ELECTROPHORESIS STUDIES OF SELECTED SPECIES OF THE GENUS RABDOTUS (GASTROPODA: BULIMULIDAE)

by

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

ABSTRACT

The foot muscle esterases of six populations of Rabdotus pallidior, one population of R. montezuma, and one population of R. harribaueri from Baja California Sur, Mexico, were compared using polyacrylamide gel electrophoresis. A comparison of several of the esterases present revealed some differences; however, further studies of a greater number of specimens are needed to determine the significance of these differences. Electrophoresis alone cannot clearly separate these species. The data provided by electrophoresis must be combined with those obtained from biochemical, anatomical, and ecological studies.

INTRODUCTION

Traditionally, species of the genus Rabdotus have been identified solely on the basis of the shell structure. A detailed study of the anatomy and conchology has recently been completed for the Baja California species of this genus (Christensen, 1978). This study has revised the genus in Baja California. However, the exact status of three closely related species is still in question. Rabdotus pallidior, R. montezuma, and R. harribaueri all have virtually identical reproductive anatomy and similar, although not identical, shell structure. It was for this reason that it was decided that electrophoresis should be used to determine whether there were other distinguishing features among the three species.

Electrophoresis has been used in systematic and genetic studies of many phyla. Electrophoresis provides biochemical information for the separation of taxa. When combined with other biochemical data and anatomical and ecological information, electrophoresis can be used to help distinguish various intra- and interspecific relationships. Electrophoresis has been used extensively in the phylum Chordata: Chen (1967) and Poluhowich and Brush (1972) for Reptilia and Amphibia; Sibley (1960) for Aves; Heckman (1972) for Osteichthyes; and Davis (1964), Petersen (1968), and

Johnson and Selander (1971) for Mammalia. Electrophoresis has been used in studies on Drosophila by Pappas, Rodrick, and Diebolt (1971); on nematodes by Gysels (1968); and on protozoa by Reeves and Bischoff (1968).

Electrophoresis has become a standard tool in the study of molluscan systematics. Michelson (1973) has compiled a list of the various media that have been used for several dozen species of molluscs. In Michelson's paper the two most favored media are starch gel and polyacrylamide gel.

In the study of molluscan systematics, starch gel electrophoresis has been used on Cepaea by Levan and Fredga (1972); Brussand and McCracken (1974); and Matteo, Schiff, and Garfield (1975); other genera studied are Helix by Wahren and Tegelström (1973) and Lacoma by Reid and Dunnill (1969). Polyacrylamide disc electrophoresis has been preferred by Davis and Lindsay (1964, 1967) for studies of Helix, by Burch and Lindsay (1968) for Bulinus, by Schwabl and Murray (1970) for Partula, and by Oxford (1973a, 1973b, 1975) for Cepaea.

A variety of tissues have been examined in these and other studies. Bedford and Reid (1969) used the crystalline styles of some marine molluscs, Schwabl and Murray (1970) used parts of the reproductive system, and Oxford (1973a, 1973b, 1975) used the hepatopancreas. However, the most common tissue studied is the foot muscle. This tissue has been used by Davis and Lindsay (1964, 1967), Davis (1967,

1968, 1972), Patterson (1968), Davis and Takada (1969), and Wu (1972). Davis and Lindsay (1967) give several reasons for using the foot muscle: it is readily removed, it gives a greater weight of proteins per volume than any other organ, it gives more distinct protein components than most other tissues, it is homogeneous, it is rarely parasitized and is easy to check for parasites, and it is characteristic of the phylum. Because the condition of the foot muscle is also independent of such conditions as age, size, and forced laboratory estivation (Davis and Lindsay 1967), I decided to study the proteins of the foot muscle using polyacrylamide disc electrophoresis.

Disc electrophoresis has several advantages over other techniques: the gels are thermostable, transparent, strong, relatively inert, non-ionic, and it is easy to alter the pore size of the gel (Ornstein 1964). In disc electrophoresis the proteins are separated on the basis of net charge, size, and tertiary structure (Maurer 1971).

The gels consist of three layers with different functions and pore sizes. The sample and spacer gels, which have relatively large pores, serve as anticonvection media and to concentrate the sample proteins. The lower or separation gel, which has small pores, serves as a sieving and anticonvection medium (Davis 1964). Each protein migrates in an electric field at a different rate according to its size, shape, and net charge. The end result is separate

discs or bands of proteins in the lower gel. The presence or absence of specific bands can then be compared to the presence or absence of these same bands in different animals. The chemical and theoretical concepts of how polyacrylamide electrophoresis works are described by Ornstein (1964), Davis (1964), and Maurer (1971).

MATERIALS AND METHODS

Davis (1973) stated that a logical starting place for a systematic study is a population. Accordingly, individuals from six populations of Rabdotus pallidior were collected in the Cape Region of Baja California Sur, Mexico. These snails were used to study the inter- and intrapopulational variation of a species. R. montezuma and R. harribaueri are harder to collect, and unfortunately, only one population of each could be studied (Table 1 and Fig. 1).

The snails were allowed to estivate in plastic or glass containers until prepared for electrophoresis. Smith (1966) reported that individuals of the same genus Rabdotus will estivate in the laboratory for up to six years, and personal experience has shown that a high mortality rate exists if the snails are kept active in terraria. Prior to an electrophoretic run the snails to be used were activated for approximately 12 hours and then placed in a freezer (-9°C). This method provided fully extended snails which were free of mucus (Brussand and McCracken 1974). After the snails were thawed, the entire foot muscle was removed with a razor blade and pressed between paper toweling to remove excess moisture. The muscle was then weighed, cut into small pieces, and hand-homogenized with glass-distilled water in an ice bath until all large pieces were homogenized.

