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CORRELATED PHENOTYPIC RESPONSES TO HABITAT DIFFERENCE IN *CEPAEA NEMORALIS* (L.)

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ABSTRACT: Using data from the Evolution Megalab Project paired samples of *Cepaea nemoralis* (L.) coming respectively from woodland and open habitats have been examined for joint response to habitat difference at different polymorphic loci. Throughout the range of the species there is a tendency for open habitat samples to have different frequencies at shell colour and pattern loci from those in neighbouring woods. In Britain, the chance that the frequency of yellow is higher in open than in wooded habitats is about 67 per cent. There is a 41 per cent chance that they will have both higher frequency of yellow and a lower frequency of unbanded at the linked banding locus. Responses of unbanded and the unlinked mid-banded locus are to a large extent independent, however. The chance that open habitats have higher yellow and a lower value for the sum of unbanded and mid-banded (effectively unbanded) is 42 per cent, while the chance that the open habitat sample is more yellow, less unbanded and less mid-banded is no more than 19 per cent. The colour, but not the banding difference was also found in the data for continental Europe. The effect of habitat acts within a polymorphic system. For Britain closely spaced sample pairs have an average frequency difference (Euclidean distance) between habitats at the three loci of about 0.26. As a result of other factors affecting the polymorphism this difference increases to 0.43 for pairs 1 km apart and 0.59 at 10 km apart. These results extend the original findings of CAIN & SHEPPARD (1954) and others but show clearly that the habitat is only part of the explanation for polymorphism in *Cepaea*.

KEY WORDS: polymorphism, *Cepaea nemoralis,* linkage, habitat, distance

INTRODUCTION

In their study of shell colour and pattern polymorphism in *Cepaea nemoralis* and its causes CAIN & SHEPPARD (1950, 1954) collected numerous samples in a region of the English midlands where mature agricultural land is separated in places by stretches of deciduous woodland. They noted that in this mosaic of land use samples from woodlands tended to have darker coloured and more uniformly patterned shells than those inhabiting hedgerows or grassland. They interpreted this association as background matching brought about by selective predation. To demonstrate the difference they plotted the frequency of yellow, as distinct from pink or brown individuals on the frequency of morphs which lacked the upper two bands on the shell, which they called "effectively unbanded". The main component of this category is completely unbanded, controlled by a dominant gene linked to the colour locus and allelic with 5-banded. At an unlinked locus a dominant allele, mid-banded, suppresses all bands but the central one on a banded shell. Other rarer phenotypes are also included. Woodland samples tended to have relatively lower frequencies of yellow and higher frequencies of effectively unbanded than those from open habitats. In their early samples CAIN & SHEPPARD (1950, 1954) found that as they moved from woodland to open conditions the change in effectively unbanded frequency is approximately as great as the change in yellow frequency. Since the effectively unbanded category was made up of a variety of genotypes controlled by unlinked loci they con-

cluded that selection acted on the phenotype rather than on any particular genetic locus, and considered that this gave support to predation as the causative agent. The alternative would be that darker morphs had a physiological advantage over pale ones in enclosed woodland conditions (LAMOTTE 1951).

In the same period some studies revealed districts where this habitat association did not operate (CAIN & CURREY 1963). Others were designed to enlarge the information on woodland, open habitat differences (e.g. CLARKE 1962, CURREY et al. 1964, CARTER 1968, GREENWOOD 1974, CAMERON & PANNETT 1985). Reviewing these results, COOK (2008) found that although the difference in frequency of colours was strongly supported, the difference in unbanded frequency was less strong and variation in mid-banded frequency was inconsistent. The Evolution Megalab (SILVERTOWN et al. 2011, WORTHINGTON et al. 2011) assembled data from across the range of the species. The data include published records (the old series) dating mostly from the 1950s to 1970s and collections made in the 21st century (the new series), mostly by the public in 2009 for an internet-based survey organized from the Open University. This again confirmed that while colour was affected by habitat, banding morphs showed no clear overall associations. This conclusion was also reached by LAMOTTE (1966) in a general analysis of populations in France.

