	0.000	0.320	0.680
31 Cardenas-10	0.033	1.000	0.000	0.000	1.000	0.000	0.000	0.467	0.533

Locality	LAP2-D	LAP2-E	EST1-A	EST1-B	EST2-A	EST2-B	EST2-C	EST2-D	EST2-E
1 Arminza	0.000	0.000	0.000	1.000	0.157	0.000	0.843	0.000	0.000
2 Munguia	0.000	0.000	0.033	0.967	0.319	0.000	0.672	0.009	0.000
3 Derio	0.000	0.000	0.000	1.000	0.155	0.034	0.810	0.000	0.000
4 Gardeliz	0.000	0.000	0.136	0.864	0.071	0.000	0.885	0.045	0.000
5 Llodio-Amu	0.000	0.000	0.007	0.993	0.066	0.000	0.897	0.037	0.000
6 Saracho	0.000	0.000	0.000	1.000	0.023	0.000	0.969	0.008	0.000
7 Orduña 1	0.000	0.000	0.011	0.989	0.000	0.000	1.000	0.000	0.000
8 Orduña-2	0.000	0.014	0.056	0.944	0.083	0.000	0.917	0.000	0.000
9 Orduña-3	0.000	0.000	0.023	0.977	0.115	0.000	0.875	0.010	0.000
10 Guinea	0.000	0.000	0.081	0.919	0.000	0.000	0.984	0.016	0.000
11 Espejo	0.000	0.000	0.299	0.705	0.130	0.000	0.870	0.000	0.000
12 Fontecha	0.000	0.000	0.009	0.991	0.023	0.000	0.977	0.000	0.000
13 M. Pastor	0.000	0.000	0.488	0.512	0.054	0.000	0.946	0.000	0.000
14 Altable	0.012	0.000	0.109	0.891	0.018	0.000	0.982	0.000	0.000
15 Tirgo	0.120	0.010	0.104	0.896	0.114	0.000	0.886	0.000	0.000
16 Herramallu	0.127	0.088	0.173	0.827	0.337	0.000	0.663	0.000	0.000
17 Ezcaray-Po	0.000	0.000	0.000	1.000	0.038	0.000	0.962	0.000	0.000
18 El Rio	0.000	0.000	0.020	0.980	0.042	0.000	0.938	0.000	0.000
19 Zaldierna	0.000	0.000	0.045	0.955	0.227	0.000	0.773	0.000	0.000
20 Anguiano	0.000	0.000	0.126	0.874	0.006	0.000	0.966	0.000	0.000
21 Altuzarra	0.065	0.016	0.397	0.603	0.125	0.000	0.875	0.000	0.000
22 Fresneda S.	0.000	0.000	0.265	0.735	0.043	0.000	0.957	0.000	0.000
23 Valvanera	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000
24 Pineda S.	0.000	0.000	0.057	0.943	0.070	0.000	0.930	0.000	0.000
25 Riocabado	0.000	0.000	0.182	0.818	0.066	0.000	0.919	0.015	0.000
26 Barbadillos	0.000	0.000	0.071	0.929	0.038	0.000	0.875	0.087	0.000
27 Ribaflecha	0.000	0.000	0.129	0.871	0.000	0.000	0.971	0.000	0.029
28 Pradillo	0.000	0.000	0.063	0.938	0.014	0.000	0.986	0.000	0.000
29 Pedroso	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000
30 Castroviejo	0.000	0.000	0.000	1.000	0.040	0.000	0.960	0.000	0.000
31 Cardenas-10	0.000	0.000	0.033	0.967	0.000	0.000	1.000	0.000	0.000

Table 2 continued

Locality	EST3-A	EST3-B	DIA-A	DIA-B	DIA-C
1 Arminza	0.770	0.230	0.427	0.500	0.073
2 Munguia	0.898	0.102	0.500	0.414	0.086
3 Derio	0.796	0.204	0.190	0.655	0.155
4 Gardeliz	0.740	0.260	0.247	0.610	0.143
5 Llodio-Amu	0.790	0.210	0.225	0.594	0.181
6 Saracho	0.789	0.211	0.356	0.508	0.136
7 Orduña 1	0.989	0.011	0.456	0.333	0.211
8 Orduña-2	0.944	0.056	0.486	0.361	0.153
9 Orduña-3	0.878	0.122	0.378	0.439	0.184
10 Guinea	0.917	0.083	0.500	0.194	0.306
11 Espejo	0.784	0.216	0.350	0.280	0.370
12 Fontecha	0.450	0.550	0.419	0.360	0.221
13 M. Pastor	0.815	0.185	0.446	0.359	0.196
14 Altable	0.806	0.194	0.527	0.409	0.064
15 Tirgo	0.888	0.112	0.264	0.482	0.254
16 Herramallu	0.768	0.232	0.453	0.311	0.236
17 Ezcaray-Po	0.962	0.038	0.275	0.563	0.162
18 El Rio	0.784	0.216	0.430	0.470	0.100
19 Zaldierna	1.000	0.000	0.381	0.214	0.405
20 Anguiano	0.082	0.918	0.594	0.294	0.112
21 Altuzarra	0.234	0.766	0.357	0.429	0.214
22 Fresneda S.	0.264	0.736	0.274	0.581	0.145
23 Valvanera	0.866	0.134	0.270	0.607	0.123
24 Pineda S.	0.792	0.208	0.156	0.822	0.022
25 Riocabado	0.114	0.886	0.406	0.384	0.210
26 Barbadillos	0.135	0.865	0.128	0.779	0.093
27 Ribaflecha	0.789	0.211	0.643	0.100	0.257
28 Pradillo	0.750	0.250	0.538	0.200	0.262
29 Pedroso	0.950	0.050	0.684	0.105	0.211
30 Castroviejo	0.984	0.016	0.325	0.341	0.333
31 Cardenas-10	0.700	0.300	0.227	0.364	0.409