Table 1. Collection Localities

Population	Locale
Rabdotus pallidior:	
Rp-1	7.0 mi from the main Todos Santos-Cabo San Lucas road and 4.2 mi above the fire tower on the road to La Burrera, alt. 1000 ft, Baja California Sur, Mexico. C. C. Christensen and J. A. Christensen, 20 Dec 1975.
Rp-2	3.1 mi S of La Piedritas (and ca. 19.8 mi S of Todos Santos) on the inland side of the Todos Santos-Cabo San Lucas road, Baja California Sur, Mexico. C. C. Christensen and J. A. Christensen, 20 Dec 1975.
Rp-3	7.7 mi E of the school in Santa Caterina, alt. 800 ft, Baja California Sur, Mexico. C. C. Christensen and J. A. Christensen, 23 Dec 1975.
Rp-4	13.5 mi NE of Santa Caterina on the road to Los Frailes, alt. 1200 ft, Baja California Sur, Mexico. C. C. Christensen and J. A. Christensen, 23 Dec 1975.
Rp-5	16.8 mi NE of Santa Caterina on the road to Los Frailes, alt. 1100 ft, Baja California Sur, Mexico. C. C. Christensen and J. A. Christensen, 23 Dec 1975.
Rp-6	5.0 mi N of Casa Huespedes Laguna and ca. 4.8 mi S of the first PEMEX station in La Ribera, alt. 200 ft, Baja California Sur, Mexico. C. C. Christensen and J. A. Christensen, 24 Dec 1975.
Rabdotus montezuma:	
Rm	2.1 mi N of El Saucito on the main road from Todos Santos to Cabo San Lucas, alt. 1500 ft, Baja California Sur, Mexico. C. C. Christensen and J. A. Christensen, 22 Dec 1975.
Rabdotus harribaueri:	
Rh	29.9 mi from Santa Caterina and 5.3 mi from Los Frailes on the road between the two locations, alt. ca. 50 ft in an arroyo 0.3 m below the road, Baja California Sur, Mexico. C. C. Christensen and J. A. Christensen, 24 Dec 1975.

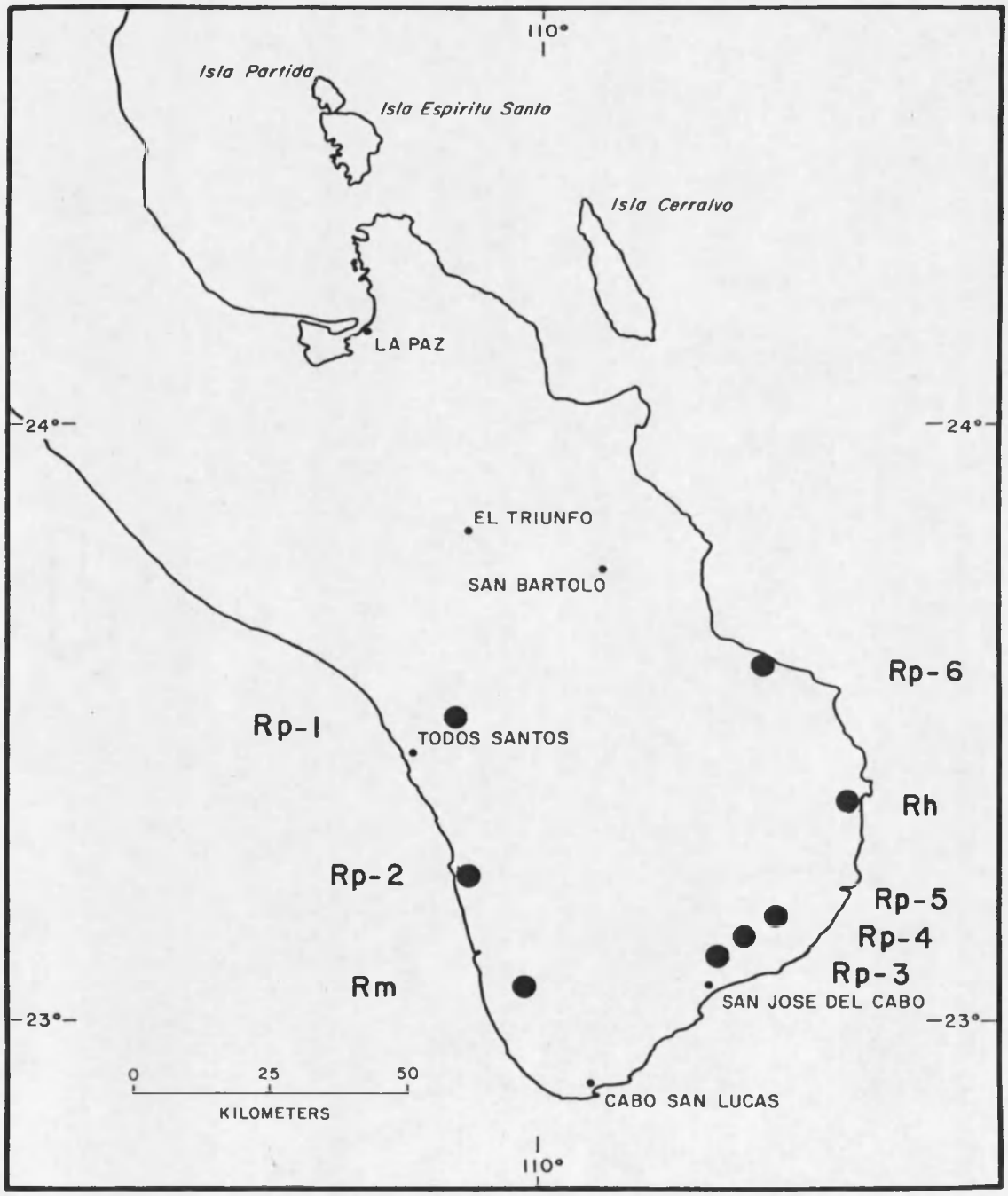

Fig. 1. Collection Sites of Rabdotus in Baja California Sur, Mexico

The amount of snail muscle and glass-distilled water used was varied from 1:10 to 1:8.5 so that the same bands from snail to snail would have approximately the same density (Davis, 1975). Next the resulting solution was transferred by pipette to a refrigerated plastic flask and homogenized in an ice bath on a Virtis 23 Homogenizer at 23,000 rpm for 5 minutes. After homogenization the solution was transferred by pipette to a refrigerated centrifuge tube and spun at maximum speed on an Adams Safeguard Centrifuge for 5 minutes. Following this procedure the supernatant was withdrawn with a 1 cc hypodermic syringe and stored in Pro-Vial plastic vials at -9°C for not longer than one month before use. This method gave consistent results. Numerous authors (Manwell and Baker 1968, Malek and File 1971, and Matteo et al. 1975) have reported that the major esterases, the proteins examined in this study, remain stable even when stored for several months at -20°C.

The electrophoretic technique used was based on that of Davis (1964) and Davis and Lindsay (1967). All chemicals and stock solutions except Fast Blue RR salt and alpha-naphthyl acetate were stored at 4°C in brown glass bottles; the above mentioned chemicals were stored at -9°C under desiccation. All solutions used are listed in Table 2.

Solutions were made up as follows: the chemicals were weighed on a torsion balance, mixed with glass-distilled water in volumetric flasks, and vacuum filtered

Table 2. Stock Solutions

Solution A (Buffer pH 9.2) Place 24 ml 1N HCl into a 100 ml volumetric flask. Add 19.15 g Tris (2-amino-2-hydroxymethyl-1,3-propanediol) and 0.23 ml Temed (N, N, N', N'-tetramethylenediamine). Fill the flask with glass-distilled water to the mark. Filter after all solutes have been dissolved and the pH checked.

