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Distribution of *Cepaea nemoralis* according to climatic regions in Spain

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121 Populations of *Cepaea nemoralis* were sampled in Spain. In an area which extends from the coast of the Bay of Biscay to 182 km inland, following the meridian 3° west of Greenwich, and which has a width of approximately 40 km. In this study, we analysed the relationships between the populations and some selective agents, such as predation by *Turdus ericetorum* and climatic factors. These latter are represented by indirect parameters, such as altitude and distance from the sea, and direct parameters such as rainfall or temperatures. Visual selection is unimportant in the sampled populations. It was observed that our area of study is subdivided into 3 zones: Zone 1. Transitional and Zone 2, which have very different phenotype frequencies. What is more, these Zones correspond to very different climatic regions. Zone 1 is located in the climatic region known as "Green Iberia". The most frequent phenotypes of *Cepaea nemoralis* are the pale ones. Zone 2 is located in "Brown Iberia", and the most frequent phenotypes are the dark ones. Therefore we believe that in the populations sampled, one or more climatic factors are operating, and presumably causing the subdivision of these populations into Zones 1 and 2.

INTRODUCTION

The land snail *Cepaea nemoralis* shows a highly varied visible polymorphism. It is one of the most widely studied organisms in terms of natural populations, over 10,000 populations, throughout its range of distribution, having been studied (Jones *et al.*, 1977).

Different authors, working both on natural and artificial populations, have suggested that climatic factors are largely responsible for the inter-colony variation of *Cepaea nemoralis* (Cain, 1968; Arnold, 1969; Jones, 1973; Bantock, 1974, 1980; Bantock and Ratsey, 1980). Similarly, phenotypes may have a direct adaptive value both in live snails and in empty shells when subjected to irradiation by artificial or natural light. In this case, dark phenotypes reach higher temperatures than pale ones (Heath, 1975; Garcia, 1978), these latter having a lower mortality rate (Lamotte, 1966). Similar results have been obtained with shells painted in various colours (Tilling, 1983).

Low temperatures favour the brown morphs of *Cepaea nemoralis*, which are more frequent in the northern part of the species distribution range (Cain and Currey, 1963; Cain, 1968; Carter, 1968; Bantock, 1974). Cain (1971) analysed subfossil samples and found that the dark forms were more abundant in periods of colder climate. The yellow phenotypes are more frequent in the warmer areas inhabited by the species (Jones *et al.*, 1977).

The southern limit of *Cepaea nemoralis* distribution is found in the Iberian Peninsula (Perrot and Perrot, 1938). This is also the area of its distribution which has the warmest climate. *Cepaea nemoralis* has been studied very little in Spain, where the main area of interest has been the Pyrenees (Lamotte, 1951, 1959, 1968; Arnold, 1968, 1969; Cameron *et al.*, 1973; Jones and Irving, 1975; Jones *et al.*, 1980; Ramos, 1984, 1985). In the rest of Spain, Harvey (1970, 1971) studied 35 populations in the northern zone, west of Bilbao, penetrating some 20 km inland. He found that the frequency of the pink morphs was higher inland than on the coast. He suggested that this could be explained by a selective effect related to differences in atmospheric humidity. He also found that the frequency of the banded morphs changed according to their distance from the sea.

The aim of this study is two-fold: firstly to furnish data about *Cepaea nemoralis* distribution in one area of Spain, and secondly to study the relationship between this species and possible selective agents such as *Turdus ericetorum*, distance from the sea, altitude, rainfall, and temperatures.

We believe that the area in which we took our samples gives many facilities for this type of study, such as the existence of two climatic regions with their respective gradients of rainfall, temperatures, altitudes, as well as being on the southern limit of the species distribution range

MATERIALS AND METHODS

The sampled area extends from Bilbao to Soria approximately following the Meridian 3°W of Greenwich. The farthest point from the sea is at a distance of 182 km in a straight line, and its width is approximately 40 km (fig. 1).

In this area, two large natural zones may be observed. The first zone is between the coast and the foothills of Puerto de Orduña in the Cantabrian Mountains. It has mild temperatures and high humidity; it is mountainous, but not of great altitude, and densely populated, especially in the area near the sea. Its vegetation is very rich and varied, with extensive pine and eucalyptus woods and wide areas of scrubland where the genus *Lilex*, *Erica* and *Rubus* predominate, and there are extensive grasslands.

The second zone is from Puerto de Orduna to the Province of Soria. Its climate is drier, and its temperatures more extreme. In its orography we may point out the mountain Sierras of La Demanda, Cameros, Urbion and Cebollera, all of which belong to the Iberian Range.

