ALLOZYMES AND AREA EFFECTS IN *CEPAEA NEMORALIS* ON THE WESTERN BERKSHIRE DOWNS

MICHAEL. S. JOHNSON*

Department of Genetics, University of Nottingham

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SUMMARY

1. Geographic variation of six loci encoding enzymes (*Est-F*, *Lap-2*, *Mdh-1*, *Gpgd*, *Pgi*, and *To-2*) and of four loci encoding shell characters (*C*, *B*, *S*, and *U*) in *Cepaea nemoralis* was examined in the western Berkshire Downs, where there is an extensive area effect for the midband gene, *U*³.

2. Significant pairwise associations of geographic variation were found for all combinations of the *U*, *S*, *Est-F*, and *Gpgd* loci. At each of these loci, an allele which is rare outside the midband area reaches moderate to high frequencies within that area. Significant associations of *C* with *U*, *S*, and *Est-F*, and of *S* with *Mdh-1* also were found. All pairwise associations reflect the area effect.

3. Multilocus geographic associations were examined by Varimax factor analysis. The midband area effect is well represented by the second Varimax axis, accounting for 20 per cent of the allelic frequency variation over all 10 loci. Variation at the *C*, *U*, *S*, *Est-F*, and *Gpgd* loci is correlated with this axis. The first Varimax axis represents structure within the midband area, accounting for 22 per cent of the allelic frequency variation, and with which several loci are correlated.

4. That several loci are associated with the midband area effect indicates that the area effect reflects substantial genetic differentiation. Whatever the cause of the area effect is, it has acted pervasively.

1. INTRODUCTION

In some regions, populations of the land snail *Cepaea nemoralis* are characterised by concentrations of particular shell morphs over large areas, irrespective of habitat background, and often with sharp transitions between adjacent areas with dissimilar morph frequencies. This phenomenon, known as "area effects", was first described in *C. nemoralis* from the Marlborough Downs in south-central England (Cain and Currey, 1963a). Subsequently, area effects have been described from several localities, most strikingly from upland chalk (Cain and Currey, 1963b; Carter, 1968; Arnold, 1971). Similar patterns of geographic variation have been found in the polynesian land snail *Partula* (Clarke, 1968; Clarke and Murray, 1971).

In order to characterise more completely the genetic basis of area effects, I have extended Carter's (1968) study of *C. nemoralis* from the Berkshire Downs by an investigation of enzyme polymorphisms.

2. MATERIALS AND METHODS

(i) *Samples*

Between September 1973, and August 1974, samples of *C. nemoralis* were collected from the western Berkshire Downs, England, at 47 sites (fig. 1),

* Present address: Department of Ecology and Evolution, State University of New York, Stony Brook, New York 11794, U.S.A.

FIG. 1.—Map of the western Berkshire Downs, showing sampling localities of *C. nemoralis*. Sample code numbers as in table 1. Solid lines are 500, 600, 700, 800, and 900 ft contours. Blackened areas are above 900 ft; hatched, 800 to 900; heavily stippled, 700 to 800; lightly stippled, 600 to 700. Dashed lines separate groups of samples discussed in text: A, western five-band area; B, western transition; C, western midband area; D, eastern midband area and transition; E, eastern non-midband area. Axes are Ordnance Survey kilometre grid references.

chosen to include the midband and five-band area effects defined by Carter (1968). Ordnance Survey Grid references and altitudes for each site are listed in table 1. Specimens from each locality were taken from as small an area as possible, always less than 30 metres in diameter. Adult individuals were marked with an identifying number and examined for allozymes and for shell banding and colour characters.

(ii) *Electrophoresis*

The snails were frozen for up to 5 months. Immediately prior to electrophoresis, dart sacs, hepatopancreas, and samples of foot were macerated separately in cold distilled water, equal volume by weight. Extracts were soaked on to filter paper inserts through a layer of filter paper.

Allozymes were separated by horizontal electrophoresis in gels of 12 per cent Electrostartch. Esterases from foot extracts, leucine aminopeptidase, malate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphoglucose isomerase, and tetrazolium oxidase were assayed, using staining recipes essentially as described by Brewer (1970). With the exception of the esterases, these enzymes were not tissue specific. Although several buffer systems were used initially, the polymorphisms included in this study were routinely scored on the Tris-EDTA-Borate system described by Markert and Faulhaber (1965), with a running time of $3\frac{1}{2}$ hours at 300 volts.

(iii) *Scoring of the characters*

The phenotypes and inheritance of the shell morphs of *C. nemoralis* are summarised by Cain *et al.* (1968). Data on four loci are presented here: colour (C^Y , yellow; C^P , pink; C^B , brown; in order of increasing dominance); bandedness (B^B , bands present, recessive to B^0 , bands absent); spread bands (S^S , spread bands, dominant to S^0 , normal bands); and midband (U^3 , single central band only, dominant to U^0 , more than one band). Since there is dominance at each locus, allelic frequencies were estimated on the assumption of Hardy-Weinberg equilibrium.