Solution B (Buffer pH 6.9) Place 25.6 ml 1M H_3PO_4 into a 100 ml volumetric flask. Add 5.7 g Tris and 0.05 ml Temed. Fill to the mark with glass-distilled water and filter after the pH has been checked.

Solution C (Monomer) Place 30 g acrylamide and 0.80 g Bis (N, N'-methylenebisacrylamide) into a 100 ml volumetric flask. Bring to a total of 100 ml with glass-distilled water. Filter.

Solution D (Monomer) Place 10 g acrylamide and 2.5 g Bis into a 100 ml volumetric flask. Add glass-distilled water to the mark. Filter after all Bis has dissolved.

Solution E (Catalyst) Dissolve 0.004 g riboflavin in 100 ml glass-distilled water. Filter.

Solution F (Upper gel concentrate) Mix one part Solution B, two parts Solution D, and one part Solution E.

Solution G (Catalyst) Dissolve 0.14 g ammonium persulfate in 100 ml glass-distilled water. Filter.

Solution H (Running Buffer pH 8.3) Dissolve 6 g Tris and 57.6 g glycine in 2 liters glass-distilled water. Adjust the pH if necessary. Add enough Bromophenol Blue to give the solution a light blue color.

Solution I (Esterase stain) Place 0.04 g alpha-naphthyl acetate and 0.07 g Fast Blue RR salt into a 100 ml graduated cylinder. Add 100 ml 0.1 M Tris-HCl buffer (6.06 g Tris and 4.1 ml concentrated HCl in 500 ml glass-distilled water). Keep away from light while stirring. Keep in dark until used. (Use within 1 hour.)

Solution H (Gel wash) Mix three parts 100% ethanol with two parts 10% acetic acid. May be stored at room temperature in a clear glass bottle.

with a Millipore 47 mm filter. The solutions were then stored at 4°C in brown glass bottles for a maximum of eight weeks, with the exception of Solution G which was always used within one week. Solutions B, D, E, and F were stored for at least 24 hours prior to use to allow for stabilization.

Just prior to an electrophoretic run Solutions A, C, F, G, and the snail supernatant were removed from storage and allowed to warm to room temperature in order to minimize the formation of air bubbles in the final gel. Standard electrophoretic glass tubes (7 mm outer diameter, 76 mm long) were then removed from a solution of 0.5 ml Kodak Photo-Flo 200 and 100 ml glass-distilled water (this solution facilitates gel removal), and thoroughly dried with paper toweling and cotton swabs. Small squares of Parafilm were placed over one opening and trimmed with a razor blade to form a tight fit. Next, the tubes were marked 13 mm from the open end with a china marker and placed Parafilm end down into a leveled rack (Fig. 2).

Next, one part Solution A, one part Solution C, and two parts Solution G were pipetted into a small beaker. The resulting lower gel solution was drawn into a 20 cc hypodermic syringe, the needle removed, one finger placed over the opening, and the plunger pulled several times to remove any trapped air bubbles.

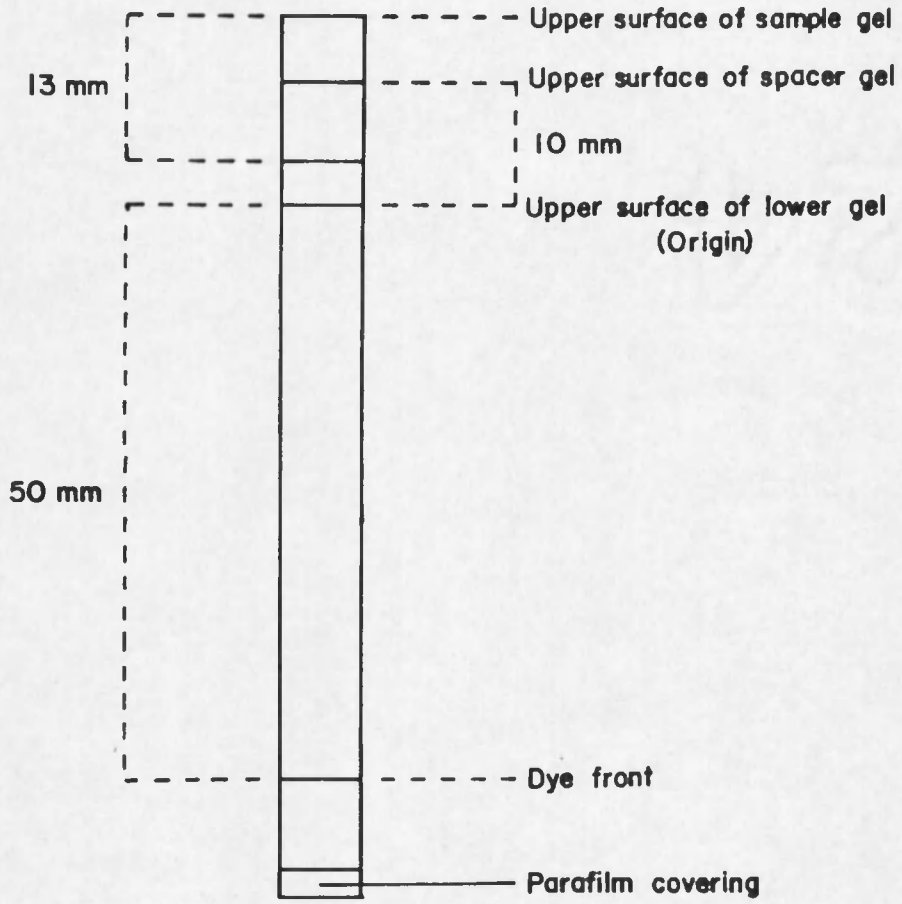

Fig. 2. Gel Layering

The glass tubes were then filled so that the top of the meniscus was level with the china mark. A small amount of glass-distilled water was slowly pipetted down the inside of the tube; care was taken to avoid any disturbance at the gel-water interface. (If turbidity was observed, the affected tube was emptied and refilled, as any disturbance at the gel-water interface may cause distortion in the bands observed.)

As the tubes were polymerized for twenty minutes, the spacer gel was prepared by mixing one part Solution F with one part glass-distilled water. After the lower gel had fully polymerized, the water layer was removed by carefully touching the surface of the water with a twisted piece of Kimwipe. Following this each tube was removed from the rack, filled with Solution F, marked 10 mm above the upper surface of the lower gel (one tube was also marked 50 mm below the surface of the gel), once again dried with Kimwipes, and returned to the same column in the rack. This time the tubes were filled so that the bottom of the meniscus was level with the china mark. They were then water layered as before. A twenty minute polymerization period followed with the rack 25 to 50 mm below two 15 watt Cool-white Sylvania fluorescent lights.