SAMPLING METHODS

121 populations of *Cepaea nemoralis* were collected, totalling 13,293 individuals. 8 populations, along the length of the Iberian range in an easterly direction, were sampled. These are considered as populations outside the studied area. In each sampling, the specimens were taken in an area of approximately 400 m^2, which is less than their panmitic unit (Lamotte, 1951). The shells were scored by the methods of Lamotte (1951). Cain and Sheppard (1954) and Arnold (1968).

Data on vegetation, *p*H, soil characteristics, climate, UTM coordinates, distance from the sea and altitude were taken for each sampling zone.

Maps from the Army Geographical Service were used, and the meteorological data was obtained from the Data Department of National Meteorological Institute and from the publications "Mapa Pluviométrico de España" (Rainfall Map of Spain) and "Atlas Climatico de España" (Atlas of Spanish Climate) (Font. 1983).

RESULTS

The frequencies of the unbanded and yellow phenotypes appear in figs. 1 and 2. It may be observed that the unbanded phenotypes are more frequent in those populations found in the northern part of the sampled area. The same thing occurs for yellow-coloured shells. Similar distributions of *Cepaea nemoralis* phenotypes with regard to the distance from the sea are shown in fig. 3. A separation of the populations into two groups may be observed. The point of separation seems to be located between km 90 and km 100. We shall call the area from the coast to this point Zone 1, and from this point to the end Zone 2.

In view of the necessity of discovering whether this division corresponds to reality or not, and whether it corresponds only to the phenotypes shown in figs. 1–3, or if all phenotypes are involved, we felt it necessary to carry out Factorial Correspondence Analysis (FCA) for the 121 populations. 14 morphs were studied for each population (Y12345, Y00345, Y00300, Y00000, Fusion Banded Yellows, Hyalozonate Yellows. Other Yellows, P12345, P00345, P00300, P00000. Fusion Banded Pinks, Hyalozonate Pinks, Other Pinks). As shown in fig. 4, the populations may be separated into two zones, and this separation is not due to one single phenotypes, as figs. 1–3 seem to suggest, but many more are involved in characterising each zone. This may be summarised by table 1.

In fig. 4, it may be observed that some Zone 1 populations are included in Zone 2 and some from Zone 2 in Zone 1 (marked on the figure with a circle).

The influence of distance from the sea and altitude on the distribution of the different phenotypes in Zones 1 and 2 was studied. It may be observed from table 2 that some phenotypes have positive association in Zone 1 and negative in Zone 2, or vice versa. This indicates that these factors cannot be the causes of the differences between the zones. Some of the apparent contradictions appear in fig. 5. The yellow shells become more frequent as the altitude increases in Zone 1, whereas in Zone 2 they get less frequent.

We have observed that in Zone 1 there is a positive association between rainfall and the pink shells and, within these, with the effectively banded shells and 12345, while the association is negative