Cepaea esterases are numerous and complex (Oxford, 1973), and only one system was scored in the present study. Esterase-F (*Est-F* locus) is the most anodal of the esterases found in extracts of the foot; it is not detectable in extracts of other tissues examined. This esterase produces a pink band when provided with a combination of alpha-naphthyl acetate and beta-naphthyl acetate as substrates, indicating preferential activity on the beta-isomer. Heterozygotes at the *Est-F* locus show two zones of activity.

The leucine aminopeptidase (*Lap-2* locus) polymorphism has been described previously, consisting of three alleles and double-banded heterozygotes (Brussard and McCracken, 1974). On the Berkshire Downs, a previously unreported null allele (*Lap-2⁰*) occurs; it is scorable in homozygotes only, these lacking activity for leucine aminopeptidase 2, but not for the monomorphic leucine aminopeptidase 1.

The anodal malate dehydrogenase (*Mdh-1* locus) and 6-phosphogluconate dehydrogenase (*6pgd* locus) polymorphisms have been described previously, with heterozygotes displaying three zones of activity (Manwell and Baker, 1968; Levan and Fredga, 1972). Three alleles at each locus were detected from the Berkshire Downs. Correspondence of these alleles with those previously described was not determined.

TABLE 1

Locality information and allelic frequencies at the loci affecting colour and banding for samples of C. nemoralis from the western Berkshire Downs. Sample numbers as in fig. 1

Sample	Ordnance grid	Alt. (ft.)	N	Allele†						Correlation of C^Y and S^S
				C^Y	C^P	C^B	B^B	S^S	U^S	
A. Five-band area										
37.	205791	650	45	0.70	0.30	—	1.00	—	—	—
19.	225793	670	69	0.83	0.17	—	1.00	0.02	—	-0.07
2.	230786	600	20	0.71	0.29	—	0.79	—	—	—
21.	238821	830	38	0.59	0.41	—	0.79	—	0.42	—
35.	247825	600	64	0.65	0.24	0.11	0.75	0.01	0.02	-0.20
40.	248826	610	18	0.66	0.34	—	0.85	—	—	—
76.	250792	630	25	0.53	0.43	0.04	0.53	—	—	—
70.	251814	720	57	0.35	0.64	0.01	0.98	0.03	—	-0.09
69.	260814	670	40	0.81	0.19	—	1.00	0.08	—	0.16
3.	263789	750	37	0.82	0.11	0.07	0.94	0.02	0.06	0.09
B. Western transition area										
34.	282841	650	46	0.83	0.13	0.04	0.99	0.03	0.07	0.15
60.	284836	740	60	0.71	0.17	0.12	1.00	0.09	0.34	0.09
6.	288812	500	19	0.56	0.41	0.03	0.82	—	0.18	—
7.	290812	530	65	0.70	0.30	—	0.82	0.05	0.10	-0.16
C. Western midband area										
61.	293823	660	37	0.90	0.09	—	0.97	0.37	0.71	0.18
58.	295841	580	55	0.76	0.24	—	1.00	0.16	0.18	0.36*
75.	297847	650	44	0.99	0.01	—	1.00	0.26	0.57	0.14
10.	298837	550	49	0.58	0.42	—	0.99	0.12	0.69	0.04
17.	298862	810	51	0.79	0.21	—	1.00	0.25	0.52	0.44**
24.	300869	600	34	0.66	0.23	0.11	0.98	0.31	0.42	0.38
57.	301839	650	47	0.98	0.02	—	0.99	0.32	0.70	0.23
55.	301845	710	21	1.00	—	—	0.98	0.39	1.00	—
54.	301849	700	42	0.99	0.01	—	1.00	0.33	0.62	0.17
26.	301868	650	48	0.59	0.39	0.02	1.00	0.40	0.40	0.47*
56.	302842	700	60	0.91	0.09	—	0.99	0.25	0.87	0.15
53.	302852	760	31	1.00	—	—	1.00	0.35	0.75	—
32.	302857	800	75	0.99	0.01	—	0.99	0.22	0.25	0.25
23.	302869	600	25	0.57	0.43	—	1.00	0.42	0.43	0.20
27.	302870	550	41	0.56	0.33	0.11	1.00	0.28	0.46	0.30
33.	306864	770	21	0.95	0.05	—	0.98	0.22	0.61	0.27
59.	307838	680	53	0.99	0.01	—	0.86	0.32	0.58	0.18
66.	310863	700	21	0.84	0.16	—	0.95	0.39	0.44	-0.05
68.	311831	660	42	1.00	—	—	0.72	0.12	0.63	—
65.	312865	710	46	0.97	0.03	—	1.00	0.34	0.38	-0.23
D. Eastern midband area and transition										
67.	315837	600	36	0.94	0.06	—	0.55	0.15	0.48	0.29
52.	319826	600	40	0.94	0.06	—	0.96	0.18	0.21	0.15
51.	319831	540	55	0.97	0.03	—	0.89	0.02	0.74	0.06
8.	325833	500	22	0.85	0.15	—	0.80	—	0.62	—
41.	328828	500	38	0.78	0.22	—	0.92	0.13	0.53	-0.16
44.	329829	500	31	0.88	0.12	—	0.93	0.36	0.57	0.20
45.	329829	500	70	0.79	0.14	0.07	0.91	0.25	0.54	0.03
62.	335858	750	49	1.00	—	—	0.99	0.05	0.48	—
63.	344813	650	40	0.96	0.04	—	0.96	0.18	0.77	0.17
64.	344851	750	29	0.95	0.05	—	0.70	0.16	0.62	-0.11
14.	347847	730	54	1.00	—	—	1.00	0.36	0.88	—
12.	371873	350	45	0.95	0.04	0.01	0.98	0.02	0.42	0.06
E. Eastern non-midband area										
39.	395848	700	29	0.89	0.11	—	1.00	0.05	—	0.17