CAMERON & COOK (2012) used nearest neighbour pairs of samples from the two habitats, using data derived from the Evolution Megalab project, to investigate the relationships more fully and to determine the geographical area over which habitat association is found. An overall colour association was amply confirmed in this more detailed analysis but there was marked geographical variation in strength of the relationship and the difference in reduced band frequency between the two habitat types was often weak or non-existent, though significant in parts of England. By contrast, in one detailed local study in Poland (OŻGO 2011) effectively unbanded was more frequent in open than in shaded habitats. There is a need to quantify the relative strengths of the effects of habitat on colour and banding, and to explore the extent to which the strong linkage between colour and the presence or absence of bands is reflected in differing disequilibria between habitats. Here we investigate more fully the extent to which differences with habitat depend on correlated effects of aspects of the phenotype.

MATERIALS AND METHODS

It is worth noting that since the mid-20th century the use of the word habitat in animal ecological studies has undergone some change in meaning. As used by, for example ELTON & MILLER (1954) and ODUM (1959), it meant the place where an organism lives, as distinct from the niche, which refers to what it does. Now, the term may have a more inclusive sense to represent all the abiotic and biotic factors that influence the organism's success (DENNIS 2010). CAIN & SHEPPARD (1950, 1954) divided their samples into those with a tree canopy (woodlands) and those from hedges, herbage and grass, which could be kept separate or grouped as open habitats. Each group was heterogeneous, influenced by a variety of physical factors, preyed upon by a range of vertebrate and invertebrate predators, together representing only a part of the full range of conditions in which *Cepaea* can exist. Collectors of Evolution Megalab data were asked to allot their samples to one of several categories, allowing us to assemble open or woodland groups for comparison. We refer to these groupings as habitats, while recognizing the underlying complexity.

The banding patterns distinguished in the new material are mid-banded and many banded. Consequently, Cain and Sheppard's effectively unbanded class has to be approximated as the sum of unbanded plus mid-banded, leaving those with the major trifasciate gene (*trifasciata, listeria*) or with multifactorial or non-genetic reduction of the upper two bands to be included among many banded. An earlier examination found that in British data at least this made little difference to the result (COOK 2008), although that may not be the case throughout the distribution of the species.

The sub-set of the Evolution Megalab data used by CAMERON & COOK (2012) in the previous analysis is re-examined. It consists of a total of 870 pairs of samples, drawn from the old and the new periods, one member of each pair being from woodland and one from an open habitat. Overall, woodland samples are the less common. The data were therefore searched to find the nearest open habitat sample to each one from woodland. In some places samples are more widely spaced than in others. The absolute upper limit included is 50 km but the great majority of pair members were much more closely spaced. Median distances apart are 0.6 km for old samples and 1.3 km for new. Where samples were recorded only as being in the same 100 m grid square, it is not possible to determine the precise distance. The three colour classes yellow, pink and brown are distinguished and within each, shells are scored as unbanded, mid-banded or many (usually 5) banded.

CAMERON & COOK (2012) scored the median difference between habitats for yellow and the three banding classes, and the number of samples in which the median in one habitat exceeded that in the other. Results were considered separately for different dates,

from different countries throughout Europe, and in some cases for subsections within countries. An excess of yellow in open habitats compared with woods was widespread but only in Britain, especially England south of 53°N, were there significant differences in both colour and banding, and in some countries the difference in banding frequency did not go in the expected direction. Different parts of Europe are represented in the old and new data sets. There was a suggestion that southern Europe responds differently from northern Europe. In view of the apparent geographical variation and of the fact that subdivision necessarily reduces sample size, we have here combined the data for both periods and made three groups: Britain (GB, 432 pairs), northern Europe (NEU, 334 pairs) and southern Europe (SEU, 104 pairs), the separation being made at 47°N. Using these groupings we have examined the relative contribution of unbanded and mid-banded, compared with yellow, to the difference with habitat, and the effect of increasing separation of habitat pairs on the level of similarity.

Comparisons of data have been made using pair-wise t-tests and analysis of variance. Rayleigh's test of polar coordinates has been used to test for non-randomness of direction of difference in frequencies between habitats and chi squared to test for heterogeneity. Disequilibrium of distribution of phenotype frequencies between loci has been calculated for pink/yellow and unbanded/banded at the linked colour and banding loci. Browns have been excluded because browns are known to have lower frequencies of banding than the other two classes. If we define frequencies of the four colour/banding phenotypes in a sample as *pu*, *yu*, *pb*, *yb* the estimated gametic disequilibrium D is

$$\sqrt{yb} - \sqrt{[(pb + yb)(yu + yb)]}.$$

Samples may differ in frequency at each of three loci. In order to assess the total phenotypic difference between a pair of samples, one from an open and one from a woodland site, we have therefore calculated the absolute difference in frequency at each locus (the Euclidian distance). This is measured as

$$\sqrt{(Y_{(o)} - Y_{(w)})^2 + (U_{(o)} - U_{(w)})^2 + (M_{(o)} - M_{(w)})^2}$$

where the letters refer to phenotype frequencies in open or wooded sites.

