REPRODUCTIVE COMPATIBILITY DESPITE LARGE-SCALE GENETIC DIVERGENCE IN *CEPAEA NEMORALIS*

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SUMMARY

Cepaea nemoralis is remarkable for its geographic variation for both shell polymorphism and allozymes. An electrophoretic study of 20 loci revealed genetic identities as low as 0.53 between English and Italian populations. Despite this genetic divergence, crosses among American, English, and Italian stocks demonstrate that populations of *C. nemoralis* are reproductively compatible on a continental scale.

This reproductive compatibility in the face of large-scale genetic divergence indicates the danger of using a genetic index to assess reproductive relationships. The decoupling of genetic divergence from speciation emphasizes the limitations of viewing the process of speciation solely in genetic terms. A comparison of *Cepaea* with another genus of land snails, *Partula*, suggests that competition may be an important determinant of the likelihood of speciation.

C. nemoralis from Lexington, Virginia, were included in the matings and electrophoretic comparisons, in order to test conflicting proposals on the origin of that population. Both sets of data favour a British, rather than an Italian, origin, but are also compatible with earlier evidence that the Lexington population is of hybrid origin.

1. INTRODUCTION

Great changes have recently taken place in our views about the genetic structure of species. It is now clear from the electrophoresis of proteins that there is a wealth of genetic variation within populations (e.g., Powell, 1975; Nevo, 1978). Other studies of natural populations have shown that under appropriate conditions genetic differentiation may proceed despite continuing gene flow (Bradshaw, 1960; Bush, 1969). Theoretical analysis supports the conclusion that species with continuous and widespread distributions are unlikely to be genetically homogenous, and may contain incipient parapatric species (Clarke, 1966; Maynard Smith, 1966; Dickinson and Antonovics, 1973; Endler, 1977).

Land snails, such as the polymorphic species *Cepaea nemoralis*, are particularly suitable for the study of genetic variation among populations (for reviews see Jones *et al.*, 1977; Clarke *et al.*, 1978). Differentiation between populations of *C. nemoralis* has been demonstrated at three separate levels. In some places, the frequencies of the genes controlling shell colour and banding depend upon the habitat in which the populations are living, largely because of selective predation on the snails (e.g., Cain and

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Sheppard, 1950, 1954). Not all populations, however, are differentiated on so small a scale. Areas with similar frequencies of particular genes affecting the shell polymorphism may extend for 10 to 25 km² and include a variety of habitats (Cain and Currey, 1963). A similar or larger scale of variation exists for genes controlling polymorphic enzymes (Johnson, 1976; Jones *et al.*, 1980; Ochman *et al.*, 1983). These "area effects" have been variously attributed to micro-climatic selection (Cain and Currey, 1963), to founder effects (Goodhart, 1963), or to local co-adaptations of the genotype (Goodhart, 1963; Clarke, 1968). Finally, there is differentiation on a larger geographical scale, such as the cline in the frequency of yellow shells which stretches across Europe (Jones *et al.*, 1977).

The wide range, limited mobility, and great variation in *Cepaea* suggest that incipient reproductive isolation might be developing between distant populations. In other polymorphic land snails, for example, reproductive isolation can be well developed between sympatric taxa connected by chains of interbreeding populations only a few kilometers in length (Clarke and Murray, 1969; Murray and Clarke, 1980).

We report here the results of matings undertaken to find out whether isolation might be detected among geographically distant populations of *C. nemoralis*, whose genetic divergence was quantified by electrophoresis of twenty enzyme loci. The results indicate reproductive compatibility despite uniquely high levels of allozymic divergence among these conspecific populations.