† Frequencies of B^o , S^o , and U^o are one minus the frequencies of their respective alternative alleles.

* $P < 0.05$

** $P < 0.01$ } From Yates Chi-square.

The phosphoglucose isomerase (*Pgi* locus) and tetrazolium oxidase (*To-2* locus) polymorphisms have not been described before. Each polymorphism consists of two alleles, with heterozygotes displaying three zones of activity.

At each of the six enzyme loci, alleles are labelled alphabetically, beginning with the allele encoding the most anodal allozyme. The genetic basis of the polymorphism has been demonstrated for *Lap-2* only (Brussard and McCracken, 1974), not including the null allele at that locus. Preliminary results indicate Mendelian segregation at the other loci as well, but the data will be presented elsewhere when complete. The phenotypes at each locus are similar to those of known genetic polymorphisms of the same enzymes in other organisms.

With the exception of *Lap-2*, allelic frequencies at the enzyme loci were calculated directly from observed genotypic frequencies. In those populations segregating for the null allele, *Lap-2* allelic frequencies were calculated on the assumption of Hardy-Weinberg equilibrium.

(iv) *Geographic associations of characters*

A medial test (Quenouille, 1972) was used to find pairwise geographic associations among the loci. To clarify and quantify patterns of geographic variation over all loci, a Varimax factor analysis (Gould, 1967) of all allelic frequencies (*i.e.* an R-mode analysis) was conducted, using the BioMed-UCLA programme at the University of Virginia Computing Center. The Varimax solution determines the principal axes of covariation among the characters, such that each character tends to load very heavily or very weakly on a given axis. Allelic frequencies were angularly transformed (Sokal and Rohlf, 1969) prior to factor analysis.

Contrary to usual procedure, the characters were not standardised. Standardisation assigns equal weight to each character, irrespective of the actual variances of the characters. Since sets of allelic frequencies are sets of equivalently scaled characters, however, it is more appropriate to weight each allele by its absolute variance in frequency.

Populations were assigned scores on each Varimax axis in the following way:

$$\text{Score} = \Sigma \sqrt{(\arcsin p_i)} \times r_i,$$

where p_i is the frequency of the i th allele, and r_i is the correlation of the i th allele with the axis in question.

3. RESULTS

(i) *Variation of allelic frequencies*

The present samples (table 1) reflect the pattern of shell morph distribution described by Carter (1968). The variation is greatest for the midband gene, which has a bimodal distribution of allelic frequencies. Samples from the Lambourn Downs (groups C and D of table 1) have high frequencies of U^3 whereas the western samples (group A) have low frequencies of this allele (fig. 2). Spread-banded shells also are common on the Lambourn Downs and rare to the west (fig. 3). Finally, most samples from the Lambourn area have high frequencies of yellow shells.

FIG. 2.—Allelic frequencies at the midband locus (U) in samples of *C. nemoralis* from the western Berkshire Downs. Frequencies are indicated by proportion of each circle shaded.

FIG. 3.—Allelic frequencies at the spread band locus (S) in samples of *C. nemoralis* from the western Berkshire Downs.

TABLE 2

Allelic frequencies at six loci encoding allozymes in samples of C. nemoralis. Sample numbers and sample sizes as in table 1