RESULTS

RELATIVE CONTRIBUTION OF BAND REDUCTION TO VARIATION WITH HABITAT

Differences between woodland and open habitats have been calculated by subtracting the open habitat frequency from the woodland frequency for each phenotype. Mean differences and t-values measuring deviation from zero are shown in Table 1. There are higher frequencies of yellow in open habitats in all regions. There are lower frequencies of unbanded and of mid-banded in GB open habitats but no significant difference in Europe. Morph frequencies are highly variable from one place to another in *C. nemoralis,* so that open, woodland pairs are to be found throughout the phenotype space when plotted as the classic Cain and Sheppard diagram, which shows yellow frequency on frequency of effectively unbanded. In order to see the trend in the data more clearly results have been plotted as yellow on the reduced banded class, but with the frequency in the woodland member of each pair placed at the origin. For yellow on unbanded in GB the trend is shown in Figure 1.

The spread of points representing different directions of change in frequency is very wide. It is clear, however, that there are more in the upper half of the diagram than in the lower and that the highest density is in the upper left, or in terms of compass points, the North-West quadrant. Using Rayleigh's test the distribution is significantly non-random ($z = 34.2$, $P<0.001$). The heterogeneity χ^2 testing the distribution of samples between the four quadrants is 16.3 ($P < 0.001$, Table 2a). The difference in mean frequency of yellow between open and woodland habitats is 0.1405 (Table 1). The difference in mean for unbanded is –0.0316 and the difference for mid-banded is –0.0214. Displacement of all three characters is significant and in the expected direc-

Table 1. Mean difference between frequencies in open habitats and woods in three regions. GB – British Isles, NEU – Europe north of 47°N, SEU – Europe south of 47°N. Asterisks indicate significance levels of tests: * – P<0.05, ** – P<0.01, *** – P<0.001

Region	n	Yellow	t	Unbanded	t	Mid-banded	t
GB	432	0.1405	10.05***	–0.0316	2.60*	–0.0214	2.06*
NEU	334	0.0482	3.06**	0.0044	0.28	–0.0019	0.17
SEU	104	0.0744	2.62*	–0.0156	0.53	0.0138	0.89

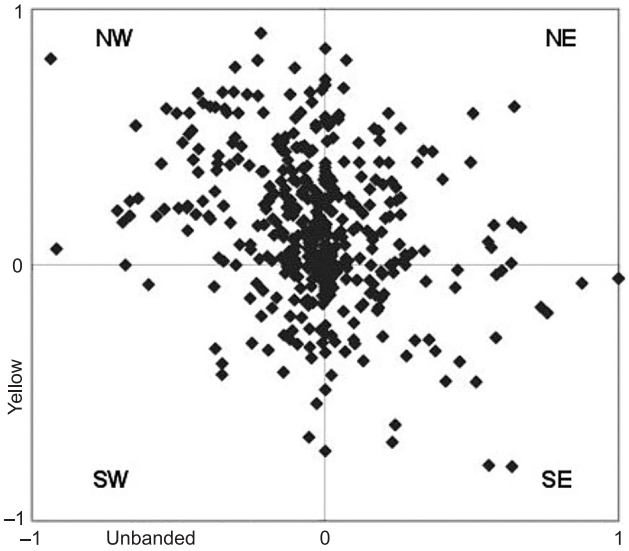

Fig. 1. Change in yellow on change in unbanded for British samples. Each point represents difference between open habitat frequency and woodland habitat frequency for a sample pair with woodland samples located at the origin

tion, with colour frequency being shifted to a greater extent than either banding category.

