ALLOZYMIC VARIATION IN A NORTH AMERICAN COLONY OF *CEPAEA NEMORALIS*

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Received 1.i.74

SUMMARY

Starch gel electrophoresis of foot-muscle extracts from over 400 individuals of the land snail *Cepaea nemoralis* collected from 10 different populations in the Lexington, Virginia, colony revealed nine scorable loci. Two of these, LAP II and PGM II, proved to be polymorphic with two and three alleles, respectively. Each of the populations surveyed was in Hardy-Weinberg equilibrium at both loci, and laboratory crosses have confirmed the basis of the polymorphism.

1. INTRODUCTION

POPULATIONS of the helicid land snail, *Cepaea nemoralis*, are especially favourable material for many studies in ecological genetics, chiefly because of their conspicuous polymorphisms in shell colour and banding patterns. A fair amount is known about the genetics of these polymorphic characters, mainly from the work of Cain and his collaborators (1968) and of Wolda (1969). In view of the recent discovery of widespread polymorphism at structural gene loci which is revealed by electrophoretic analysis (Selander and Kaufman, 1973), one might have expected a spate of papers dealing with biochemical polymorphism in this species. However, this was not the case; when we began our studies on the ecological genetics of populations of *Cepaea* which had been introduced into North America, the only published account of electrophoretic variation in *Cepaea* was that of Manwell and Baker (1968) in which they reported on isozymes of various dehydrogenases and esterases.

Recently, Levan and Fredga (1972) have described isozyme patterns in 13 enzymes in three species of land snails, including *Cepaea nemoralis* and *C. hortensis*. They felt that single-locus codominant Mendelian inheritance occurred in two of the patterns they observed, but they did not perform actual genetic crosses to confirm their model. More recently, Oxford (1973) has reported the results of breeding experiments which explain the rather complex inheritance of esterase isozymes in *C. nemoralis*.

Here we present data from 10 North American populations which describe two additional polymorphisms in that species, along with statistical and breeding data which firmly establish the allelic nature of the variation at the two loci.

2. MATERIALS, METHODS AND RESULTS

Mature and large immature *C. nemoralis* were collected for allozyme analysis from 10 sites in the Lexington, Virginia, area in June 1972 and in March and April 1973. At each site small areas were searched thoroughly for snails, usually by two collectors, so that the more cryptic shell morphs would not be excluded from the collection.

These snails were prepared for electrophoresis by placing them alive in

plastic boxes and freezing them at −20° C. for several hours. When treated in this way the snails died in an extended position, and it was a simple matter to cut off the foot and head for enzyme extraction. Other methods of killing proved to be unsatisfactory due to the large amount of mucus that was secreted by stressed snails. The foot-muscle tissue was minced into several pieces and placed into a 75-mm Nalgene centrifuge tube containing 1:2 weight:volume of cold buffer (0·1M Tris, 0·001M EDTA, 5×10^{-5}M NADP, with *p*H adjusted to 7·0 with HCl). This mixture was sonicated for 30 seconds with a Bronwill Biosonic IV and centrifuged at 4° C. for 20 minutes at 15,000 r.p.m. The supernatant was removed and stored at −80° C. until electrophoresis. No diminution of enzyme activity was noted even after several months of storage at this temperature.

Horizontal starch gel electrophoresis and staining procedures similar to those described by Selander *et al.* (1971) were employed to demonstrate the presence of anodally migrating proteins presumably encoded by nine structural gene loci. The buffer systems used and the enzymes assayed are as follows: lithium hydroxide (Selander *et al.*, 1971, Buffer 2), glutamate oxalate transaminase (GOT I); continuous tris-citrate (Buffer 4), 6-phosphogluconate dehydrogenase (6-PGD I); continuous tris-citrate (Buffer 5), leucine aminopeptidase, two loci (LAP I, LAP II), phosphoglucomutases, two loci (PGM I, PGM II); tris-versene-borate (Buffer 6), glucose-6-phosphate dehydrogenase (G-6-PD I); and tris-maleate (Buffer 9), isocitrate dehydrogenase, two loci (IDH I, IDH II). Loci were numbered and alleles designated alphabetically in order of decreasing anodal mobility.

Seven of the above nine loci showed no variation in more than 450 individuals; but two, PGM II and LAP II, proved to be polymorphic with two and three alleles, respectively. In each of the 10 populations surveyed, the proportion of phenotypes showed no significant deviation from Hardy-Weinberg expectation (table 1).

TABLE 1

	PGM II					LAP II							
Population	*AA*	*AB*	*BB*	N	P*	*AA*	*AB*	*BB*	*AC*	*BC*	*CC*	N	P*
1	19	50	22	91	$0·9 > p > 0·5$	6	1	0	30	0	42	79	$0·9 > p > 0·5$
2	13	47	29	89	$0·9 > p > 0·5$	4	3	0	33	3	45	88	$p > 0·995$
3	11	25	13	49	$p > 0·995$	4	0	0	14	1	28	47	$0·9 > p > 0·5$
4	9	11	4	24	$p > 0·995$	1	1	0	13	1	8	24	$0·5 > p > 0·1$
5	5	10	8	23	$0·9 > p > 0·5$	1	0	0	12	2	9	24	$0·5 > p > 0·1$
6	4	5	15	24	$0·1 > p > 0·05$	3	0	0	6	0	15	24	$0·5 > p > 0·1$
7	6	9	9	24	$0·9 > p > 0·5$	0	2	1	6	8	7	24	$0·9 > p > 0·5$
8	8	12	4	24	$p > 0·995$	2	0	0	8	7	7	24	$0·5 > p > 0·1$
9	7	9	11	27	$0·5 > p > 0·1$	0	1	0	5	2	7	15	$p > 0·995$
10	3	35	55	93	$0·9 > p > 0·5$	9	0	0	44	0	42	95	$0·9 > p > 0·5$
			Total	468							Total	444	

* P is the probability that the genotypic proportions observed correspond to those expected under Hardy-Weinberg equilibrium. The probabilities are those associated with the corresponding value of the G statistic, corrected for continuity when appropriate. The frequency of LAP II^A is combined with LAP II^B for analysis.