Sample	*Est-F	Lap-2				Mdh-1			6pgd			*Pgi	*To-2
	a	a	b	c	0	a	b	c	a	b	c	b	b
<i>A. Five-band area</i>													
37.	0.11	0.21	0.60	—	0.19	0.96	0.04	—	—	1.00	—	0.99	0.90
19.	0.04	0.43	0.25	—	0.32	0.66	0.34	—	—	1.00	—	0.99	0.99
2.	0.02	0.28	0.28	—	0.45	0.64	0.33	0.03	0.02	0.98	—	1.00	0.86
21.	—	0.28	0.60	—	0.12	0.17	0.28	0.56	—	1.00	—	0.86	0.67
35.	—	0.72	0.28	—	—	0.39	0.34	0.27	—	1.00	—	0.83	0.56
40.	—	0.61	0.39	—	—	0.50	0.50	—	—	1.00	—	0.86	0.67
76.	—	0.25	0.55	—	0.20	0.48	0.44	0.08	—	0.98	0.02	0.74	0.72
70.	—	0.50	0.36	0.01	0.13	0.19	0.03	0.78	—	1.00	—	0.99	0.78
69.	—	0.47	0.28	0.03	0.22	0.44	0.28	0.29	—	1.00	—	1.00	0.76
3.	0.04	0.13	0.71	—	0.16	0.55	0.45	—	—	1.00	—	1.00	0.90
<i>B. Western transition area</i>													
34.	0.03	0.20	0.80	—	—	0.63	0.23	0.14	—	0.95	0.05	0.99	0.92
60.	0.03	0.37	0.60	0.03	—	0.72	0.28	—	0.03	0.97	—	0.97	0.82
6.	—	0.38	0.62	—	—	0.65	0.15	0.20	—	0.76	0.24	1.00	0.87
7.	0.04	0.20	0.44	0.04	0.31	0.58	0.11	0.32	0.01	0.88	0.11	0.91	0.84
<i>C. Western midband area</i>													
61.	0.24	0.20	0.80	—	—	0.55	0.15	0.30	0.01	0.59	0.39	0.97	0.74
58.	0.24	0.07	0.66	—	0.27	0.65	—	0.35	—	0.70	0.30	0.99	1.00
75.	0.03	0.16	0.84	—	—	0.40	—	0.60	—	0.68	0.32	1.00	0.99
10.	0.42	0.15	0.85	—	—	0.44	0.36	0.20	—	0.44	0.56	1.00	0.66
17.	0.15	0.08	0.77	0.01	0.14	0.92	—	0.08	—	0.83	0.17	1.00	0.93
24.	0.06	0.04	0.63	0.16	0.17	0.83	0.02	0.15	—	0.84	0.16	1.00	0.74
57.	0.24	0.03	0.97	—	—	0.85	—	0.15	—	0.67	0.33	1.00	0.91
55.	0.40	0.45	0.55	—	—	0.81	—	0.19	—	0.71	0.29	1.00	1.00
54.	0.16	0.28	0.31	—	0.41	0.68	—	0.32	—	0.49	0.51	1.00	1.00
26.	—	0.06	0.47	0.18	0.29	0.71	0.02	0.26	—	0.57	0.43	1.00	0.79
56.	0.42	0.09	0.91	—	—	0.82	—	0.18	—	0.56	0.44	1.00	0.88
53.	0.21	0.33	0.49	—	0.18	0.79	—	0.21	—	0.61	0.39	1.00	0.97
32.	0.25	0.15	0.85	—	—	0.86	0.05	0.09	—	0.41	0.59	0.99	0.99
23.	0.12	0.21	0.42	0.02	0.35	0.80	—	0.20	—	0.84	0.16	1.00	0.78
27.	0.02	—	0.79	0.05	0.15	0.57	0.23	0.20	—	0.77	0.23	0.99	0.68
33.	0.31	0.10	0.66	0.02	0.22	0.71	0.02	0.26	—	0.79	0.21	1.00	1.00
59.	0.10	0.24	0.37	—	0.24	0.95	—	0.05	—	0.68	0.32	1.00	0.96
66.	0.26	0.20	0.53	0.06	0.22	0.69	—	0.31	—	0.98	0.02	1.00	0.95
68.	—	—	0.49	0.08	0.44	0.94	0.01	0.05	—	0.07	0.93	1.00	1.00
65.	0.49	0.08	0.66	0.01	0.26	0.68	0.01	0.30	—	1.00	—	1.00	0.88
<i>D. Eastern midband area and transition</i>													
67.	0.12	0.18	0.58	—	0.24	0.10	0.89	0.01	—	0.92	0.08	0.97	0.61
52.	0.05	0.26	0.30	0.02	0.42	0.22	0.71	0.06	0.10	0.69	0.21	1.00	0.51
51.	—	0.37	0.36	—	0.27	0.34	0.34	0.32	—	0.75	0.25	0.92	0.71
8.	—	0.73	0.27	—	—	0.32	0.36	0.32	—	1.00	—	1.00	0.91
41.	—	0.46	0.30	0.07	0.16	0.75	0.24	0.01	—	0.93	0.07	0.86	0.87
44.	0.06	0.19	0.29	0.08	0.44	0.61	0.32	0.06	—	1.00	—	0.92	0.83
45.	0.06	0.12	0.35	0.02	0.51	0.65	0.25	0.10	—	0.99	0.01	0.97	0.88
62.	0.08	0.44	0.41	—	0.16	0.12	—	0.88	0.01	0.79	0.20	0.67	0.67
63.	0.12	0.42	0.58	—	—	0.92	—	0.08	—	1.00	—	1.00	0.55
64.	0.09	0.35	0.33	—	0.32	0.52	0.16	0.33	—	1.00	—	0.88	0.88
14.	0.01	0.26	0.28	—	0.46	0.61	0.11	0.28	—	1.00	—	0.91	0.83
12.	—	—	0.53	0.32	0.15	0.58	0.40	0.02	0.08	0.92	—	0.86	0.56
<i>E. Eastern non-midband area</i>													
39.	0.02	0.10	0.90	—	—	0.50	0.09	0.41	—	1.00	—	0.86	1.00

* Frequencies for one allele only are given for those loci segregating for two alleles.