CAIN & SHEPPARD's (1950, 1954) proposal was based on the direction of change rather than its magnitude. To examine this we can consider the number of data points falling in the four quadrants. In Table 2a the results for GB have been arranged in a clockwise direction with the critical upper left quadrant (NW on Fig. 1) at the left of the table. Using these figures there are 288 cases (179 + 109) in 432, or 67 per cent, in which yellow is at a higher frequency in the open than in the woodland habitat. The fraction in which the open habitat member of a pair is both more yellow and less unbanded than the woodland member is 0.414. Similarly, the fraction that is both more yellow and less mid-banded is 0.338 but the heterogeneity of distribution of points between quadrants is not significant ($\chi^2 = 3.14$, $P > 0.05$). The fraction that is both more yellow and has fewer unbandeds and mid-bandeds (approximately the effectively unbanded category) is 0.419, so that effectively unbanded is no improvement on unbanded alone. When yellow is plotted on mid-banded Table 2b allots the 288 cases where the open habitat has the higher frequency of yellow to different groups according to whether they are more or less unbanded and more or less mid-banded. Only 80 cases from the original 432 have the open habitat yellow frequency higher and the unbanded and mid-banded frequencies both lower than in woodland.

These calculations cannot usefully be applied to either of the European groupings. For NEU the difference in unbanded is almost zero but positive (the mean frequency of unbanded is higher in open habitats than in woods) and in SEU likewise the difference in mid-banded is near zero but positive. The data we have available from Europe do not support a response by unbanded or mid-banded to habitat difference.

PHENOTYPE ASSOCIATIONS WITHIN HABITATS

Among samples from each habitat considered separately, there are both broad geographical trends in mean morph frequencies and associations between morph frequencies at different loci (Table 3). As expected (SILVERTOWN et al. 2011) mean frequencies of yellow and unbanded increase from GB through NEU to SEU in both habitats. Within GB both unbanded and midbanded are negatively correlated to yellow in both habitats, while the two effectively unbanded morphs associate positively. Although significant, the strength of these associations is not great. In NEU, the morphs appear to vary independently, while in SEU the relationship between yellow and unbanded is positive. These differences might reflect selective forces

Table 2. Distribution of values for differences between pairs (GB data) using the quadrant system shown in Fig. 1. In Table 2a, the NW quadrant has yellow (Y) > 0, and the banding category, unbanded (U), mid-banded in banded (M), or the combination of both (U + M) < 0; in the NE quadrant, Y > 0 and banding category ≥ 0; in the SE, Y ≤ 0, banding category ≥ 0; and in the SW both Y ≤ 0 and banding category < 0. Table 2b shows the distribution of values of U and M among pairs where Y > 0 (NW and NE quadrants). The χ^2 values test heterogeneity with respect to the two-way classification in the four quadrants.

Table 2a)

	Total	NW	NE	SE	SW	χ^2	fraction in NW
Y on U	432	179	109	84	60	16.30***	0.414
Y on M	432	146	142	84	60	3.14	0.338
Y on U+M	432	181	107	79	65	12.28***	0.419

Table 2b)

	U < 0	U ≥ 0	totals
M < 0	80	66	146
M ≥ 0	99	43	142
totals	179	109	288

Table 3. Mean phenotype frequencies in each habitat in the three regions, and the relationships between them. The last three columns show correlation coefficients between pairs of morphs and their significance. Significance levels as for Table 1

Table 3a) woods

	n	mean Y	mean U	mean M	r(Y,U)	r(Y,M)	r(U,M)
GB	432	0.3725	0.2642	0.3330	–0.271***	–0.135**	0.124**
NEU	334	0.4953	0.2844	0.3146	–0.041	–0.199***	0.008
SEU	104	0.6377	0.3169	0.1924	0.400***	0.059	0.260**

Table 3b) open habitats

	n	mean Y	mean U	mean M	r(Y,U)	r(Y,M)	r(U,M)
GB	432	0.5141	0.2326	0.3071	–0.197***	–0.167***	0.249***
NEU	334	0.5435	0.2884	0.3088	0.026	–0.054	–0.088
SEU	104	0.7121	0.3013	0.1992	0.344***	0.061	0.176

Table 4. Mean values of estimated gametic disequilibrium for different pairs of loci in the different habitats and regions. Values in the t(diff) column measure significance of differences between habitats, the other t-values measure differences from D = 0. Significance levels as for Table 1

Table 4a) colour/banding

	n (pairs)	D open	SE	t	D woods	SE	t	t(diff)
GB	302	0.0050	0.0027	1.84	0.0140	0.0033	4.26***	2.12*
NEU	289	0.0200	0.0037	5.41***	0.0201	0.0035	5.71***	0.03
SEU	80	0.0021	0.0045	0.46	–0.0080	0.0061	1.32	1.33