This study was also designed to investigate a second question. *C. nemoralis* has been introduced to several localities in North America, the best-known introduction being that at Lexington, Virginia (Howe, 1898; McConnell, 1935; Brussard, 1975; Richards and Murray, 1975). There is some doubt about the source of the founders of this population. Howe (1898) believed that the material came from Italy, but he also noted a possible alternative source in Great Britain. Brussard (1975) argued that electrophoretic data for two polymorphic loci favour an Italian origin, since the Lexington population was allozymically different from three samples collected in Wales. There is evidence, however, of significant change in the Lexington populations since Howe's time, notably the disappearance of brown shells, a phenotype characteristic of northern Europe. A comparison of the patterns of reproductive compatibility and genetic similarity of Lexington snails with animals from Britain or Italy, might help to trace their origin. To these ends our crosses used American, English, and Italian snails.

2. MATERIALS AND METHODS

The American stock was taken from the "Redwood" site (intersection of Interstate Highway 64 and US 11) in Lexington, Virginia. The English stock came from the sand dunes at Berrow in Somerset. The Italian stock was collected at Santa Croce near Pavia. An additional Italian sample from near Florence was used for the electrophoresis but not for matings.

All the animals used in the matings were collected as juveniles and maintained in isolation until maturity. Animals were given serial numbers as they matured and were assigned to matings by means of a table of random numbers. Twenty-four individuals from each source population were crossed

as follows:

- 8 American $\times$ English
- 8 English $\times$ Italian
- 8 Italian $\times$ American
- 4 American $\times$ American
- 4 English $\times$ English
- 4 Italian $\times$ Italian

Matings were established in the winter of 1977 and the spring of 1978 and were maintained until December 1979. The numbers of young from each mating were counted and six young from each productive mating were reared for a second generation. These young were mated as follows:

- 8 American/English $\times$ American/English
- 8 English/Italian $\times$ English/Italian
- 8 Italian/American $\times$ Italian/American
- 4 American /American $\times$ American/American
- 4 English/English $\times$ English/English
- 4 Italian/Italian $\times$ Italian/Italian

The second generation was crossed so as to avoid inbreeding and to equalise as much as possible the contributions of offspring from the original matings. They were established in the spring of 1979 and maintained until January 1980. The numbers of offspring were recorded. Animals dying during the course of the experiments were replaced from virgin stocks.

All mated pairs were maintained on sandy loam in 20 cm flower pots, each covered with a sheet of glass. They were fed oatmeal and lettuce, and were provided with lumps of natural chalk. The snails oviposited in the soil and eggs were allowed to hatch in the pots. Emerging young were counted and preserved, except for the individuals chosen for rearing. These were transferred to 10 $\times$ 10 $\times$ 2 cm plastic boxes. Moistened toilet paper in the bottom of the boxes maintained a humid atmosphere, and the young snails were fed a diet of oatmeal, lettuce, and powdered natural chalk.

Samples from these matings and from the Florence population were examined by conventional starch-gel electrophoresis for variation in 15 enzymes, representing 20 gene loci. The enzymes examined and the electrophoretic buffers used are shown in table 1.

Allelic designations indicate relative electrophoretic mobilities of the corresponding allozymes, and wherever possible correspond with those used by Jones *et al.*, (1980). The inheritance of allozymes for several of these loci has been confirmed previously (Brussard and McCracken, 1974; Johnson, 1979). Examination of F_1 and F_2 offspring from the interpopulational crosses confirmed the inheritance of allozyme differences between the populations.

Based on the 20 loci, unbiased estimates of genetic identity, I , and genetic distances (Nei, 1978) were calculated for all pairs of the four populations.