While the spacer gel was polymerizing, a solution of one part snail supernatant and two parts Solution F was mixed in small vials. As three different snails were

usually run at a time, care was taken to label properly each vial. The remaining supernatant was returned to the freezer. (Matteo, Schiff, and Garfield [1975] have reported that repeated freezing and thawing do not harm the major esterases.) After twenty minutes the spacer gel had turned white-opaque, and the water was then removed with Kimwipes. Each tube was once again washed with Solution F, dried, and returned to the rack. The tubes were then filled to the top (ca. 0.15 ml) with one of the sample solutions and placed under the lights for another twenty minutes. During this final polymerization the lower chamber of the electrophoretic unit was filled with Solution H and placed inside a 4°C refrigerator.

After polymerization was completed, each tube was removed from the rack, pushed through the rubber stoppers of the upper chamber (Fig. 3), and placed into the correspondingly numbered slot of the upper chamber. The Parafilm coverings were now removed and replaced with a drop of Solution H. The rest of Solution H was poured into the upper chamber, the lid fitted tightly on top, and the entire structure placed on top of the lower chamber which was already inside the refrigerator. After the wires in the lid were connected to a Heathkit power supply unit (Model IP-32), the unit was allowed to warm up while the temperature in the refrigerator returned to 4°C. Once the temperature reached 4°C, the power unit was turned on and adjusted to

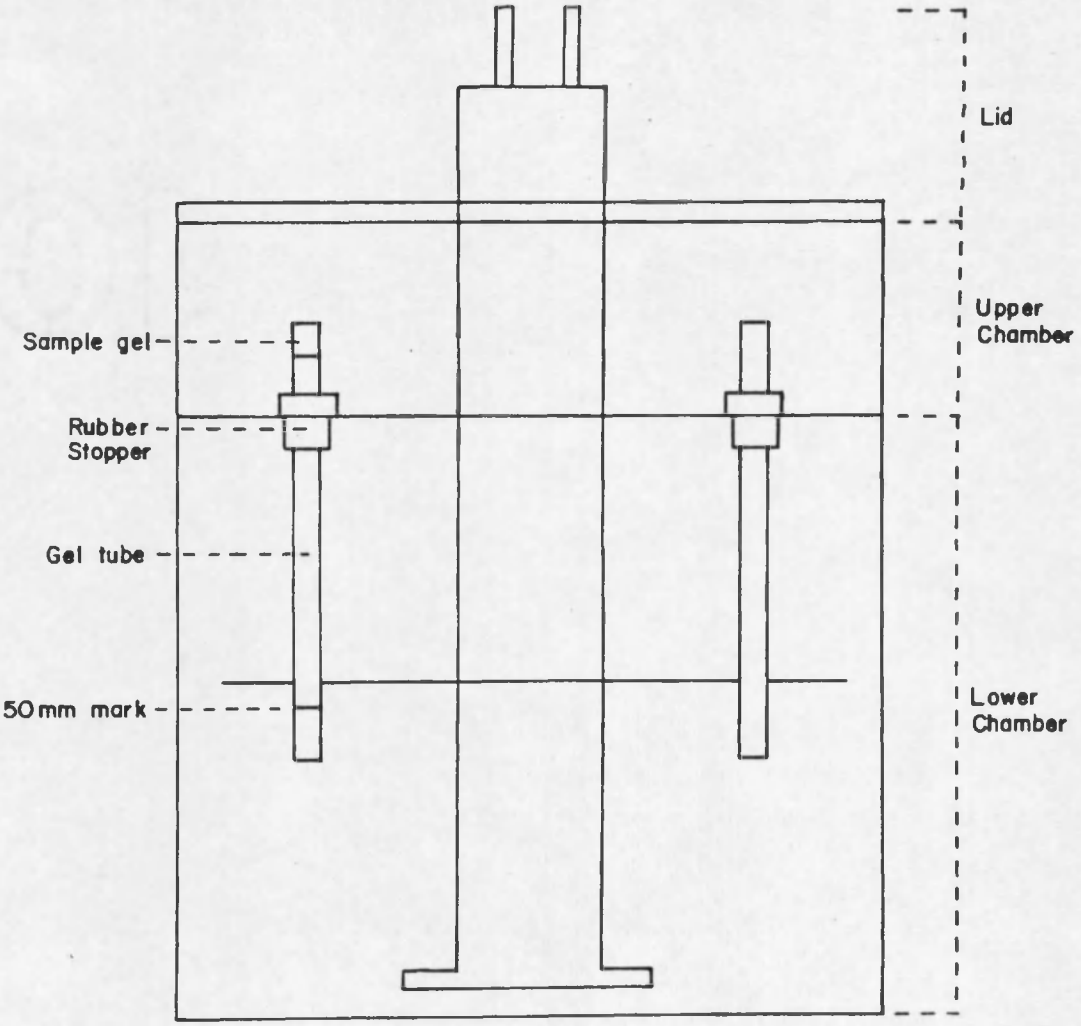

Fig. 3. Electrophoretic Chamber

5 mamps per tube or a total of 60 mamps as twelve tubes were always run at one time. Each run, the time required for the dye front to reach the 50 mm mark, required the current to be on for approximately 0.5 hour.

After the electrophoretic run was completed, the power supply unit was turned off, the lid disconnected, and the upper chamber removed from the refrigerator. The Solution H from the upper chamber was poured off and stored at 4°C in a brown glass bottle. Each tube was removed from the chamber, and the gel loosened by carefully running a stream of glass-distilled water from a 20 cc hypodermic syringe between the glass tube and the gel. Following removal of the gel, the dye front was marked by inserting a small piece of nylon line at that point.

Each gel was then placed into a numbered 100 mm test tube, covered with Solution I, and incubated at 37°C for 2.5 hours; stains similar to Solution I have been used by Markert and Hunter (1959), Burch and Lindsay (1968), Patterson (1968), Coles (1969), and Flowerdew and Crisp (1975). While the gels were in the stain, the rest of Solution H, from the lower chamber, was poured into a second brown glass bottle and stored at 4°C. After 2.5 hours all of Solution I was poured off and discarded; the gels rinsed with glass-distilled water and replaced into the test tubes. The test tubes were then filled with Solution J, the gel wash, and incubated at 37°C overnight. In the morning the gels were

removed from the test tubes, rinsed with glass-distilled water, transferred to leveled 75 mm test tubes, and covered with 10% acetic acid. This storage procedure was carried out one tube at a time in order to avoid any accidental mixing of the gels.