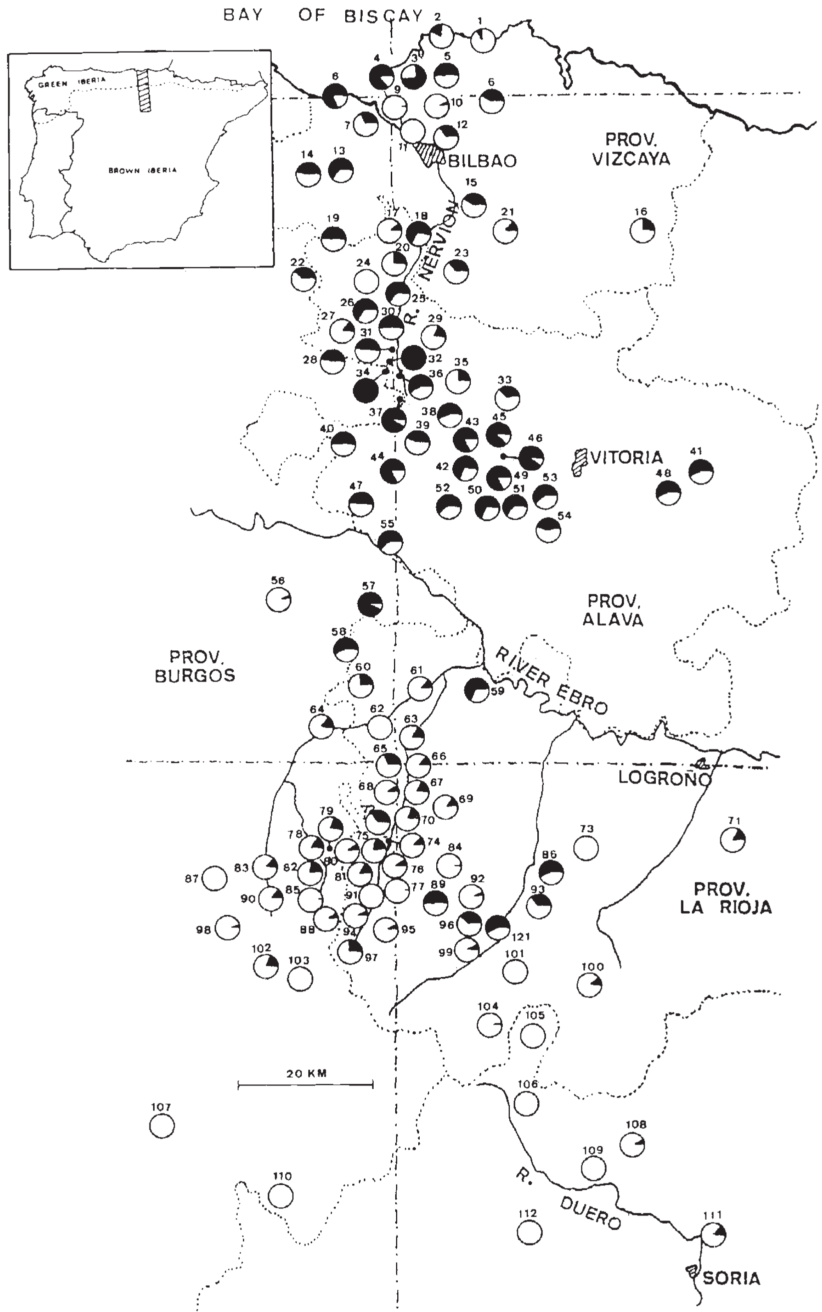

Figure 1 Geographical distribution of *Cepaea nemoralis* populations for phenotypes unbanded. (Unbanded frequency in black.)

with the yellow shells and, within these, with the effectively unbanded shells and 00000. The same associations may be observed with the minimum temperatures of the year, and the mean maxima and minima of each month, the annual mean of daily temperatures and the annual mean of mean monthly temperatures (fig. 6).

In Zone 2, the rainfall is positively associated with the pink shells and, within these, with the effectively unbanded shells and 00300, and

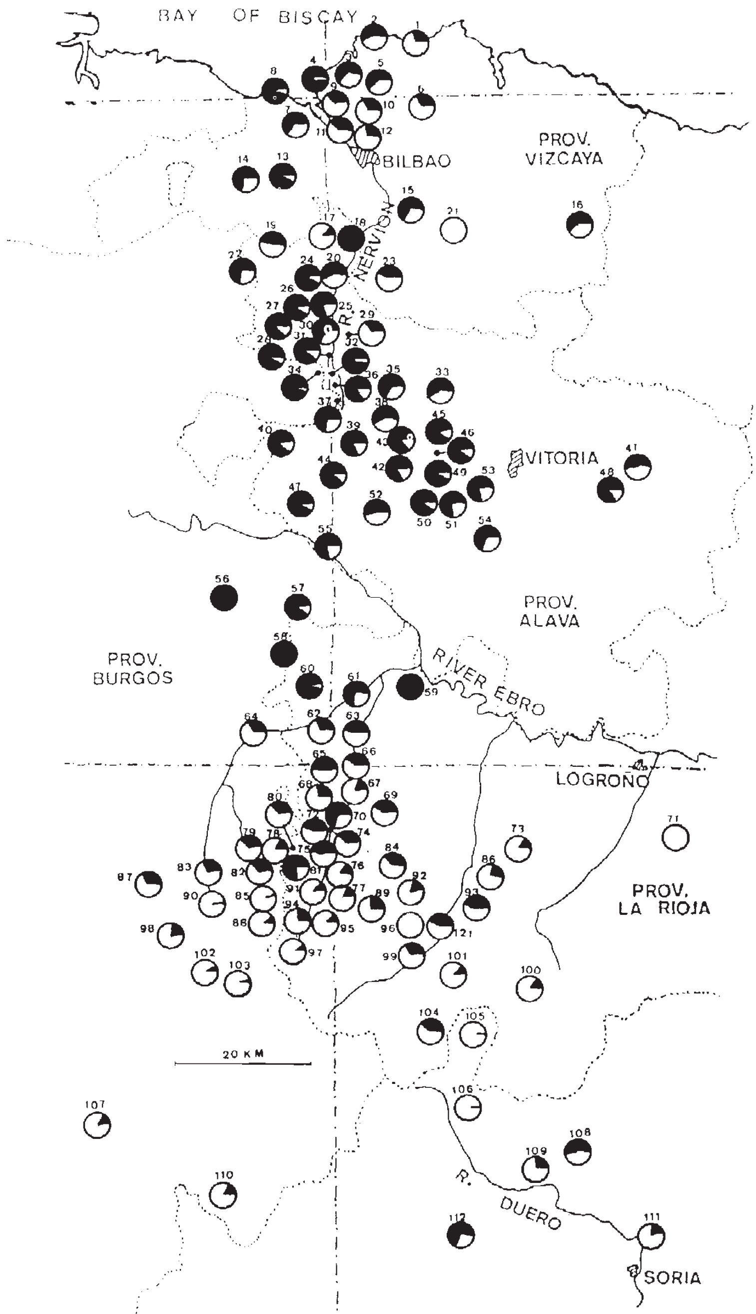

Figure 2 Distribution of yellow-shelled phenotypes in the area of study. (Yellow frequency in black.)

negatively with the yellow shells and, within these, with the banded shells, fusion banded and 12345. No association exists with temperatures.