As in the shell characters, there is considerable geographic variation in the allozymes (table 2). The clearest patterns of variation are for *Est-F* and *6pgd*. The *Est-F^a* and *6pgd^c* alleles reach moderate to high frequencies in the area of high frequencies of *U³* and *S^S*, but have negligible frequencies in the western samples (figs. 4 and 5). Such marked geographic concentrations are not evident for the other allozymes.

(ii) *Geographic associations of allelic frequencies*

The similarity of patterns of geographic variation at the *U*, *S*, *Est-F*, and *6pgd* loci evidenced by figs. 2 to 5 is statistically significant (table 3). These four loci form a group, within which all pairs show significant associations of allelic frequencies. At each of these loci, the allele correlated with the midband area effect is rare to absent outside that area. The frequency of *C^Y* is also significantly associated with *U³*, *S^S*, and *Est-F^a*, but *C^Y* also occurs at high frequencies outside the midband area. The only other significant pairwise associations are between *S* and both *Lap-2* and *Mdh-1*.

All of the statistically significant associations reflect the area effect on the Lambourn Downs. Allelic frequencies among the loci are neither strongly nor independently correlated, however. Calculation of partial correlation coefficients among *U*, *S*, *Est-F*, and *6pgd* revealed significant independent correlations between *U³* and *S^S* and between *S^S* and *Est-F^a* only. Rather than a close correlation between pairs of loci, there is a tendency for certain alleles to be relatively common in the same general area.

To clarify and quantify this complex of interlocus combinations, Varimax axes of covariation in the frequencies of all 25 alleles were computed (table 4). Only the first two axes need be considered, as only they produced obvious geographical groupings. Both axes relate to the area effect. The midband area effect is well represented by the Varimax II axis, with which the midband locus correlates strongly. Colour and spread-band also load heavily, and *Est-F* and *6pgd* are the only other loci with correlations exceeding 0.5. This axis represents 20 per cent of the allelic frequency variation, averaged over the 25 alleles.

The Varimax I axis indicates a pattern of covariation *within* the midband area, representing an average of 22 per cent of the variation in allelic frequencies. Variation at the *S*, *Est-F*, and *6pgd* loci is correlated with Varimax I at approximately the same level as with Varimax II. Varimax I represents an association of alleles along a ridge running south from White Horse Hill (*i.e.* south of sample 17) in the western half of the midband area. In this more restricted region, populations are nearly fixed for *B^B*, *Pgi^b*, and *To-2^b*, have relatively high frequencies of *Mdh-1^a* and *Lap-2^b*, and have low frequencies of *Mdh-1^b* and *Lap-2^a* (group C, tables 1 and 2).

Combination of the two axes provides a particularly clear picture of geographic variation in the ten loci (fig. 6). Varimax II separates the midband area effect of the Lambourn Downs from the five-band area effect to the west. Varimax I separates the populations in the western portion of the midband area from all other populations, both east and west. Five geographically contiguous and non-overlapping groups of populations

Fig. 4.—Allelic frequencies at the esterase-F locus (*Est-F*) in samples of *C. nemoralis* from the western Berkshire Downs.

Fig. 5.—Allelic frequencies at the 6-phosphogluconate dehydrogenase locus (*6pgd*) in samples of *C. nemoralis* from the western Berkshire Downs.

TABLE 3
Pairwise geographic associations among the loci

	<i>B</i>	<i>S</i>	<i>U</i>	<i>Est-F</i>	<i>Lap-2</i>	<i>Mdh-1</i>	<i>6pgd</i>	<i>Pgi</i>	<i>To-2</i>
<i>C</i>	—	+	+++	+	—	—	—	—	—
<i>B</i>		—	—	—	—	—	—	—	—
<i>S</i>			+	+++	+	+++	++	—	—
<i>U</i>				++	—	—	+	—	—
<i>Est-F</i>					—	—	+	—	—
<i>Lap-2</i>						—	—	—	—
<i>Mdh-1</i>							—	—	—
<i>6pgd</i>								—	—
<i>Pgi</i>									—

+++ , $P < 0.001$; ++ , $P < 0.01$; + , $P < 0.05$; — , non-significant.

