NOTES AND COMMENTS

INHERITANCE AND GEOGRAPHIC VARIATION OF ALLOZYMES IN *CEPAEA NEMORALIS*

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SUMMARY

Laboratory crosses confirm the inheritance of allozymes at six loci in *Cepaea nemoralis*. There is considerable geographic variation within Britain for these allozymes, with each locus having high frequencies of alternative alleles in some populations. The average population has only 45 per cent of the total genic variation in the British populations examined.

1. INTRODUCTION

IN an earlier study (Johnson, 1976), electrophoretic variants of six enzymes in *Cepaea nemoralis* were assumed to be inherited. Although such an assumption is likely to be correct, several examples of non-genetic variation of isozymes in *C. nemoralis* (Oxford, 1975, 1978; Gill, 1978*a*, *b*) and in other organisms (*e.g.* Baldwin and Hochachka, 1970; Bolaffi and Booke, 1974; Flowerdew and Crisp, 1976; Shaklee *et al.*, 1977) emphasise the importance of direct confirmation of inheritance. For *C. nemoralis*, such confirmation is presently available only for allozymes of leucine aminopeptidase and hepatopancreas esterases (Brussard and McCracken, 1974; Oxford, 1973). In this note, I present evidence for the inheritance of allozymes at six loci. I also present data on geographic variation of allelic frequencies in British populations, which help place previous studies in perspective.

2. MATERIALS AND METHODS

In addition to the previously described 46 samples from the western Berkshire Downs (Johnson, 1976), I analysed six samples representing the major portion of the range of *C. nemoralis* in Britain: Seahouses, Northumberland; Spurn Head, Yorkshire; Rhosneiger, Anglesey; Point of Air, Flintshire; Collston Bassett, Nottinghamshire; and Pendeen Watch, Cornwall. Genic variation of these samples was partitioned into within- and between-population components, using Nei's (1973) method. For this purpose, samples numbered 69 and 75 from Johnson (1976) were taken as representative of the Berkshire Downs.

Snails were frozen for up to 5 months prior to electrophoresis. Variation for foot esterase (*Est-F*), leucine aminopeptidase (*Lap-2*), malate dehydrogenase (*Mdh-1*), phosphoglucose isomerase (*Pgi*), and tetrazolium oxidase (*To-2*) was detected as described previously (Johnson, 1976).

For genetic studies, immature snails from the wild were isolated and reared to maturity. Mated pairs were kept in flower pots, and the offspring

reared on a diet of rolled oats, carrots, and natural chalk. Parents and offspring were frozen for up to 5 months with no adverse effects on the scoring of allozyme genotypes.

3. Results and discussion

As summarised in table 1, the crosses confirm the inheritance of the allozymes. Clearcut segregations were found for all loci except *Pgi*, for which no heterozygous parents were available. The data for *Pgi* are consistent with Mendelian inheritance, but are not conclusive. The data for *Lap-2* are similar to those of Brussard and McCracken (1974), except that their crosses did not include the null allele, *Lap-2*^{O}. Unfortunately, we cannot be certain which individuals were heterozygous for *Lap-2*^{O} in the

Table 1

Inheritance of allozymes in laboratory crosses of Cepaea nemoralis

Parental phenotypes	Number of matings	Phenotypes and number of offspring				
Est-F		*a*		*ab*		*b*
b × *b*	2	0		0		31
a × *ab*	1	8		11		0
b × *ab*	2	0		46		36
Lap-2		*a*		*ab*		*b*
b × *b*	5	0		0		59
a × *ab*	1	6		5		0
b × *ab*	2	0		44		37
a × *b*(0)*	1	8		7		0
ab × *b*(0)*	2	32		22		58
b × 0	1	0		0		8
Mdh-1		*a*	*ab*	*ac*	*bc*	*c*
a × *a*	2	16	0	0	0	0
a × *ab*	2	57	53	0	0	0
a × *ac*	1	25	0	20	0	0
ac × *ac*	1	4	0	6	0	2
ac × *bc*	1	0	10	12	14	18
6pgd		*b*		*bc*		*c*
b × *b*	7	63		0		0
b × *bc*	1	14		11		0
b × *c*	1	0		16		0
bc × *c*	2	0		10		12
Pgi			*ab*		*b*	
a × *b*	1		19		0	
b × *b*	8		0		71	
To-2		*a*		*ab*		*b*
a × *ab*	1	6		5		0
a × *b*	1	0		40		0
ab × *b*	2	0		60		44
b × *b*	3	0		0		98

* These parents are presumed to be *Lap-2*^{bO} heterozygotes.

present crosses. However, several of the crosses indicate the presence and segregation of this allele. The genetic data thus confirm that non-genetic variation such as found by Oxford (1975, 1978) and Gill (1978*a*, *b*) does not interfere with scoring of genotypes for these six loci.