Another characteristic studied in *Cepaea nemoralis* is the colour of the lip. The majority of white lipped snails are found in Zone 2. The frequency of the white lip seems to increase with distance from the sea and with altitude (fig. 7). No associations have been found either with rainfall or temperatures.

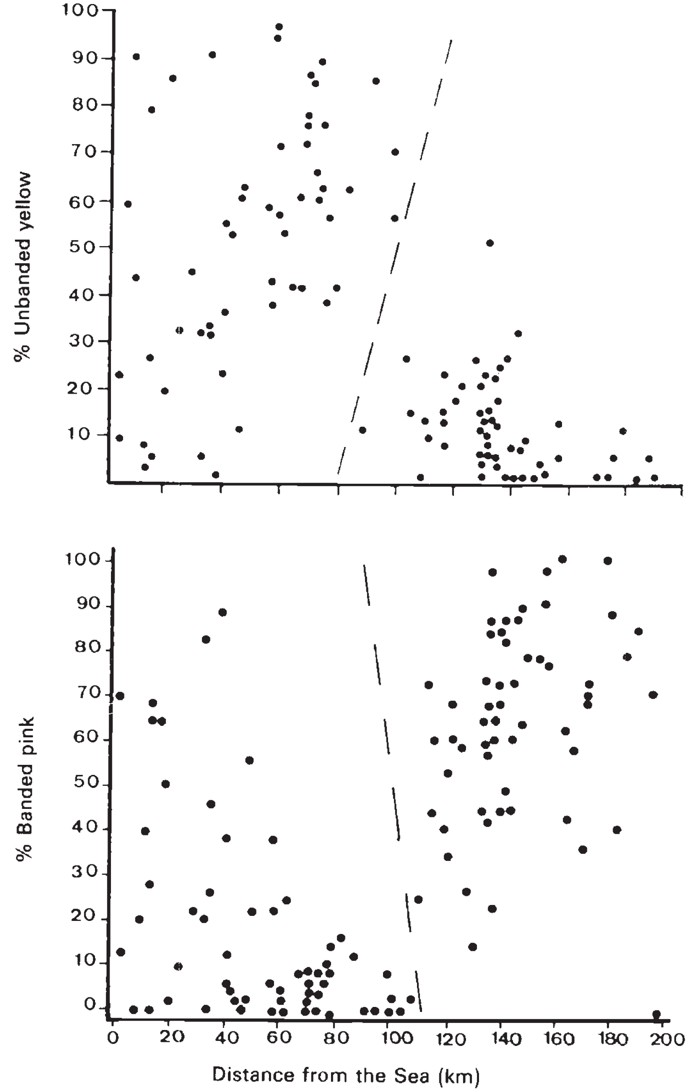

Figure 3 Representation of banded pink and unbanded yellow *Cepaea nemoralis* with respect to their distance from the sea (kmS).

DISCUSSION AND CONCLUSIONS

Great variability has been observed in the populations which we have studied (Mazón, 1984). There may be several explanations for this fact, as proposed by Jones *et al.* (1977) in the review "polymorphism in *Cepaea*: A problem with too many solutions?"

In our study we have selected some of those factors we believe may exercise influence on the maintenance of polymorphism.

Visual selection by *Turdus ericetorun* acts as a selective agent in some areas of England (Sheppard, 1951; Cain and Sheppard, 1954; Clarke, 1960). In our area of study predation by *Turdus ericetorum* has very little importance, as it appears only in a very small number of populations, and usually with very few shells predated per population. These results agree with those of Lamotte (1950, 1966) in some areas of France. As in France, the song thrush is a winter visitor to Zone 2, but is found in Zone 1 all year round.