TABLE 4
Varimax factor loadings for each of the 25 alleles

Allele	Axis I	Axis II	Allele	Axis I	Axis II
<i>C^V</i>	—0.073	0.792	<i>Lap-2^c</i>	0.134	—0.044
<i>C^P</i>	0.025	—0.780	<i>Lap-2^o</i>	—0.074	0.060
<i>C^B</i>	0.000	—0.414	<i>Mdh-1^a</i>	0.665	0.131
<i>B^B</i>	0.684	0.039	<i>Mdh-1^b</i>	—0.620	—0.292
<i>B^o</i>	—0.684	—0.039	<i>Mdh-1^c</i>	—0.096	0.132
<i>S^S</i>	0.533	0.669	<i>6pgd^a</i>	—0.254	0.048
<i>S^o</i>	—0.533	—0.669	<i>6pgd^b</i>	—0.264	—0.384
<i>U^S</i>	0.118	0.875	<i>6pgd^c</i>	0.410	0.513
<i>U^o</i>	—0.118	—0.875	<i>Pgi^a</i>	—0.748	—0.077
<i>Est-F^a</i>	0.533	0.523	<i>Pgi^b</i>	0.748	0.077
<i>Est-F^b</i>	—0.533	—0.523	<i>To-2^a</i>	—0.592	—0.150
<i>Lap-2^a</i>	—0.511	—0.176	<i>To-2^b</i>	0.592	0.150
<i>Lap-2^b</i>	0.505	0.083	—	—	—

can thus be recognised on the basis of Varimax scores and geographical position:

- The westernmost populations, representing Carter's (1968) five-band area, with low scores on both Varimax axes;
- A relatively narrow western zone of transition;
- The western midband area, with very high scores on both Varimax axes;
- The eastern midband area and transition, also with high scores on Varimax II, but with lower scores on Varimax I;
- The eastern non-midband area, represented by sample 39.

These groups are indicated in tables 1 and 2 and in each of the figs.

The exact delineation of the groups is arbitrary, but the general trends are clear. Maximum differentiation is between the non-midband areas (groups A and E) and the western populations of the midband area (group C). Scores on both Varimax axes are highest in group C. Transition from the peculiarity of this group of populations is sharp in the west, and gradual in the east. The two Varimax axes agree in the overall structure of the area effect; population scores on the two axes are correlated within group C ($r = 0.62$, $P < 0.01$) and within groups C and D combined ($r = 0.60$, $P < 0.01$).

FIG. 6.—Scores on the first two Varimax axes for samples of *C. nemoralis* from the western Berkshire Downs. Sample code numbers and groupings as in fig. 1.

While geographic concentrations of several alleles focus on the same area, the extent of the concentrations varies among the loci. The extreme western portion of the midband area has peculiar frequencies at the *Lap-2*, *Mdh-1*, *Pgi*, and *To-2* loci. The *Est-F^a* and *Gpgd^c* alleles are more extensively associated with the midband area, and *S^S* even more so. The *U³* allele maintains high frequencies much farther eastward than do the others.

Scores on both Varimax axes are associated with altitude. Both high and low scores on Varimax II tend to be from high altitudes; intermediate scores tend to be from lower elevations. In other words, both the midband and five-band areas are uplands, while the transition samples are from lower sites. Within groups C and D, Varimax II scores are moderately correlated with altitude ($r = 0.38$, $P < 0.01$).

On Varimax I, the five-band area is similar to the transition and eastern midband areas, so there is no overall association of this axis with altitude. High scores on this axis are associated with the highest portions of the midband area, however. Within group C, there is a positive correlation of Varimax score with altitude ($r = 0.61$, $P < 0.01$). The lowest samples from this group, samples 27 and 10, are approximately 3.3 km apart, but have similar scores on Varimax I. On the other hand, sample 27, near the bottom of White Horse Hill, is only 0.9 km from sample 17, near the top, and these two samples represent the extreme scores on Varimax I within group C.

(iii) *Linkage disequilibrium*

In view of the geographical associations of allelic frequencies, an attempt was made to find intrapopulation associations of loci, although any such associations would have to be strong in order to be detected by the generally small sample sizes. Only *C* and *S* show a consistent direction of linkage disequilibrium. As reported by Carter (1968), there is an excess of yellow-spread banded shells from the midband area effect (table 1). Fifteen of 17 samples segregating at both loci in group C show an excess of yellow-spreads ($P < 0.05$, sign test). Twenty-two of 26 samples from groups C and D combined show this excess ($P < 0.05$). Only four of eight samples from groups A and B follow this pattern, the number expected from chance alone. Although the area of linkage disequilibrium corresponds with the midband area, the intensity of disequilibrium, as measured by product-moment coefficients of correlation (table 1), is not correlated with scores on either Varimax axis within the area effect.

4. DISCUSSION

The clearest and most important point to emerge from the present data is that the "midband" area effect on the Lambourn Downs represents much more than a peculiarly high and extensive concentration of the U^3 allele. Geographic concentrations of S^S , $Est-F^a$, and $6pgd^c$ (figs. 3 to 5), are strikingly associated with the area effect. The association of S^S , $Est-F^a$, and $6pgd^c$ with alleles at the *Lap-2*, *Mdh-1*, *Pgi*, and *To-2* loci as represented by the Varimax I axis, further indicates extensive genetic divergence between the midband and non-midband areas.

This finding agrees with evidence from *Partula* that area effects represent marked genetic differentiation (Clarke, 1968). The association of visual with non-visual polymorphisms indicates that the study of shell morph frequencies is not of narrow application. In this regard, it is also interesting that there are associations of allozymes and shell morphs on a larger geographic scale in American colonies of *Cepaea* (Brussard, 1975).