At a later date each gel was run three times on a Photovolt densitometer (Model 525); the tracings of each gel were then measured with a ruler calibrated in 0.5 mm increments (Davis 1968), and an Rf value (the ratio of the distance from the origin to a protein band over the distance from the origin to the dye front) obtained for all visible bands close to the dye front. An Rf value tends to minimize differences in homologous bands due to different run lengths (Davis and Lindsay 1967). The Rf mean and standard deviation (Wu 1972) were calculated for each band used, and a grand mean and standard deviation of each of these bands were determined for every snail. The presence or absence of a band in each snail was scored.

RESULTS

Davis and Lindsay (1967) have suggested that at least 10 specimens per population are needed in order to show all variations within a population and to establish a pattern. Ten snails from population Rp-4 of Rabdotus pallidior were run; unfortunately, due to mortality and the inability to collect large numbers of R. harribaueri and R. montezuma, and the other populations of R. pallidior, only three or four individuals could be used.

The esterase stain provided 12 to 15 bands. Of these only those closest to the dye front were measured as the staining technique failed to stain consistently the slower moving proteins. Davis and Lindsay (1967), Davis (1968), Burch and Lindsay (1968), and Schwabl and Murray (1970) have shown that these faster moving bands are more distinct and reliable.

The grand mean and standard deviations calculated are presented in Appendix A. The presence or absence of a band is shown in Fig. 4, Fig. 5, Fig. 6, Table 3, and Table 4. This study can only show that the bands are analogous; it cannot show that the bands are homologous (Davis 1967).