The FCA represents the populations and phenotypes according to the distance χ^2. In fig. 4 we have the graphic representation of populations and 14 phenotypes together on axes 1 and 2 of the

Table 1 Principal significant differences (*) in phenotypes between Zone 1 and 2 of *Cepaea nemoralis*, according to parametric and non-parametric tests

Phenotypes	Zone 1 (%)	Zone 2 (%)
Yellow	70·8	32·4*
Pink	28·4	66·7*
Yellow 12345	16·8	12·8
Yellow 00345	1·1	0·5
Yellow 00300	7·0	2·7*
Yellow 00000	41·0	7·2*
Fusion banded yellow	1·5	7·2*
Hialozonate yellow	0·5	0·3
Pink 12345	10·6	26·7*
Pink 00345	0·4	1·6
Pink 00300	4·6	12·5*
Pink 00000	9·3	6·6
Fusion banded pink	1·7	15·0*
Hialozonate pink	0·9	0·4
Five-banded	27·4	39·5*
Mid-banded	11·6	15·3
Unbanded	50·2	13·9*
Fusion banded	3·2	22·2*
Effectively banded yellow	21·2	21·6
Effectively banded pink	13·2	45·5*
Effectively unbanded yellow	49·6	10·8*
Effectively unbanded pink	15·2	21·2
Effectively banded	34·4	67·1*
Effectively unbanded	64·7	32·1*
Banded yellow	29·8	25·2
Banded pink	19·1	60·1*
Banded	48·9	85·3*
00345	1·4	2·1

Table 2 Sign change of the significant correlation coefficients of some *Cepaea nemoralis* phenotypes for Zones 1 and 2

Distance from the sea Phenotypes	Zone 1	Zone 2
Yellow unbanded	+	−
Pink 12345	−	+
Unbanded	+	−
Banded pink	−	+
Banded	−	+
Yellow	+	−
Effectively banded pink	−	+
Effectively unbanded yellow	+	−
Altitude		
Yellow unbanded	+	−
Pink 00300	−	+
Unbanded	+	−
Banded pink	−	+
Banded	−	+
Effectively unbanded pink	−	+
Yellow	+	−

FCA. These axes explain 50·81 per cent of the variance. Axis 1 may be interpreted in two ways: firstly as the axis which separates Zone 1 and 2, if we take into account the distribution of the populations, and secondly taking phenotypes into account, from which it may be observed that this axis reflects the shell darkening of *Cepaea nemoralis*, *i.e.*, 1) on the side (left) it is pale in colour, understanding pale to mean phenotypes Y00000, Y00300, effectively unbanded yellows, etc. 2) on the other side (right), the phenotypes are darker (fusion banded pink, fusion banded yellow, pink 12345, etc.). In short, we may say that axis 1 separates the populations in Zones 1 and 2 according to the darkening of the shell.

The interpretation of axis 2 is more difficult, although it also seems to reflect the degree of darkening of the shells, but in this case for the pink phenotypes, though the fusion banded yellows are also included. The sampled populations from the eastern region of the Iberian range are situated in the upper right-hand part of fig. 4, forming a separate group.

From all the analyses carried out, we may conclude that two different zones exist in the area which we sampled, and moreover that in zone 1 the most frequent phenotypes are the pale-coloured ones, whereas in Zone 2 they are dark (figs. 1–4).

Between Zones 1 and 2 there is a series of populations with intermediate characteristics which we have called "Transitional populations".

Most of the populations of Zone 1 which are classified in Zone 2, according to FCA, correspond to woodland populations, which indicates that these populations are darker in colour than those of the grasslands, this may also be observed in figs. 1 and 2, where populations 1, 9, 10, 11, and 17 were sampled in woodland areas. This is in accord with the preference of dark snails for shaded places (Lamotte, 1959, 1966; Jones *et al.*, 1977), although this should be confirmed by a sampling of a higher number of woodland populations in Zone 1.

The division of the area of study into Zones 1 and 2 coincides with the existence in the Iberian-Peninsula of two climatic regions. Zone 1 is included in the so-called "Green Iberia", which has a Western European climate, and the same pattern all year round: mild winters, cool summers, moist air and abundant clouds bringing frequent rain (Font, 1983). However, Zone 2 is included in "Brown Iberia", and its climate is Mediterranean, with hot, dry summers, harsh winters, abundant sunshine, very irregular rainfall and drastic changes from summer to winter. The Transitional Zone coincides with the change of climatic zones.