Figure 3 Hierarchy of populations for reciprocal neighbour method of six factorial axes with explained variance of 83.3 per cent.

GLO-A, PGM2-B, PGD-B, and the southern area by GLO-B, PGM3-B and DIA-B.

The main significant differences between north and south appear in table 4, the results of which agree generally with the previous analyses.

Using Nei and Prevosti's genetic distance analysis, a dendrogram was constructed, (fig. 4), in which the north/south division is also seen. In this analysis, a series of populations may be observed which are classified at times in the north and at others in the south (12-16). Geographically, they are in the north/south separation area, which leads us to believe they correspond to a transitional area.

Heterozygosity is high in all populations. It ranges from 13.5 per cent in population 24 (Pineda) and 29.6 per cent in population 13 (M. Pastor), with the average being 21.5 per cent. In the northern area ($H=0.231$) it is significantly higher than in the south (0.201) ($t=2.5$, $P=0.019$).

Although samples from the southern area are at greater mean altitude (863 m) than those in the

Table 3 Hierarchical *F*-statistic for *Cepaea nemoralis* in Spain. Samples representing 31 localities (demes) (F_{DR}) in 8 regions (F_{RT})

Locus	Freq.	F_{DR}	F_{RT}	F_{DT}
PGD				
A	0.061	0.141	0.030	0.167
B	0.439	0.121	0.034	0.151
C	0.500	0.147	0.012	0.158
Mean		0.135	0.024	0.155
PGM-2				
A	0.058	0.060	0.151	0.203
B	0.440	0.119	0.021	0.138
C	0.449	0.118	0.049	0.161
D	0.001	0.019	0.002	0.021
E	0.002	0.002	0.002	0.004
Mean		0.113	0.047	0.154
PGM-3				
A	0.078	0.229	0.000	0.206
B	0.902	0.182	0.000	0.171
C	0.020	0.017	0.000	0.028
Mean		0.183	0.000	0.169
SOD				
A	0.001	0.006	0.001	0.007
B	0.402	0.112	0.063	0.167
C	0.597	0.110	0.063	0.166
Mean		0.111	0.063	0.167
SOD-C				
A	0.997	0.004	0.005	0.009
GLO				
A	0.328	0.053	0.018	0.070
B	0.509	0.056	0.024	0.079
C	0.163	0.052	0.000	0.050
Mean		0.054	0.016	0.069
IDH-1				
A	0.985	0.157	0.000	0.140
B	0.012	0.141	0.000	0.123
C	0.003	0.056	0.004	0.059
Mean		0.140	0.000	0.125
LAP-1				
A	0.950	0.107	0.049	0.150
LAP-2				
A	0.027	0.174	0.000	0.146
B	0.151	0.079	0.030	0.107
C	0.808	0.096	0.028	0.121
D	0.010	0.044	0.049	0.090
E	0.004	0.043	0.008	0.051
Mean		0.094	0.024	0.116
EST-1				
A	0.906	0.145	0.016	0.159
EST-2				
A	0.077	0.069	0.028	0.095
B	0.001	0.014	0.002	0.017
C	0.913	0.061	0.024	0.084
D	0.007	0.032	0.004	0.036
E	0.002	0.021	0.000	0.017
Mean		0.062	0.024	0.085
EST-3				
A	0.704	0.357	0.029	0.376
DIA				
A	0.195	0.059	0.010	0.068
B	0.421	0.055	0.061	0.113
C	0.384	0.033	0.014	0.046
Mean		0.051	0.031	0.080
Total		0.123	0.030	0.149

Table 4 Significant differences in mean allelic frequencies between northern and southern zone of sampled area. (*t* of Student and probability)

Allele	North	South	<i>t</i>	<i>P</i>
PGD				
B	0.553	0.242	3.34	0.002
C	0.398	0.582	2.68	0.012
PGM-2				
C	0.413	0.566	2.13	0.042
SOD				
C	0.706	0.508	2.92	0.007
GLO				
A	0.409	0.261	3.56	0.001
B	0.410	0.589	3.93	0.000
LAP-1				
A	0.088	0.007	4.50	0.000
B	0.886	0.923	3.25	0.003
LAP-2				
C	0.879	0.750	2.7	0.011

north (416 m), we found no correlation between altitude and heterozygosity. However, there do exist significant correlations between enzymatic loci and altitude (PGM2-B; PGM2-C; GLO-B) (fig. 5).