3. RESULTS

(i) *Matings*

The results of the 72 matings among the three different stocks are shown in tables 2 and 3. It is quite clear that there is nothing like complete

TABLE 1

Enzymes assayed and buffer systems used in the study of genetic variation in Cepaea nemoralis

Enzyme	Locus	Tissue*	Quaternary structure [†]	Electrophoretic buffer [‡]
Esterase	<i>Est-1</i>	F	monomer	TEB
Glucose-6-phosphate dehydrogenase	<i>G6pd</i>	F	multimer	TEB
Glutamate-oxaloacetate transaminase	<i>Got-1</i>	H	no data	TM
	<i>Got-2</i>	H	no data	TM
Indophenol oxidase	<i>Ipo-2</i>	F	dimer	TEB
Isocitrate dehydrogenase	<i>Idh-1</i>	H	dimer	TM
	<i>Idh-2</i>	H	no data	TM
Lactate dehydrogenase	<i>Ldh</i>	F	no data	TEB
Leucine aminopeptidase	<i>Lap-1</i>	H	monomer	TEB
	<i>Lap-2</i>	H	monomer	TEB
Leucylproline peptidase	<i>Lpp</i>	H	dimer	LiOH
Leucyltyrosine peptidase	<i>Ltp</i>	H	monomer	LiOH
Malate dehydrogenase	<i>Mdh-1</i>	H	dimer	TC
	<i>Mdh-2</i>	H	no data	TC
Mannosephosphate isomerase	<i>Mpi</i>	H	monomer	TC
Nucleoside phosphorylase	<i>Np</i>	F	trimer	TEB
6-Phosphogluconate dehydrogenase	<i>6Pgd</i>	H	dimer	TC
Phosphoglucose isomerase	<i>Pgi</i>	H	dimer	TC
Phosphoglucomutase	<i>Pgm-2</i>	H	monomer	TM
	<i>Pgm-3</i>	H	monomer	TM

* F = foot; H = hepatopancreas.

[†] Based on isozyme patterns of heterozygotes.[‡] TEB = buffer 6, TM = buffer 9, TC = buffer 4, LiOH = buffer 2 of Selander *et al.* (1971).

TABLE 2

Matings among stocks of Cepaea nemoralis from America, England, and Italy, giving means and standard errors of numbers of offspring per attempted mating and per mating that produced at least some offspring

Type	No. of matings	Nos. of offspring		No. of successful matings	Nos. of offspring	
		x	S.E.		x	S.E.
American/English	8	209.25	52.67	7	239.14	50.08
English/Italian	8	81.00	27.87	6	108.00	29.44
Italian/American	8	171.00	31.88	8	171.00	31.88
American/American	4	167.00	24.51	4	167.00	24.51
English/English	4	215.00	84.00	3	286.67	61.95
Italian/Italian	4	213.00	60.07	4	213.00	60.07

reproductive isolation between any of the populations. Only four of the 36 matings of the first generation failed to produce any offspring at all, and they are scattered over three groups, two experimental and one control. The second generation was rather less successful than the first for experimentals and controls. However, at least some of each class of hybrid crosses produced young, and the numbers of young per mating exceeded those from the controls. These results imply that populations of *C. nemoralis* maintain genetic compatibility on a scale of many hundreds of kilometres.

TABLE 3

Matings among the offspring of the matings recorded in table 2, giving means and standard errors of numbers of offspring per attempted mating and per mating that produced at least some offspring

Type	No. of matings	Nos. of offspring		No. of successful matings	Nos. of offspring	
		x	S.E.		x	S.E.
F ₂ American/English	8	77.88	35.89	5	124.60	46.38
F ₂ English/Italian	8	18.13	14.33	2	72.50	42.50
F ₂ Italian/American	8	41.13	30.46	3	109.67	69.99
F ₂ American/American	4	0	0	0	0	0
F ₂ English/English	4	88.00	45.11	3	117.33	48.46
F ₂ Italian/Italian	4	10.50	10.50	1	42.00	—

There is a great deal of variation within each group of matings; even when matings that have produced no offspring are excluded, the numbers of offspring per mating show two- to eightfold differences within each group. As the standard errors averaged 22 per cent of their respective means, it is difficult to assess the real extent of differences between the groups. For example, analysis of variance using all the six types of matings as treatments failed to detect any significant heterogeneity among the matings ($F_{5,30} = 1.40$; or, excluding unproductive matings, $F_{5,26} = 1.84$). Comparison of each group of experimental crosses with the corresponding "parental" groups of controls showed significantly fewer offspring only for the English $\times$ Italian matings ($t_{14} = 2.40$; $P < 0.05$). This difference did not extend to the second generation, however.