Opposite associations were found for the same phenotypes between Zones 1 and 2 as regards

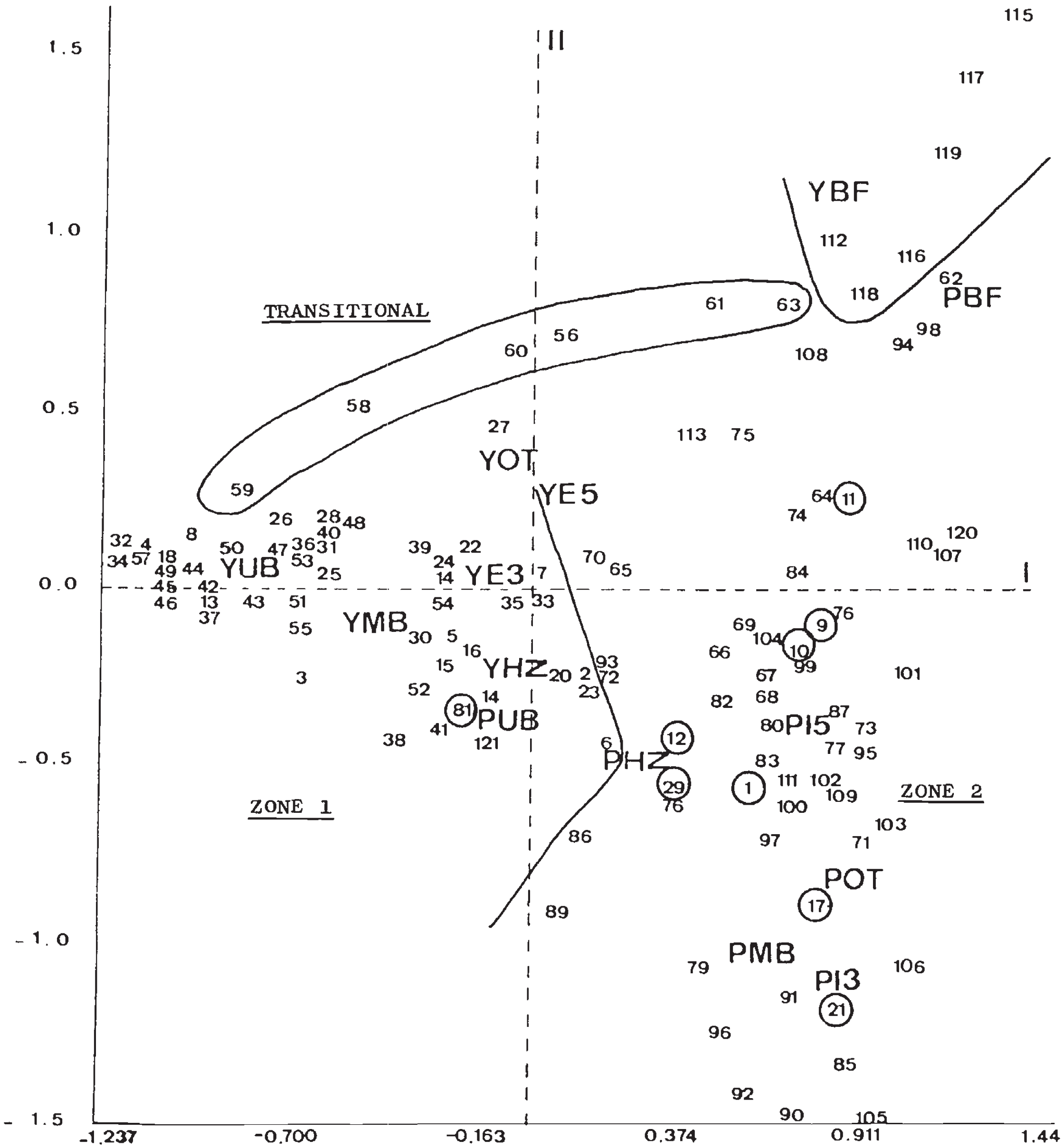

Figure 4 Simultaneous representation of *Cepaea nemoralis* populations and phenotypes on axes 1 and 2, obtained through Factorial Correspondence Analysis (FCA). (YE5 = Yellow 12345; YE3 = Yellow 00345; YMB = Yellow 00300; YUB = Yellow 00000; YBF = Fusion Banded Yellows; YOT = Other Yellows; PI5 = Pink 12345; PI3 = Pink 00345; PMB = Pink 00300; etc.)

altitude and distance from the sea. Similarly, in Zone 1 several associations were found between certain morphs and climatic parameters such as rainfall and temperatures, this last point may be due to the fact that the climatic conditions of this zone are mild, and without sudden changes, which may lead to greater association between morphs association with temperature is nil, or very low. This may be interpreted as being due to the drastic changes in weather pattern between the seasons in "Brown Iberia". Furthermore, this area is very mountainous, which can give rise to microclimate phenomena like the accumulation of cold air in the valley-bottoms, snails with dark phenotypes