Table 4b) colour/mid-banded in banded

	n (pairs)	D open	SE	t	D woods	SE	t	t(diff)
GB	261	–0.0021	0.0035	0.59	0.0029	0.0033	0.89	1.04
NEU	264	0.0029	0.0031	0.94	0.0072	0.0038	1.88	0.88
SEU	73	–0.0053	0.0051	1.02	0.0074	0.0041	1.82	1.93

acting independently on each locus, or selection favouring certain combinations in individual snails. Hence, it is of interest to estimate the disequilibrium D between alleles at the different loci. The results are given in Table 4a. There is a significant difference between habitats only in GB. In northern Europe and Britain mean values are positive and relatively high, in three cases differing significantly from the null expectation of zero. A similar comparison has been made for comparison of the unlinked colour and mid-banded (Table 4b). In this case both browns and unbandeds of other colours have been excluded. No estimates differ significantly from zero, so there is random assortment.

VARIATION IN DIFFERENCE WITHIN PAIRS IN RELATION TO DISTANCE

Throughout its range morph frequencies in *C. nemoralis* are highly variable from one place to another, so that differences in frequencies between habitats are likely to be confounded with geographical variation (CAMERON & PANNETT 1985). To investigate the relationship between habitat effects and geographical variation we first partitioned the total variance in colour, banded and mid-banded frequencies into components measuring between-habitat difference and difference between the means for each pair. The results are shown in Table 5. The between habitat component for the colour category yellow is significant in all regions. The two banding categories show a significant habitat effect only for GB. There is always significant heterogeneity between sample pairs, at a similar level in all regions, indicating that they have been picked from a set of populations with heterogeneous frequencies. In the present data there is a wide range of distances between members of a pair. It is therefore possible that members of habitat pairs that are closely spaced will exhibit predominantly a habitat effect, which may be overlaid by other factors as distance between members of pairs increases.

Regression analysis has been carried out on Euclidean distances to investigate how overall difference in frequency between members of pairs varies with their

Table 5. Results of analysis of variance showing significance of differences between habitat type and pair means (location) for different phenotypes and regions. Significance levels as for Table 1

Category	Total cases	F(habitat)	P	F(location)	P
Yellow					
GB	864	100.99	***	2.89	***
NEU	668	9.34	**	2.74	***
SEU	208	6.87	**	2.90	***
Unbanded					
GB	864	6.74	**	3.05	***
NEU	668	0.08		2.05	***
SEU	208	0.28		2.58	***
Mid-banded					
GB	864	4.25	*	3.94	***
NEU	668	0.03		2.89	***
SEU	208	0.79		2.52	***

Table 6. Regression of difference in frequency between habitat types and morphs on logarithm of geographic separation of pair members. Rows labeled combined give Euclidean distance. n – number of pairs; b – regression coefficient; t – t-test of b = 0; a (10) – projected frequency difference at 10 km; a – intercept (at 1 km); a (0.1) – projected frequency difference at 0.1 km

Category	n	b	SE(b)	t	a(10)	a	SE(a)	a(0.1)
GB combined	**432**	**0.0726**	**0.0066**	**10.99**	**0.592**	**0.426**	**0.0109**	**0.261**
GB Y		0.0408	0.0060	6.77	0.359	0.265	0.0099	0.173
GB U		0.0441	0.0054	8.19	0.292	0.191	0.0089	0.089
GB M		0.0215	0.0048	4.53	0.207	0.158	0.0079	0.108
NEU combined	**328**	**0.0423**	**0.0064**	**6.64**	**0.462**	**0.366**	**0.0121**	**0.270**
NEU Y		0.0281	0.0055	5.13	0.271	0.207	0.0104	0.144
NEU U		0.0174	0.0052	3.37	0.227	0.188	0.0098	0.149
NEU M		0.0137	0.0043	3.16	0.169	0.137	0.0083	0.106
SEU combined	**104**	**0.0560**	**0.0130**	**4.29**	**0.428**	**0.313**	**0.0307**	**0.197**
SEU Y		0.0387	0.0105	3.69	0.255	0.176	0.0250	0.098
SEU U		0.0287	0.0109	2.63	0.243	0.183	0.2530	0.122
SEU M		0.0048	0.0064	0.74	0.104	0.096	0.0150	0.089