DISCUSSION

In this study, an electrophoretic analysis was made of ten organs of *Cepaea nemoralis* for all loci. The organs selected were those that gave best resolution. For example, the albumen gland for SOD, and the kidney for DIA, were rejected, since stains could be seen on the electrophoretic plate, and no bands were produced. In general, the hepatopancreas and the ovotestis were the organs that gave best band resolution. For GLO the digestive apparatus was used, as the rest of the organs probably expressed various loci whose alleles migrated very closely, making it very difficult to determine the different electromorphs. SOD-C is characteristic of the reproductive system. On the other hand, enzymes such as anodal β -GAL were tested, but not included in the analysis because the electrophoretic variation pattern could not be determined.

Biochemical polymorphism of *Cepaea nemoralis* presents great variability between populations, which is similar to what happens with the visible polymorphism; but this is not so great as in the Pyrenees. No do we see here the fixing in nearby populations of alternative alleles (Caugant *et al.*, 1982; Ochman *et al.*, 1983). In this paper, new enzymes are introduced, such as β -GAL, ARYL, DIA and GLO.

Figure 4 Dendrogram formed by UPGMA algorithm using Prevosti distance.

The heterogeneity of F_{DR} does not seem to support random genetic drift as the cause of the genetic differentiation between localities. Both the APC and the dendrogram constructed on the basis of Nei and Prevosti's distance divide the area into two zones. This could be, on a smaller scale, the same phenomenon as observed by Ochman *et al.* (1987).

Heterozygosity is higher than average for *Cepaea nemoralis* (0.134) or for molluscs (0.148),

Figure 5 Representation of allele C of locus PGM2 for altitude in the North of Spain.

but does not reach the values of 0.644 found for *Modiolus auriculatus* or 0.635 for *Cerithium caeruleum* (Selander and Ochman, 1983; Nevo, 1984).

The northern zone of our sampling area is characterised by an Atlantic climate—very rainy, with mild temperatures—while the southern zone has a continental climate—very dry, with extreme temperatures—and the average altitude in the south is greater than in the north.

Heterozygosity is significantly higher in the north than the south. This could be in accordance with the situation described by Nevo (1984) for molluscs and other taxa in which heterozygosity diminishes in those species which live in extreme areas (arid or extra-mesic). Furthermore, this process in *Cepaea nemoralis* could be of greater importance, since in the sampling area we are approaching the southern limit of its distribution.

Valdez-Forsans (1983) also found a reduction in heterozygosity in the highest areas of the Ariege valley in the French Pyrenees. In the southern Pyrenees Caugant *et al.* (1982) found no correlation between heterozygosity and altitude, but the higher populations were significantly less heterozygous than those from localities at medium or low elevations. This decrease in heterozygosity in unfavourable conditions could be a general process in *Cepaea nemoralis*. This could agree with the niche width variation hypothesis.

There are a series of coincidences between the visible (Mazon *et al.*, 1987) and biochemical polymorphism studied in the same area: (a). The subdivision of the populations into two zones according to biochemical polymorphism coincides with the subdivision observed for the visible. Furthermore, the point of division is the same. Population 3 (Derio) was classified in the southern zone for visible polymorphism and for biochemical polymorphism when geographically it corresponds to the north. The intermediate area between the two zones (transitional zone) is also the same for both polymorphisms. (b) The southern zone is more heterogeneous than the north for both visible and biochemical polymorphisms. This may be seen clearly in the dispersion of the populations in the APC and in the greater distances observed in the dendrograms for the southern zone. A relationship between visible and biochemical polymorphism exists in some cases (Johnson, 1976) and not in others (Jones *et al.*, 1980; Caugant *et al.*, 1982; Ochman *et al.*, 1983).

The concordance of climatic zones with the zones of biochemical polymorphism leads us to ask the question, are the enzymatic loci subject to

climatic selection? In our case, we feel that some loci may indeed be subject to selection, since the loci which contribute most to the formation of the zones are some of those which are correlated with altitude (PGM, GLO). On the other hand, others, which have great variance (EST3, PGM3, SOD) are not so important in the formation of zones, although it is difficult to understand the selection mechanism upon these loci.

At microgeographical level, adaptive effects have been observed for enzymatic loci in snails, which are probably due to microclimate diversifying selection (Nevo, 1982). However, in Ochman's large scale study (1987) he finds very few correlations between allele frequency changes and environmental components, although selection cannot be excluded. On the other hand, on a small scale there is evidence for the action of selection. We are extending this study to other areas, and extending the number of enzymatic loci to include those studied by other authors.

To sum up, the coincidence of the subdivision created by visible and biochemical polymorphism, the different heterozygosity in each zone, the correlation of some loci with altitude and heterogeneity of F_{DR} are good arguments in favour of the differentiation of these two zones into two possible "geographical races", possibly due to climatic selection.

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