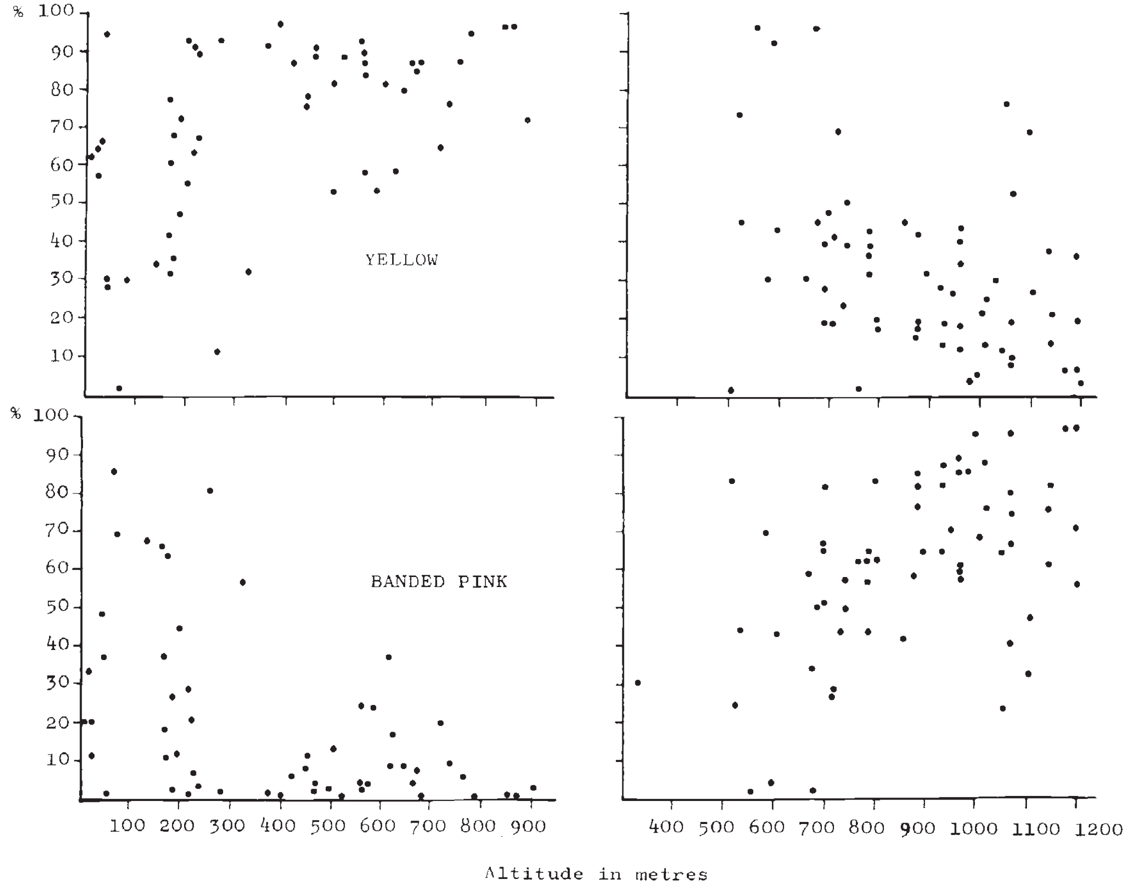

Figure 5 Variations of banded pinks and yellow shells with altitude in Zones 1 and 2. (Zone 1 on the left, Zone 2 on the right)

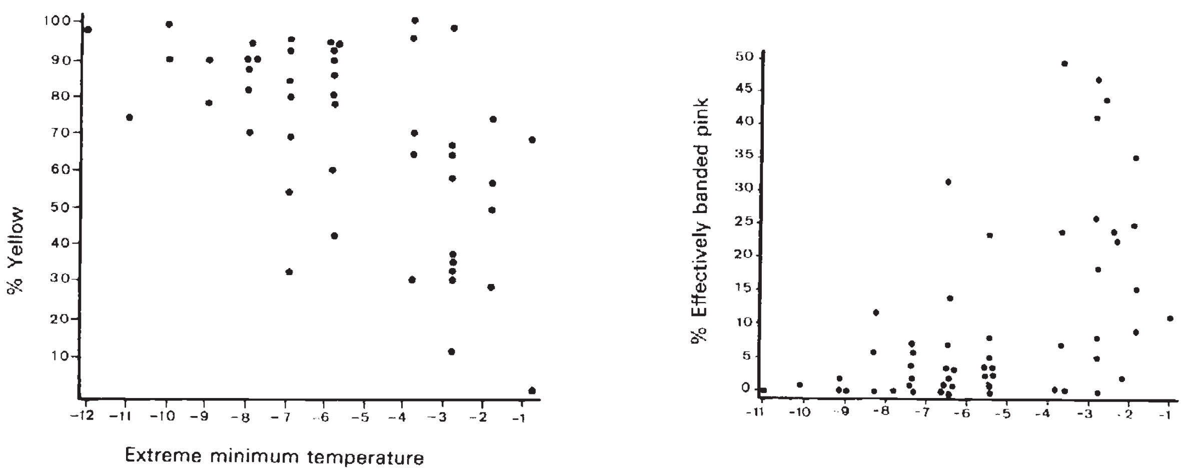

Figure 6 Representation of effectively banded pink and yellow shells of *Cepaea nemoralis* in Zone 1 for the extreme minimum temperatures of the year.