distance apart. If habitat has a characteristic effect on frequency but widely-spaced pairs are more different from each other than closely-spaced pairs then the regression of frequency difference on distance between members of a pair should be non-zero and positive. The regression analyses are given in Table 6. The result for GB, combined values is illustrated in Fig. 2. There is clearly an increasing trend in all three regions, least expressed in SEU and by the mid-banded morph. For combined values the relation is steeper for GB than for the other regions (comparison of GB and NEU, $t = 3.30$, $P<0.05$). Comparison of intercept values shows that the difference between habitats is greatest for GB (for comparison of GB with NEU and SEU $t = 3.68$ and 3.47 respectively, $P<0.001$ and <0.01). The average distance between members of a pair is also higher in Europe than in Britain. For GB the regression line passes through 0.26 at 0.1 km. While this difference (between closely spaced samples) is not entirely due to the effect of habitat alone (some pairs showing a direction of change opposite to the general trend), it is less than that seen at greater distances. At 1 km the difference has increased to 0.43. It continues to rise with increasing distance between pairs but will level out since the species is polymorphic for these phenotypes throughout its range.

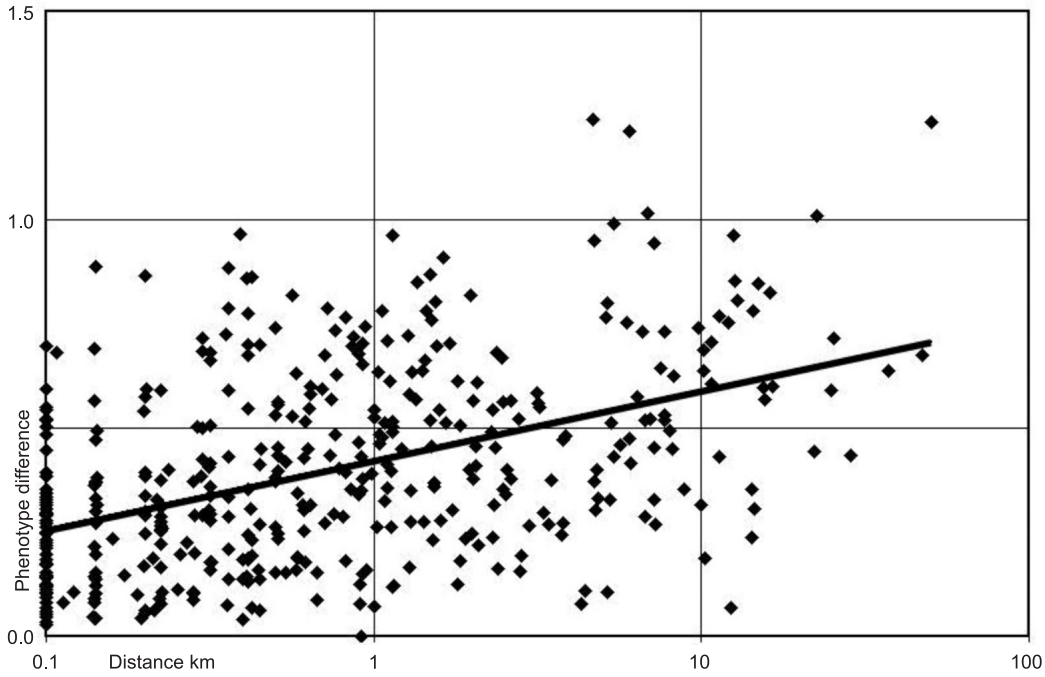

Fig. 2. Relation between total phenotypic difference between samples in a woodland, open habitat pair and distance apart of the pair. Data for GB, 3 phenotypes

DISCUSSION

As this and other studies show (summaries in COOK 1998, 2008, SILVERTOWN et al. 2011, CAMERON & COOK 2012), morph frequencies in *Cepaea nemoralis* vary with habitat, but to differing extents and in different ways according to region. This variation may involve more than one locus, and the aim of this study was to examine the interaction of variation among them and the effect of distance on the relationship. Clearly, several processes are involved and the interactions of variation at different loci are important. The result here confirms that the two unlinked band-reducing phenotypes show association with habitat, sometimes individually and sometimes together within Britain. For continental Europe the difference in yellow frequency between habitats is demonstrated but there is no evidence of an effect on the banding categories. These differences relate to the long-standing debate about the relative importance of selection by predators and the effects of microclimatic differences between habitats (COOK 1998). Although there is certainly evidence for selective predation (CAIN & SHEPPARD 1954) a number of local studies suggest that microclimate is involved (LAMOTTE 1966, OŻGO 2011, OŻGO & BOGUCKI 2011).