In addition to verifying the genetic pervasiveness of the Lambourn area effect, the patterns of covariation among the 10 polymorphic loci provide a clearer description of the area effect than do frequencies of U^3 alone. This more complete picture of the area effect is relevant to, but does not resolve, the controversial question of the cause(s) of area effects.

Goodhart (1963) and Wright (1965) suggested an origin of area effects from small isolated founder populations which developed differently co-adapted gene complexes. One argument against an important influence of isolation has been that the area effects for different loci are not concordant (Cain and Currey, 1963a). To judge from the larger number of loci examined here, however, there is much covariation among the loci in the western Berkshire Downs, as one would expect if the history of the area effect were one of differentiation in isolation, followed by secondary contact.

From the Marlborough Downs north and eastwards across the Berkshire Downs, there is a decrease in the intensity of area effects, paralleling a decrease in altitude and topographic variation (Carter, 1968). Within the western Berkshire Downs, this association is further evident from the correlation of Varimax scores with altitude. Carter (1968) felt that the

correlation of area effects with regions of marked topography argued against a role of isolation. The reverse conclusion would seem as likely.

The distribution of *C. nemoralis* on the Marlborough and western Berkshire Downs is limited topographically. The low-lying areas often contain *C. hortensis*, but not *C. nemoralis*. The gap between eastern and western samples in the present study (western portion of area B, fig. 1) is such an area (Carter, 1968; personal observations). Restriction of *C. nemoralis* to higher ground could result in patterns of isolation, which would be most marked in the areas of sharp and elevated topography; *i.e.* in those areas with the most striking area effects. While only a suggestion, the consistency of the Lambourn area effect with possible patterns of historical isolation at least precludes the use of this area effect as evidence against a role of isolation.

If isolation played a role in the development of the Lambourn area effect, however, that role must have been long ago. Isolation across the present gap is not the cause of the area effect, because the transition to the midband area occurs east of the gap, rather than on either side of it. Also, subfossils indicate that the distribution of *C. nemoralis* has contracted, while that of *C. hortensis* has expanded, since the hypsithermal, about 4500 B.C. (Currey and Cain, 1968; Cain, 1971). Thus, the distribution of *C. nemoralis* on the Downs is probably more restricted at present than for more than 6500 years, and any role of isolation must have been during an earlier period of range contraction.

To suggest a role of isolation need not be to suggest a role of random divergence, as was done by Goodhart (1963) and Wright (1965), because isolation favours divergence through selection as well. Indeed, the patterns of variation among the loci suggests an inadequacy of random divergence. While the area of maximum differentiation of the covariant loci is in the western portion of the midband area, there are differential eastward extensions among the loci. This nested pattern of distribution would not be expected from random drift, suggesting a role of selection.

The nature of the actual selective mechanisms remains obscure, however. The covariation among several loci suggests that, if environmental selection is the main determinant of allelic frequencies, the effective parameter of the environment must act in a fundamental and pervasive manner. Climate is the most obvious such aspect of the environment. There is considerable distributional (Lamotte, 1959; Cain and Currey, 1963*a*, *b*); Arnold, 1968; Cain *et al.*, 1968; Jones, 1973) and possibly some direct (Richardson, 1974; Bantock, 1974) evidence for climatic determination of colour morph frequencies in *Cepaea*. Among the allozymes, however, only *Est-F* is correlated with shell colour in the western Berkshire Downs, and this correlation does not hold microgeographically.

There is some evidence for climatic selection with respect to shell banding (Arnold, 1968; Currey and Cain, 1968; Cain, 1971), but a large scale trend is not evident (Jones, 1973). The most striking area effects for midbanded shells occur in highly exposed uplands, subject to environmental extremes. In the Lambourn area, this general association is further reflected by correlations of Varimax scores with altitude. In this regard, it is particularly interesting that U^3 increases on Liddington Castle (Cain and Currey, 1963*a*) and Charlbury Hill (sample 21, table 1), both highly exposed hilltops well outside the midband area. On Charlbury Hill, at least, there are not parallel responses at the other loci associated with the midband area effect.

Also, the correlations of Varimax scores with altitude hold only in limited portions of the study area. Thus, until environmental parameters can be locally measured, and correlated with the microgeographic distribution of allelic frequencies, the role of climatic selection will remain an elusive possibility.

The difficulty of finding environmental correlates with area effects in *Cepaea* and in *Partula* led Clarke (1966, 1968) to postulate an important role of coadaptation in the origin of area effects. Evidence for coadaptation would include linkage disequilibrium and genetic disturbance in the transition zone between area effects. There is evidence for such genetic disturbance in *Partula taeniata* (Clarke, 1968). In the present study, there is consistent linkage disequilibrium between *C* and *S* within the midband area, but not outside that area. Whether larger samples would reveal disequilibrium among the other covariant loci remains to be seen. Experimental evidence that the observed linkage disequilibrium is maintained by selection is clearly desirable.