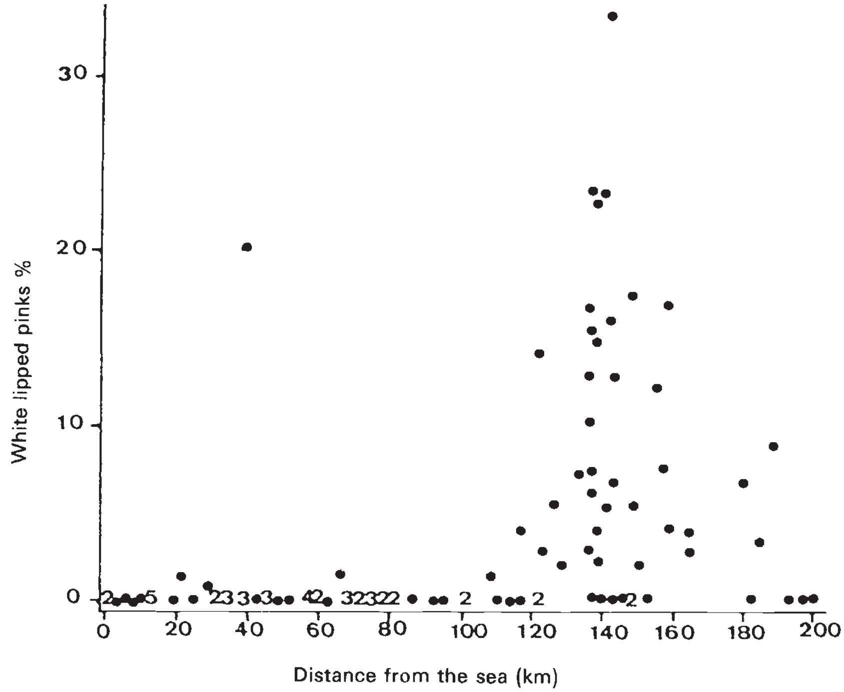

Figure 7 Representation of white lipped *Cepaea nemoralis* with respect to the distance from the sea.

being found in these places (Cain, 1968; Cain and Currey, 1963).

Similar results were found by Cameron, Carter and Haynes (1973) North and South of the Pyrenees. Between Noguera Ribagorzana and valley of Jueu the morphological frequencies for colour and banding go in opposite directions. Furthermore, in the valleys located to the North of the Pyrenees they found a high level of consistency between the results, while on the southern side there are apparent inconsistencies. These are attributed to the Mediterranean climate, which produces great microclimatic differences. Zone 1 would seem to behave like zone North of the Pyrenees, and Zone 2 like the southern zone.

When rainfall increases there is a tendency for the yellow phenotypes to decrease, and for the pinks to increase in both zones, these results are similar to those found in Brittany by Guerrucci-Henrion (1966). This is highly important, since rainfall decreases with respect to the distance from the Sea in Zone 1, and increases again in Zone 2. The influence of rainfall on the degree of shell darkening is very uneven, depending on the zone, since in Zone 1, the banded pinks are positively associated with it, while in zone 2 the association is positive with pinks 00300. However, the unbanded yellows are negatively associated in Zone 1, and the banded yellows are positively associated in Zone 2. In may be observed that the yellows are negatively associated with some of the temperatures. This seems to indicate that the yellows are more resistant to the colder conditions in this Zone, which is in accord with Lamotte (1966). Although Jones *et al.* (1977) say that the yellows are more frequent in the warmer part of the species range, this possible disagreement may be due to the fact that in our zone they are also correlated with rainfall, the yellow shells being found in the coldest and driest part of Zone 1.

According to Jones *et al.* (1977), the white lipped allele is linked to very wet areas. However, in the area which we studied, this is not so. The white lip is mainly located in Zone 2, which is drier and colder than Zone 1.

All this suggests that the distribution of our populations in Zones 1, intermediate and 2 is not due to any one factor, but rather to the conjunction of several factors which would cause zonal variation, one factor will only manifest itself when this supposes extreme conditions for the survival of *Cepaea nemoralis*. This could be the reason why various authors have found that the same phenotype is correlated with opposing climatic parameters, hot-cold, dry-wet, (see Table 1 of Jones *et al.*, 1977).

We do not know if the effects of climatic are due to the adaptive value of the shell phenotypes or to changes in habitat produced by the climate, although there exists a series of studies which suggest that the phenotypes have an adaptive value (Heath, 1975; Garcia, 1978; Bantock, 1980, Tilling, 1983).

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