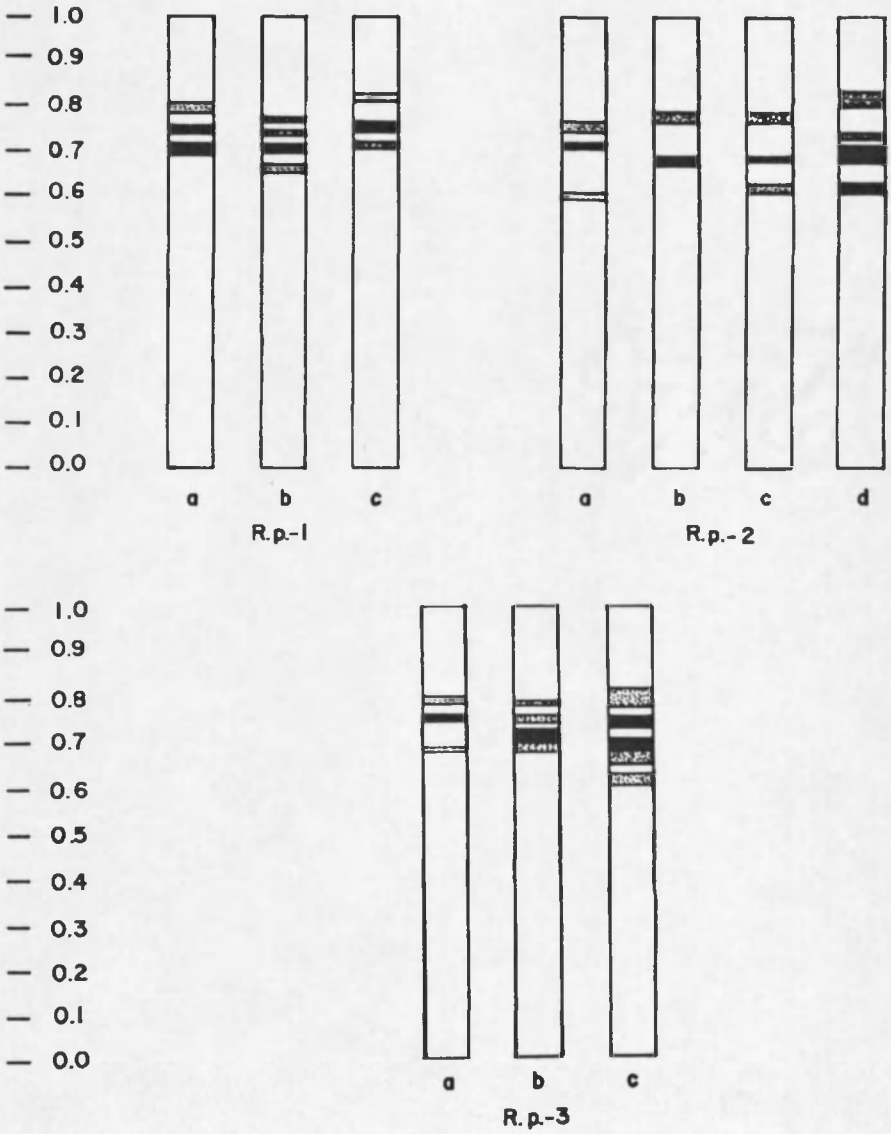

Fig. 4. Esterase banding of Rabdotus pallidior, Rp-1, Rp-2, Rp-3

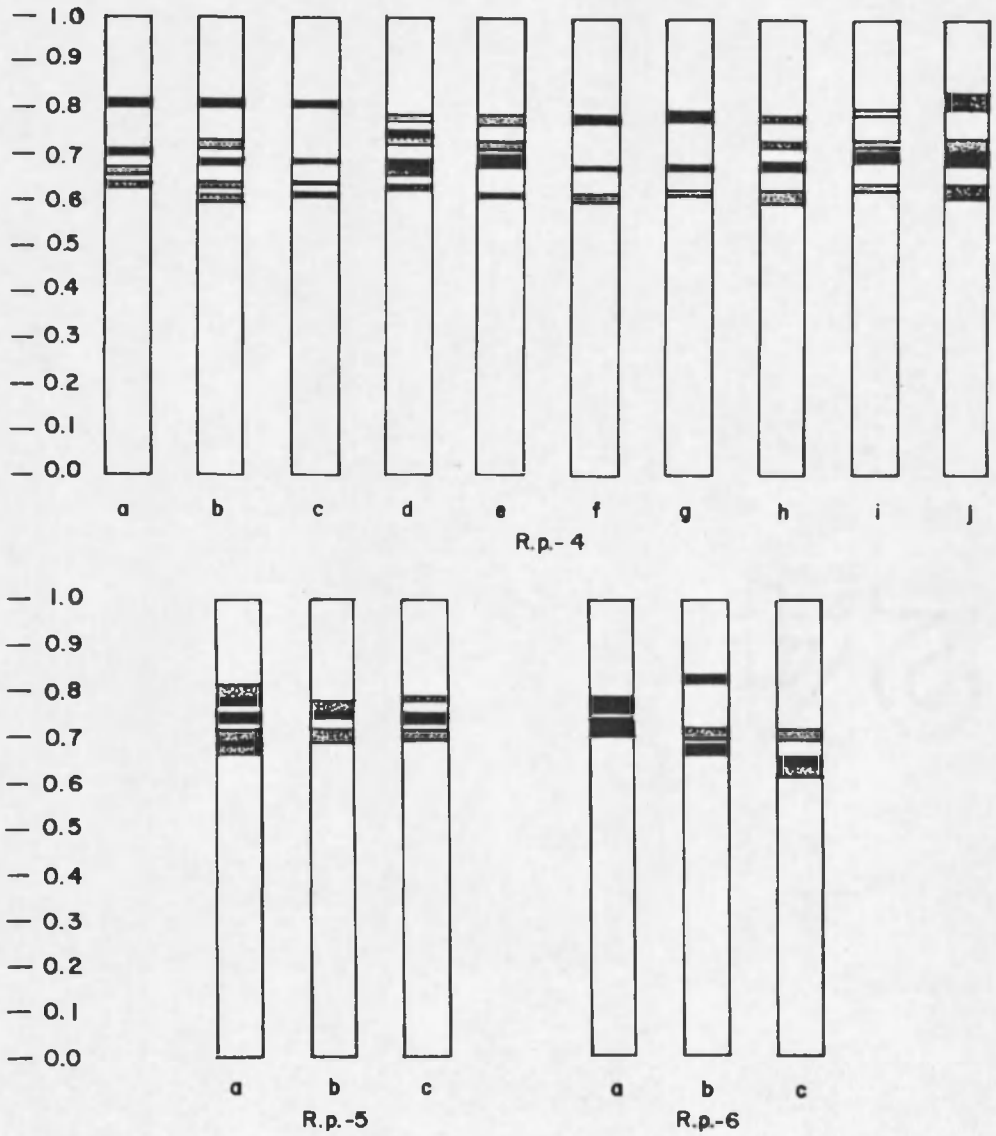

Fig. 5. Esterase banding of Rabdotus pallidior, Rp-4, Rp-5, Rp-6

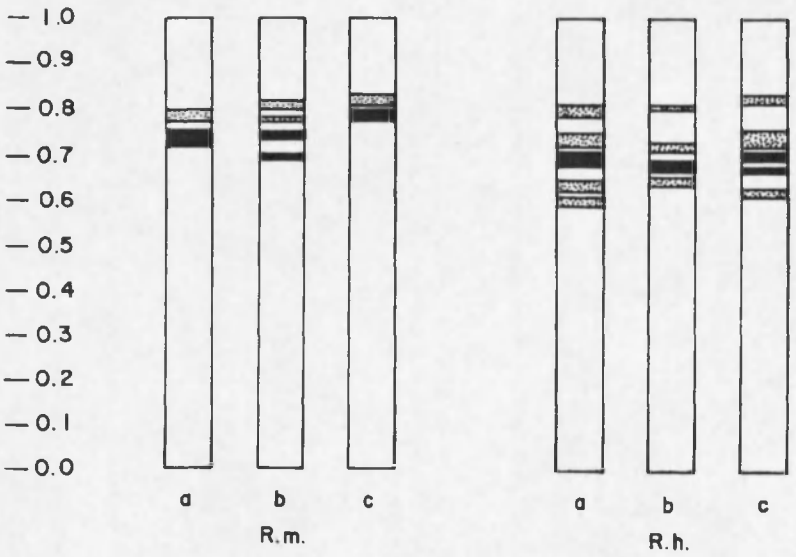

Fig. 6. Esterase banding of Rabdotus montezuma and Rabdotus harribaueri

Table 3. Presence or Absence of Esterase Bands

Specimen	Est 1	Est 2	Est 3	Est 4	Est 5	Est 6	Est 7
Rp-1							
a	-	-	-	+	+	-	+
b	-	-	+	+	+	+	-
c	-	-	-	+	+	-	+
Rp-2							
a	+	-	-	+	+	-	-
b	-	-	-	+	-	-	+
c	-	+	-	+	-	-	+
d	-	+	-	+	+	-	+
Rp-3							
a	-	-	-	+	-	+	+
b	-	-	+	+	+	+	-
c	-	+	+	+	+	-	+
Rp-4							
a	-	+	+	+	-	-	+
b	-	+	+	+	+	-	+
c	-	+	+	+	-	-	+
d	-	+	+	+	+	+	+
e	-	+	-	+	+	-	+
f	-	+	-	+	-	-	+
g	-	+	-	+	-	-	+
h	-	+	-	+	+	-	+
i	-	+	-	+	+	-	+
j	-	+	-	+	+	-	+
Rp-5							
a	-	-	+	+	+	+	+
b	-	-	-	+	+	+	-
c	-	-	-	+	+	+	-
Rp-6							
a	-	-	-	+	+	-	-
b	-	-	+	+	-	-	+
c	-	+	+	+	-	-	-
Rh							
a	-	+	+	+	+	-	+
b	-	-	+	+	+	-	+
c	-	+	+	+	+	-	+
Rm							
a	-	-	-	-	+	+	-
b	-	-	-	+	+	+	+
c	-	-	-	-	-	+	+

Table 4. Review of Esterase Bands

	Est 1	Est 2	Est 3	Est 4	Est 5	Est 6	Est 7
Esterase present in some individuals of all populations	-	-	-	+	+	-	+
Scarest esterase	+	-	-	-	-	-	-
Esterase found in all individuals of *Rabdotus pallidior*	-	-	-	+	-	-	-
Esterase found in all individuals of *Rabdotus harribaueri*	-	-	+	+	+	-	+
Esterase found in all individuals of *Rabdotus montezuma*	-	-	-	-	-	+	-

DISCUSSION AND CONCLUSIONS

Seven proteins can be identified in the region of the gel closest to the dye front. These proteins are designated Est_1, Est_2, ..., Est_7 with Est_1 being closest to the origin. The density of a band was not a determining factor because the density may be variable from individual to individual (Ukoli 1974).

The snails from locality one of Rabdotus pallidior always had Est_4 and Est_5. (See Appendix A for the Rf values.) Of the three individuals run, two had Est_7, one had Est_3 and Est_6, and none had Est_1. In the population from locality two all four snails had Est_4, three had Est_7, two had Est_2, two had Est_5, one had Est_1, and none had Est_3 or Est_6. Only Est_4 was always present in the snails from locality three. However, two of the three snails had Est_3 and Est_5, two had Est_6, and two had Est_7. One had Est_2 and none had Est_1. In the population from locality four all ten individuals had Est_2, Est_4, and Est_7. Six had Est_5, four had Est_3, one had Est_6, and none had Est_1. For the population from locality five all three individuals had Est_4, Est_5, and Est_6. One snail also had Est_3 and Est_7. None had Est_1 or Est_2. In the population from locality six all three snails had Est_4. Two snails had Est_3, one had Est_2, and one had Est_5.