(ii) *Electrophoresis*

Variation was found at 16 of the 20 loci examined (table 4). Within populations, polymorphism was detected at 30 to 50 per cent of the loci, while individual heterozygosity averaged 11.6 per cent. In addition to this extensive variation within populations, there is considerable divergence among the populations. The average genetic identity among the four populations is only 0.65, with a range of 0.53 to 0.83 (table 5). These low genetic identities result from the fixation or near-fixation of alternative alleles in different populations (table 4; fig. 1).

The most similar of the populations are Berrow and Lexington, which show large genetic divergence from the Santa Croce population. The snails from Florence are genetically intermediate among the others, rather than being particularly similar to those from Santa Croce.

4. DISCUSSION

(i) *Genetic divergence and speciation*

The matings clearly demonstrate a high degree of genetic compatibility between distant populations: any postmating isolation, such as that which might exist between English and Italian snails, is weak. These results are compatible with the apparent absence of barriers to interbreeding in the laboratory between English and Pyrenean snails (Cook, 1967; Cain *et al.*,

TABLE 4

Allelic frequencies at 16 loci in 4 populations of Cepaea nemoralis. Calculations of average heterozygosity include an additional 4 monomorphic loci. Sample sizes in parentheses

Locus	Allele	Berrow, England (11)	Lexington, Virginia (10)	Santa Croce, Italy (13)	Florence, Italy (10)
<i>Est-1</i>	100	0.15	0.11	0.88	1.00
	97	0.85	0.89	0.12	—
<i>G6pd</i>	103	—	0.07	—	0.10
	100	1.00	0.93	0.96	0.90
<i>Ipo-2</i>	97	—	—	0.04	—
	130	—	1.00	0.96	0.85
<i>Idh-1</i>	100	1.00	—	0.04	0.15
	100	1.00	1.00	0.73	1.00
<i>Idh-2</i>	92	—	—	0.27	—
	100	0.80	0.25	0.81	0.85
<i>Lap-1</i>	78	0.20	0.75	0.19	0.15
	100	1.00	1.00	1.00	—
<i>Lap-2</i>	90	—	—	—	1.00
	125	—	—	0.96	—
	120	—	—	0.04	—
	105	—	0.30	—	0.70
	95	1.00	—	—	0.30
	90	—	0.70	—	—
<i>Lpp</i>	100	1.00	0.20	1.00	—
	97	—	—	—	1.00
	88	—	0.80	—	—
	116	—	—	0.69	—
<i>Ltp</i>	100	1.00	0.70	0.31	0.95
	96	—	0.30	—	0.05
<i>Mdh-1</i>	100	0.50	1.00	—	—
	90	—	—	1.00	1.00
	60	0.50	—	—	—
	115	—	0.05	1.00	—
<i>Mpi</i>	100	1.00	0.95	—	1.00
	110	0.11	—	0.54	0.25
<i>Np</i>	100	0.89	1.00	0.46	0.75
	125	—	—	1.00	—
<i>6Pgd</i>	115	—	—	—	1.00
	100	1.00	1.00	—	—
<i>Pgi</i>	100	0.05	0.05	1.00	0.55
	50	0.95	0.95	—	0.45
<i>Pgm-2</i>	150	0.23	0.11	0.08	—
	100	0.77	0.89	0.92	1.00
<i>Pgm-3</i>	200	0.10	0.10	0.88	0.50
	100	0.90	0.90	0.12	0.50
Average Heterozygosity (20 loci)		0.095	0.118	0.121	0.129

1968), and support the current taxonomic treatment of *C. nemoralis* as a single species:

This genetic compatibility prevails despite a degree of genetic divergence which is unprecedented within a species. For perspective, approximately 80 per cent of electrophoretic comparisons between conspecific populations from a wide range of plants and animals have revealed genetic identities

TABLE 5

Average genetic identities (above diagonal) and genetic distances (below diagonal) between populations of *Cepaea nemoralis*, based on 20 loci

	Berrow, England	Lexington, Virginia	Santa Croce, Italy	Florence, Italy
Berrow		0.829	0.532	0.655
Lexington	0.188		0.532	0.665
Santa Croce	0.631	0.631		0.676
Florence	0.423	0.409	0.391

FIG. 1. Frequency distribution of loci with respect to genetic identity in comparisons among four populations of *Cepaea nemoralis*.

greater than 0.95; fewer than 2 per cent of such comparisons have values of I lower than 0.85 (Thorpe, 1982). Thus, even the I of 0.83 between populations of *C. nemoralis* from Berrow and Lexington is unusually low. The identities of less than 0.55 between these populations and that from Santa Croce are well below the modal value (approximately 0.75) for comparisons among congeneric species (Thorpe, 1982). Indeed, the genetic distances among the populations of *C. nemoralis* overlap the estimates obtained between humans and chimpanzees (King and Wilson, 1975; Bruce and Ayala, 1979).

This large allozymic divergence within a species emphasizes the possible hazards involved in using genetic similarities to infer reproductive relationships. For example, Thorpe (1982: p. 151) proposed that, "If allopatric populations of dubious status have genetic identities below about 0.85 it is improbable that they should be considered conspecific, while nominate species with I values above about 0.85 should be considered doubtful if there is no other evidence of their specific status". Although this criterion was derived empirically, exceptions are sufficiently common to require caution. For example, reproductive isolation with little allozymic divergence has been found in birds (Avice and Aquadro, 1982), gophers (Patton *et al.*,

1972), fish (Avice *et al.*, 1975; Kornfield *et al.*, 1979), snails (Johnson *et al.*, 1977, ms), *Drosophila* (Sene and Carson, 1977), and plants (Gottlieb, 1973; Crawford and Smith, 1982), indicating that great genetic similarity does not always indicate conspecific status. The data for *C. nemoralis* demonstrate that the genetic measure is also fallible at the other end of the scale; even conspecific populations may show very low genetic identities. Thus, taxonomic decisions based entirely on the degree of genetic similarity are subject to gross error, and allozymes must be treated with the same care as any other type of data.

In addition to their taxonomic implications, the low genetic identities within *C. nemoralis* raise the question of what determines whether speciation will occur. The genetic structure of *C. nemoralis* would appear to be intrinsically favourable for allopatric speciation. Variation within populations is great, and variation among populations is unusually large. In addition to the continental scale of divergence shown in this study, strong genetic subdivision occurs even within small regions (Johnson, 1976, 1979; Jones *et al.*, 1980; Ochman *et al.*, 1983). Furthermore, "area effects" for allozymes often are concordant among loci (Johnson, 1976; Ochman *et al.*, 1983), perhaps suggesting incipient speciation. Although area effects have been cited as the early stages of an important mode of speciation (*e.g.*, White, 1978) the genetic compatibility among populations of *C. nemoralis* emphasizes the difficulty of equating geographic patterns of differentiation with incipient speciation.