While the effect of habitat on variation at more than one locus is evident in Britain, its strength is variable (CAMERON & COOK 2012), and its predictive power is low. The probability that the next pair of samples an observer examines will exhibit the expected pattern is not high. When yellow is plotted on unbanded for the paired sample data from Britain, the numbers of samples in the four quadrants indicate a probability of 0.67 (against the random expectation of 0.5), that the open habitat sample has more yellows than the woodland sample. The probability that it is more banded is 0.55, while the probability that it is both is no more than 0.414 (against the random expectation of 0.25). When yellow frequency is related to effectively unbanded the probability shows no increase (at 0.419). CAIN & SHEPPARD (1950, 1954) were fortunate in their location when they showed a clear distinction in both characters between the two habitat types. Within Britain, some regions show a much weaker connection between habitat and morph frequencies (CAMERON & COOK 2012). Outside Britain, only the association of yellow frequencies and habitat is maintained, and it is generally not so strong. As in Britain, there are many pairs showing a contrary trend. While some of these discordant results may be a consequence of drift or founder effects dependent on the history of closely associated populations (CAMERON & DILLON 1984), they also result when distances between members of a pair are increased (see below).

Where there are trends for variation with habitat at more than one locus, these may arise either as independent selection on each, or as a result of selection favouring particular combinations. The latter is easier

to detect when the loci are tightly linked and a selective event may have a lasting effect over several generations, as in colour and banding, than where loci are unlinked, as with mid-banded and the colour and banding loci. In no case here are there significant differences in the distribution of mid-banded in banded between pink and yellow shells. By contrast, disequilibria in the distribution of unbanded between colours show a significant excess of unbanded in pink in three out of six comparisons, and are in the same direction in the others. Following CAIN & SHEPPARD (1954), a disequilibrium favouring pink unbanded and yellow banded might be expected in woods, where the pink shells matched the background, and yellow shells with bands broke up the solid mass of conspicuous colour; it might be less powerful in the open, where banding might provide protection regardless of colour. This expectation is met in broad surveys where samples with high frequencies of the double dominant or the double recessive combinations (pink unbanded or yellow banded) have a higher frequency of positive D values than those in which single dominant combinations are in excess (COOK 2005). There is, however, also the possibility that migration between colonies with unlike frequencies may mimic this effect. In the present case the disequilibrium is greater in British woods than in the open, although only marginally significant. Strikingly, however, the disequilibrium is strongest in northern Europe (and the same in each habitat), where there is no evidence of a habitat effect on unbanded alone. Southern Europe shows no evident trend, and in other studies the disequilibrium tips towards excess of unbanded in yellow southwards (CAMERON et al. 2011).

This and other evidence suggests that there may be selective forces operating on more than one locus independently of habitat. There are significant among sample associations between morphs at different loci, in reverse directions in Britain and southern Europe, and these are similar in both habitats. These relate to the large scale changes across the range; even within a habitat, the circumstances that favour yellow in southern Europe also favour unbanded (high temperatures and insolation), while the reverse is true in Britain. Within each region, however, analysis of variance shows that among pair variation is significant even when there is also a significant habitat effect. As shown by CAMERON & PANNETT (1985) these effects are found at very local scales. Members of a pair tend to have similar morph frequencies, reflecting common ancestry. While founder effects may account for some spatial patterns, the region-wide correlations between morphs at different loci mentioned above suggest a range of selective regimes is to be found within each region. Whatever the cause, microgeographical variation is widespread (COOK 1998).

The difference between woodland and open samples is most obviously a direct consequence of habitat when the paired samples are closely spaced. Difference in frequencies increases with distance apart, indicating that the influence of habitat is progressively submerged within other factors affecting morph frequencies. There are obvious implications for any study seeking to expose relationships with habitat. Pairs less than 1 km apart are needed to disentangle the effects of habitat from a multitude of other factors that can affect morph frequencies.

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