In the population of R. harribaueri studied, all three snails had Est_3, Est_4, Est_5, and Est_7. Two of the snails had Est_2, and none had Est_1 or Est_6. All three individuals of R. montezuma had Est_6. Two snails had Est_5, two had Est_7, one had Est_4, and none had Est_1, Est_2, or Est_3.

The three species can be separated, although there seems to be as much variation among the populations of R. pallidior as among the three species. (Davis and Lindsay 1967; Burch and Lindsay 1968; Reid and Dunnill 1969; Davis 1972, 1973; and Levan and Fredga 1972 have also found intraspecific variation in molluscs. Wright and File 1968 found even greater intraspecific differences than those between species of the same group.) Rabdotus montezuma never had Est_1, Est_2, or Est_3 but always had Est_6. (Est_6 was common in only two populations of R. pallidior.) R. harribaueri always had Est_4, Est_5, and Est_7. R. pallidior always had Est_4. In addition the population from locality one never had Est_1 or Est_2 and always had Est_5. Est_1 and Est_2 were also missing in the population from locality five, and while Est_5 was always present so was Est_6. The population from locality two was missing Est_3 and Est_6. All other populations had at least one individual with one or the other of these esterases. The only esterase missing from the snails from locality three was Est_1. The only other population with just Est_1 missing was the one from locality four. The population from locality four always had Est_2 and

Est_7. No other population always had Est_2. Est_1 and Est_6 are missing from the snails from locality six. No other population is completely missing these esterases.

In conclusion, their virtually identical reproductive anatomy and very similar shell morphology indicate that these snails have a relatively recent common ancestor. The esterases of the foot muscle also support this theory. Est_4, Est_5, and Est_7 were present in at least one individual from every population. It may be that the common ancestor had these esterases. R. harribaueri would then be the closest to the original form (100% of the individuals had all three of these esterases), with R. pallidior next (42%) and finally R. montezuma (33-1/3%).

These figures together with other data indicate that R. harribaueri and R. pallidior are more closely related to each other than to R. montezuma. In addition to the presence of Est_4, Est_5, and Est_7, Est_1 appeared 0% of the time in R. harribauer, 4% of the time in R. pallidior, and 0% of the time in R. montezuma. Est_2 was found 66-2/3% of the time in R. harribaueri, 54% of the time in R. pallidior, and 0% of the time in R. montezuma. Est_3 was found 100% of the time in R. harribaueri, 38% of the time in R. pallidior, and 0% of the time in R. montezuma. Est_6 appeared 0% of the time in R. harribaueri, 27% of the time in R. pallidior (Est_6 was common in only two populations and completely missing in two other populations), but was found 100% of the time in R.

montezuma. These findings for the separation of R. harribaueri, R. pallidior, and R. montezuma are consistent with the findings of Christensen (1978). However, more individuals of the populations of R. pallidior, and more individuals and populations of R. harribaueri and R. montezuma must be studied. Additional information is needed to clearly separate R. pallidior and R. harribaueri by means of electrophoretic data and to determine the significance of intraspecific variation in these snails. The presence of Est_6 in other populations of R. montezuma must also be confirmed.

APPENDIX A

Rf VALUES, STANDARD DEVIATIONS, AND PERCENTAGE OF BANDS PRESENT

Population	Band	Specimen	Specimen	Specimen	Percentage present
Rp-1		a	b	c	
	7	0.794±0.008	--	0.816±0.006	66-2/3
	6	--	0.771±0.003	--	33-1/3
	5	0.754±0.008	0.747±0.007	0.762±0.010	100
	4	0.708±0.010	0.699±0.004	0.719±0.009	100
	3	--	0.666±0.010	--	33-1/3
	2	--	--	--	0
	1	--	--	--	0
Rp-2		a	b	c	
	7	--	0.778±0.010	0.779±0.011	
	6	--	--	--	
	5	0.759±0.010	--	--	
	4	0.717±0.011	0.685±0.011	0.696±0.004	
	3	--	--	--	
	2	--	--	0.627±0.009	
	1	0.611±0.007	--	--	
		d			
	7	0.817±0.017			75
	6	--			0
	5	0.741±0.009			50
	4	0.704±0.016			100
	3	--			0
	2	0.629±0.010			50
	1	--			33-1/3
Rp-3		a	b	c	
	7	0.794±0.007	--	0.793±0.021	66-2/3
	6	0.755±0.007	0.784±0.005	--	66-2/3
	5	--	0.749±0.009	0.741±0.010	66-2/3
	4	0.685±0.006	0.718±0.015	0.694±0.015	100
	3	--	0.690±0.011	0.673±0.018	66-2/3
	2	--	--	0.616±0.012	33-1/3
	1	--	--	--	0

Population	Band	Specimen	Specimen	Specimen	Percentage present
Rp-4		a	b	c	
	7	0.808±0.008	0.814±0.009	0.813±0.007	
	6	--	--	--	
	5	--	0.722±0.006	--	
	4	0.704±0.004	0.685±0.006	0.687±0.004	
	3	0.665±0.008	0.635±0.007	0.640±0.006	
	2	0.632±0.006	0.607±0.008	0.612±0.008	
	1	--	--	--	
		d	e	f	
	7	0.783±0.009	0.780±0.011	0.789±0.005	
	6	0.748±0.011	--	--	
	5	0.733±0.007	0.724±0.008	--	
	4	0.688±0.008	0.687±0.013	0.676±0.004	
	3	0.662±0.006	--	--	
	2	0.631±0.003	0.613±0.005	0.610±0.009	
	1	--	--	--	
		g	h	i	
	7	0.789±0.008	0.786±0.006	0.799±0.007	
	6	--	--	--	
	5	--	0.728±0.008	0.732±0.008	
	4	0.679±0.008	0.684±0.012	0.701±0.008	
	3	--	--	--	
	2	0.622±0.007	0.614±0.014	0.631±0.008	
	1	--	--	--	
		j			
	7	0.827±0.018			100
	6	--			10
	5	0.729±0.018			60
	4	0.700±0.017			100
	3	--			40
	2	0.626±0.017			100
	1	--			0