The crosses also allowed partial analysis of linkage relationships among these loci and the shell colour and banding loci. No evidence of linkage was found for any of the combinations tested (table 2). The sample sizes were adequate to detect recombination frequencies of less than about 30 per cent.

TABLE 2

Tests for linkage between pairs of allozyme and shell colour and banding loci

	Est-F	*Lap-2*	*Mdh-1*	*6pgd*	*To-2*
Colour/Banding	n.s.*	—	n.s.	—	n.s.
Midband	—	n.s.	n.s.	n.s.	—
Mdh-1	n.s.	n.s.	—	—	n.s.

* n.s.: no detectable linkage. —: no information.

TABLE 3

Allelic frequencies for six allozyme loci in samples of Cepaea nemoralis. *Sample sizes in parentheses*

Allele	Sea-houses (56)	Spurn Head (48)	Rhos-neiger (84)	Point of Air (55)	Collston Bassett (80)	Pendeen Watch (56)	Berkshire Downs Range*
Est-F							
a	0·74	0·50	0·76	0·19	0·21	0·08	0 to 0·42
b	0·26	0·50	0·24	0·81	0·79	0·92	1·00 to 0·58
Lap-2							
a	0·99	0·53	—	0·79	0·82	0·04	0 to 0·73
b	0·01	0·45	1·00	0·21	0·18	0·96	0·25 to 0·90
c	—	0·02	—	—	—	—	0 to 0·32
0	—	—	—	—	—	—	0 to 0·46
Mdh-1							
a	0·28	0·44	—	0·97	0·21	0·61	0·17 to 0·96
b	0·32	0·56	0·16	—	—	0·12	0 to 0·50
c	0·39	—	0·84	0·03	0·79	0·28	0 to 0·56
6pgd							
a	—	—	—	—	—	—	0 to 0·10
b	1·00	1·00	1·00	1·00	1·00	1·00	0·07 to 1·00
c	—	—	—	—	—	—	0 to 0·93
Pgi							
a	0·91	1·00	0·16	0·25	0·46	1·00	0 to 0·17
b	0·09	—	0·84	0·75	0·54	—	0·83 to 1·00
To-2							
a	0·95	0·90	0·10	0·46	0·07	0·62	0 to 0·44
b	0·05	0·10	0·90	0·54	0·93	0·38	0·56 to 1·00

* Range of frequencies for samples of 40 or more individuals.
Data from Johnson (1976).

Among the population samples, the striking observation is the large amount of geographic variation (table 3). For each of the six loci, there are high frequencies of alternative alleles in some populations. Thus, although in many species, a single sample may characterise the genetic composition of the entire species (Avise, 1974), this is clearly not the case in *C. nemoralis*. In fact, only 45 per cent of the observed genic variation for the six loci studied is represented in the average population. Even small regions may not be adequately represented by a few populations, as shown by the marked variation in the samples from the Berkshire Downs, collected from an area less than 10 km across (table 3; Johnson, 1976).

This marked geographic variation emphasises the need for caution in the interpretation of allozyme variation in *Cepaea*. For example, Manwell and Baker (1968), on the basis of inadequate geographic sampling, proposed introgression from *C. hortensis* as the source of some genetic variants in a population of *C. nemoralis*. Such a conclusion could be valid only after full characterisation of populations of *C. nemoralis* in the absence of *C. hortensis*.

More interestingly, Brussard (1975) hypothesised that two groups of North American populations of *C. nemoralis* represent separate source populations in northern and southern Europe. For supporting evidence, he examined three Welsh populations, including Rhosneiger, and found them to conform with the expected northern type. However, sampling from any of several areas in Britain would have given contrary results, and the causes of geographic variation in American populations are unclear.

Finally, although local correlations in allelic frequencies at different loci can occur (Johnson, 1976), the picture across Britain as a whole is one of generally independent variation of the allozyme loci (table 3). Despite considerable variation in allelic frequencies at each of the polymorphic loci studied, distinct racial variation is absent. Given the large genetic differences between populations, an obvious question is, why has speciation occurred so seldom in *Cepaea*?

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