A breeding experiment was done to investigate the heritability of alleles at the polymorphic loci. Twenty pairs of large immature *C. nemoralis* were isolated in 6-inch clay flower pots half-filled with soil. Each pot was covered with a glass plate and placed in a shallow pan of water. The snails were provided with lettuce, carrots, spinach, tropical fish food (various brands) and an abundance of pulverised limestone, and were maintained on a 16-hour photoperiod at 27° C.

Since it is necessary to kill the snails for electrophoresis, matings had to be arranged between snails of unknown allozyme phenotypes. Snails of similar size were paired; shell morphs, although noted, were not considered.

The absence of a darkened and/or reflected lip about the shell aperture was the criterion used in establishing whether or not individuals were immature (Pilsbry, 1939).

Eggs, when they appeared, were placed on moist filter paper in 100 × 20 mm glass petri dishes and incubated at room temperature. Newly hatched snails were reared in similar conditions.

A total of 10 egg clutches from seven different pairs were obtained. Three clutches hatched within 2 weeks; the others never did and were, presumably, infertile. Cross C1 × C2 yielded 38 eggs on 12th June 1973; 36 hatched and 13 survived until electrophoresis was performed on 24th October 1973. Cross C5 × C6 yielded 59 eggs on 12th July 1973; 46 hatched and 12 survived until electrophoresis. The young are very fragile; and most of the mortality occurred soon after hatching, usually due to drowning in moisture condensed on lettuce leaves and on the tops of the petri dishes. To avoid the high mortality of previous crosses, the offspring of cross C15 × C16 were prepared for electrophoresis immediately after hatching. Data were obtained from this cross on 51 individuals which hatched from a clutch of 57 eggs.

Sample preparation of the small snails differed somewhat from the procedure for adults and large immatures. Those with a live weight above 50 mg were placed in 75-mm Nalgene centrifuge tubes with an equal volume:weight of the Tris-EDTA buffer (0·1 ml of buffer was added to tubes with individuals weighing between 50 and 100 mg). The whole snail was ground up in the tube with a thin glass rod. The sample was then sonicated, centrifuged and stored as above. Young snails weighing less than 50 mg were smashed with a pestle in a porcelain spot plate in 0·04-0·05 ml buffer, depending on the size of the individual. Using this sample preparation, LAP and PGM phenotypes were obtained for snails as small as 3·4 mg. The sample was taken up in 75-mm capillary tubes, centrifuged at 10,000 r.p.m. for 2 minutes and stored as above.

The results of the breeding experiment are shown in table 2; these results clearly demonstrate that the phenotypes seen on our gels represent

TABLE 2

Cross	Parental shell phenotypes	Parental allozyme phenotypes	Progeny phenotypes: PGM II			LAP II		
			AA	*AB*	*BB*	*AA*	*AC*	*CC*
C1 × C2	P(12)3(45) × Y00000	PGM II: *AB* × *BB*	0	5 (6·5)*	8 (6·5)			
		LAP II: *CC* × *CC*				0	0	13 (13)
C5 × C6	Y00300 × Y10345	PGM II: *AB* × *AB*	2 (3)	6 (6)	4 (3)			
		LAP II: *CC* × *AC*					4 (6)	8 (6)
C15 × C16	Y12345 × Y123(45)	PGM II: *AA* × *AA*	51 (51)					
		LAP II: *CC* × *AC*					26 (25·5)	25 (25·5)

* Numbers in parentheses are expected values. None of the deviations approaches statistical significance.

allelic variation at these loci. The correspondence in natural populations of observed phenotypes to those expected under Hardy-Weinberg equilibrium further confirms the allelic nature of this variation, although with our sample sizes, the possibility that considerable selection may be operating on these allozymes is certainly not excluded.

We have sampled *C. nemoralis* at nine other localities in North America (Brussard, in preparation) and have found only one additional polymorphic locus: PGM I has a second, slower migrating allele in the colony at London, Ontario. This variation is also in Hardy-Weinberg equilibrium and suggests

a single-locus, diallelic model, but breeding data are not yet available to confirm this interpretation.

3. Discussion

Levan and Fredga (1972) reported that the population of *C. nemoralis* which they sampled from southern Sweden was monomorphic at both the PGM II and LAP II loci. Similarly, Manwell and Baker (1968) found the PGM II locus to be invariant in snails from two localities in southwest England, but that 6-PGD, which is monomorphic at Lexington, was segregating at three alleles in these populations. This apparent discrepancy is best explained by evidence that the Lexington colony did not have a northern European origin, but rather was accidentally introduced from Italy (Brussard, in preparation). We predict that at least some Italian populations of *C. nemoralis* will contain the same alleles as those found at Lexington.

Acknowledgments.—This research is supported by National Institutes of Health grant GM18329 and National Science Foundation grant GB26224. Dr Robert K. Selander has given advice on numerous occasions; his help is gratefully acknowledged.

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