If speciation is not closely coupled to the amounts and patterns of genetic divergence, it follows that it must be considered as a process in its own right, and not simply an incidental of overall genetic change (Cracraft, 1982). Since populations of *C. nemoralis* have not become reproductively isolated, despite an apparently favourable genetic subdivision, it is likely that speciation in *Cepaea* is under the control of extrinsic factors. A comparison with patterns of speciation in another genus of land snails, *Partula*, is suggestive in this regard. Like *C. nemoralis*, species of *Partula* are outcrossing hermaphrodites with low vagility and high heterozygosity. Species of *Partula* on the island of Moorea, however, have very high genetic identities suggesting that reproductive isolation has developed through relatively simple genetic changes (Johnson *et al.*, 1977, ms). In common with other groups that have radiated on oceanic islands, *Partula* on Moorea has evolved in a depauperate fauna. In contrast, *Cepaea* belongs to the highly diverse European snail fauna, suggesting that competition and the availability of ecological niches could be important factors determining the amount and rate of speciation. Although the possible importance of competition for speciation has been recognized (*e.g.*, Hutchinson, 1968; Rosenzweig, 1978; Littlejohn, 1981), most recent discussions of the diversity of modes of speciation have emphasised the patterns of genetic change (Bush, 1975; White, 1978; Templeton, 1981). Since genetic variation and subdivision are poor predictors of speciation, however, it may be worthwhile to give greater emphasis to the ecological perspective in searching for controls of speciation.

(ii) *Origin of the Lexington colony*

The American *C. nemoralis* were included in our comparisons in order to investigate the origin of the Lexington colony. Although reproductive compatibility between populations was generally high, the directions of

differences in the crosses were consistent for both the F_1 and F_2 . The mean number of offspring per mating can be ranked as:

American $\times$ English $>$ America $\times$ Italian $>$ English $\times$ Italian.

This suggests that the American (Lexington) population might be more compatible with the English (Berrow) than with the Italian (Santa Croce) population. The electrophoretic data are consistent with this pattern, as the Lexington population shows greater similarity to Berrow than to either of the Italian populations. Thus, the quantitative evidence marginally favours an English, rather than Italian, origin of the Lexington population.

It is also essential, however, to consider the qualitative evidence, that is, the sharing of particular alleles. Brussard's (1975) view that the Lexington population came from Italy was based on the absence of brown shells (commonly found only in northern populations of *C. nemoralis*) and on differences between Lexington and Welsh populations in allelic composition at the *Lap-2* locus. The absence of an allele from a derived population is not very informative, because the allele might have been lost after colonisation. Indeed, the brown-shelled morph formerly occurred in the Lexington population (Howe, 1898) strengthening the suggestion of an English origin.

On the other hand, the presence of the *Lap-2*¹⁰⁵ and *Lap-2*⁹⁰ alleles (= *Lap-2*^a and *Lap-2*^c of Brussard, 1975) at Lexington and their absence from the Welsh populations argue against a Welsh origin. However, both alleles have been found in other British populations (Johnson, 1979), and the allele was not found in either of the Italian populations examined in the present study. Thus, Brussard's (1975) data are compatible with an English origin of the Lexington population.

In the present study, two additional alleles, *Mdh-1*¹⁰⁰ and *6Pgd*¹⁰⁰ were found in Lexington and Berrow, but not in the Italian populations, again favouring a northern origin. Conflicting evidence, however, is provided by the *Ltp*⁹⁶ allele, which was detected only at Lexington and Florence. Furthermore, the *Lpp*⁸⁸ allele, which is common at Lexington, was not found in any of the other populations, leaving us without any evidence about its source.

The difficulty of establishing the origin of the Lexington population stems from the fact that fixation or near-fixation of alternative alleles may occur over a few km (Johnson, 1976; Jones *et al.*, 1980; Ochman *et al.*, 1983). Although there may be concordance among loci in a particular area, there need not be on a larger scale (Johnson, 1979). Thus, unless a comprehensive survey of geographic variation reveals alleles distinctly characteristic of particular regions, the origin of the Lexington population will remain obscure. Finally, the conflicting electrophoretic evidence and the intermediate performance of the American $\times$ Italian matings raise the possibility that both of Howe's (1898) theories of introduction might be correct, and that the Lexington population owes its origin, and its extensive variability, to hybridisation between stocks introduced from both Italy and Great Britain.

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