An important difficulty in the controversy on the causes of area effects is that the major hypotheses are not mutually exclusive. Indeed, one would expect much synergism between isolation, environmental selection, and coadaptation. According to one's preconception, the data here reported can be taken as evidence for several combinations of these three factors. In any case, the demonstration of pervasive genetic change associated with the Lambourn area effect makes area effects more fascinating, if not less enigmatic.

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5. REFERENCES

- ARNOLD, R. W. 1968. Climatic selection in *Cepaea nemoralis* (L.) in the Pyrenees. *Phil. Trans. Roy. Soc. B*, 253, 549-593.
- ARNOLD, R. W. 1971. *Cepaea nemoralis* on the east Sussex South Downs, and the nature of area effects. *Heredity*, 26, 277-298.
- BANTOCK, C. R. 1974. Experimental evidence for non-visual selection in *Cepaea nemoralis*. *Heredity*, 33, 409-412.
- BREWER, G. J. 1970. *An Introduction to Isozyme Techniques*. Academic Press, New York.
- BRUSSARD, P. F. 1975. Geographic variation in North American colonies of *Cepaea nemoralis*. *Evolution*, in press.
- BRUSSARD, P. F., AND MCCracken, G. F. 1974. Allozymic variation in a North American colony of *Cepaea nemoralis*. *Heredity*, 33, 98-101.
- CAIN, A. J. 1971. Colour and banding morphs in subfossil samples of the snail *Cepaea*. In *Ecological Genetics and Evolution*, pp. 65-92 (ed. R. Creed). Blackwell, Oxford.
- CAIN, A. J., AND CURREY, J. D. 1963a. Area effects in *Cepaea*. *Phil. Trans. Roy. Soc. B*, 246, 1-83.
- CAIN, A. J., AND CURREY, J. D. 1963b. Area effects in *Cepaea* on the Larkhill Artillery Ranges, Salisbury Plain. *Zool. J. Linn. Soc. Lond.*, 45, 1-15.
- CAIN, A. J., SHEPPARD, P. M., AND KING, J. M. B. 1968. The genetics of some morphs and varieties of *Cepaea nemoralis* (L.). *Phil. Trans. Roy. Soc. B*, 253, 383-396.
- CARTER, M. A. 1968. Area effects and visual selection in *Cepaea nemoralis* (L.) and *Cepaea hortensis*. *Phil. Trans. Roy. Soc. B*, 253, 397-446.
- CLARKE, B. 1966. The evolution of morph ratio clines. *Am. Natur.*, 100, 389-402.

- CLARKE, B. 1968. Balanced polymorphism and regional differentiation in land snails. In *Evolution and Environment*, pp. 351-368 (ed. E. T. Drake). Yale University Press, New Haven.
- CLARKE, B., AND MURRAY, J. 1971. Polymorphism in a polynesian land snail, *Partula suturalis vexillum*. In *Ecological Genetics and Evolution*, pp. 51-64 (ed. R. Creed). Blackwell, Oxford.
- CURREY, J. D., AND CAIN, A. J. 1968. Climate and selection of banding morphs in *Cepaea* from the climatic optimum to the present day. *Phil. Trans. Roy. Soc. B*, 253, 483-498.
- GOODHART, C. B. 1963. "Area effects" and non-adaptive variation between populations of *Cepaea* (Mollusca). *Heredity*, 18, 459-465.
- GOULD, S. J. 1967. Evolutionary patterns in pelycosaurian reptiles: a factor-analytic study. *Evolution*, 21, 385-401.
- JONES, J. S. 1973. Ecological genetics and natural selection in molluscs. *Science*, 182, 546-552.
- LAMOTTE, M. 1959. Polymorphism of natural populations of *Cepaea nemoralis*. *Cold Spring Harb. Symp. Quant. Biol.*, 24, 65-84.
- LEVAN, G., AND FREDGA, K. 1972. Isozyme polymorphism in three species of land snails. *Hereditas*, 71, 245-252.
- MANWELL, C., AND BAKER, C. M. A. 1968. Genetic variation of isocitrate, malate, and 6-phosphogluconate dehydrogenases in snails of the genus *Cepaea*—Introgressive hybridisation, polymorphism, and pollution? *Comp. Biochem. Physiol.*, 26, 195-209.
- MARKERT, C. L., AND FAULHABER, I. 1965. Lactate dehydrogenase isozyme patterns of fish. *J. Exp. Zool.*, 159, 319-332.
- OXFORD, G. S. 1973. The genetics of *Cepaea* esterases. I. *Cepaea nemoralis*. *Heredity*, 30, 127-139.
- QUENOUILLE, M. H. 1972. *Rapid Statistical Calculations*, 2nd edition. Hafner, New York.
- RICHARDSON, A. M. M. 1974. Differential climatic selection in natural populations of land snail *Cepaea nemoralis*. *Nature*, 247, 572-573.
- SOKAL, R. R., AND ROHLF, F. J. 1969. *Biometry*. W. H. Freeman, San Francisco.
- WRIGHT, S. 1965. Factor interaction and linkage in evolution. *Proc. Roy. Soc. B*, 162, 80-104.