Population	Band	Specimen	Specimen	Specimen	Percentage present
Rp-5		a	b	c	
	7	0.802±0.014	--	--	33-1/3
	6	0.780±0.011	0.764±0.006	0.780±0.009	100
	5	0.744±0.011	0.748±0.006	0.744±0.012	100
	4	0.702±0.012	0.702±0.014	0.705±0.011	100
	3	0.673±0.011	--	--	33-1/3
	2	--	--	--	0
	1	--	--	--	0
Rp-6		a	b	c	
	7	--	0.824±0.012	--	33-1/3
	6	--	--	--	0
	5	0.766±0.021	--	--	33-1/3
	4	0.723±0.021	0.709±0.011	0.705±0.009	100
	3	--	0.677±0.009	0.654±0.007	66-2/3
	2	--	--	0.628±0.012	33-1/3
	1	--	--	--	0
Rm		a	b	c	
	7	--	0.812±0.015	0.831±0.011	66-2/3
	6	0.784±0.016	0.799±0.012	0.792±0.009	100
	5	0.741±0.015	0.744±0.007	--	66-2/3
	4	--	0.703±0.008	--	33-1/3
	3	--	--	--	0
	2	--	--	--	0
	1	--	--	--	0
Rh		a	b	c	
	7	0.803±0.013	0.808±0.008	0.831±0.012	100
	6	--	--	--	0
	5	0.736±0.012	0.724±0.011	0.743±0.016	100
	4	0.701±0.014	0.685±0.010	0.704±0.012	100
	3	0.642±0.010	0.652±0.012	0.679±0.009	100
	2	0.607±0.010	--	0.624±0.009	66-2/3
	1	--	--	--	0

REFERENCES

Bedford, J. J., and M. S. Reid. 1969. Gel electrophoresis of proteins in the crystalline styles of 6 species of certain Mollusca. Comp. Biochem. Physiol. 29: 659-664.

Brussand, P. E., and G. F. McCracken. 1974. Allozymic variation in a North American colony of Cepaea hemorobis. Heredity 33(1):98-101.

Burch, John B., and Gene K. Lindsay. 1968. Electrophoretic analysis of esterases in Bulinus. Ann. Rept. Amer. Malac. Union for 1967:39-40.

Chen, P. S. 1967. Separation of serum proteins in different amphibian species by polyacrylamide gel electrophoresis. Experientia 23:483-485.

Christensen, Carl Cummer. 1978. A revision of the genus Rabdotus (Pulmonata: Bulimulidae) in Baja California, Mexico. Ph.D. dissertation, University of Arizona.

Coles, G. C. 1969. Enzyme electrophoresis and speciation of Schistosoma intermediate hosts. Parasitology 61:19-25.

Davis, Baruch J. 1964. Disc electrophoresis: method and application to human serum proteins. Ann. N. Y. Acad. Sci. 121(2):404-427.

Davis, George M. 1967. The systematic relationship of Pomatiopsis lapidaria and Oncomelania hupensis formosana (Prosobranchia: Hydrobiidae). Malacologia 6(1):1-143.

Davis, George M. 1968. A systematic study of Oncomalania hupensis chiui (Gastropoda: Hydrobiidae). Malacologia 7(1):17-70.

Davis, George M. 1972. Geographic variation in Semisulcospira libertina (Mesogastropoda: Pleuroceridae). Proc. Malac. Soc. Lond. 40(1): 5-32.

Davis, George M. 1973. Integrated approach to molluscan systematics. Malc. Rev. 6:42-43.

Davis, George M. 1975. Department of Malcology, Academy of Natural Science, Philadelphia, personal communication.

Davis, George M., and Gene K. Lindsay. 1964. Disc electrophoresis in the study of molluscan systematics. Ann. Rept. Amer. Malac. Union for 1964:20-21.

Davis, George M., and Gene K. Lindsay. 1967. Disc electrophoretic analysis of moluscan individuals and populations. Malacologia 5(2):311-334.

Davis, George M., and T. Takada. 1969. Oncomelania hupensis nosophora: electrophoretic separation of foot proteins of laboratory-reared and field collected specimens. Experimental Parasitology 25:193-201.

Flowerdew, M. W., and D. J. Crisp. 1975. Esterase heterogeneity and an investigation into racial differences in the cirripede Balanus balanoides using acrylamide gel electrophoresis. Marine Biology 33:33-39.

Gysels, H. 1968. Electrophoretic observations on the protein composition of free-living and plant-parasitic nematodes, with a special reference to some components showing a digestive activity. Nematologica 14:489-496.

Heckman, James Robert. 1972. Developmental, immunological and distributional properties of lactate dehydrogenase isozymes in trout. Diss. Abst. Int. 33(1):66-B. 72:19317.

Johnson, Walter E., and Robert K. Selander. 1971. Protein variation and systematics in kangaroo rats (genus Dipodomys). Syst. Zool. 20(4):377-405.

Levan, G., and K. Fredga. 1972. Isozyme polymorphism in three species of land snails. Hereditas 71: 245-252.

Malek, Emile A., and Sharon K. File. 1971. Electrophoretic studies on the digestive gland esterases of some biomphalarid and lymnaeid snails. Bull. Wld. Hlth. Org. 45:819-825.

Manwell, C., and C. M. A. Baker. 1968. Genetic variation of isocitrate, malate and 6-phospho-gluconate dehydrogenases in snails of the genus Cepaea--introgressive hybridization polymorphism and pollution? Comp. Biochem. Physiol. 26:195-209.

Markert, C. C., and R. L. Hunter. 1959. The distribution of esterases in mouse tissues. J. Histochem. Cytochem. 7:42-49.

Matteo, Martha R., Robert Schiff, and Linda Garfield. 1975. The non-specific esterases of the marine snail Littorina littorea: histochemical characterization. Comp. Biochem. Physiol. 50A:141-147.

Maurer, H. Rainer. 1971. Disc electrophoresis and related techniques of polyacrylamide gel electrophoresis, 2nd ed. Walter de Gruyter, Berlin.

Michelson, E. H. 1973. Electrophoretic techniques in malachology: applications and pitfalls. Malac. Rev. 6:44-47.

Ornstein, L. 1964. Disc electrophoresis. I. Background and theory. Ann. N. Y. Acad. Sci. 121(2):321-349.

Oxford, G. S. 1973a. The genetics of Cepaea esterases 1. Cepaea nemoralis. Heredity 30:127-139.

Oxford, G. S. 1973b. Molecular weight relationships of the esterases in Cepaea nemoralis and Cepaea hortensis (Mollusca: Helicidae) and their genetic implications. Biochem. Genet. 8:365-382.

Oxford, G. S. 1975. Food induced esterase phenocopies in the snail Cepaea nemoralis. Heredity 35(3):361-370.

Pappas, Peter W., Gary F. Rodrick, and John R. Diebolt. 1971. Protein and enzyme variation in Drosophila. Comp. Biochem. Physiol. 40B:1029-1035.

Patterson, Charlotte M. 1968. Studies on Succineidae. Ann. Rept. Amer. Malac. Union for 1967:31-32.

Petersen, Michael K. 1968. Electrophoretic blood-serum patterns in selected species of Peromiscus. Amer. Midl. Naturalist 79(1):130-148.

Poluhowich, John J., and Alan H. Brush. 1972. An electrophoretic study of Sphenodon proteins. Comp. Biochem. Physiol. 41B:281-285.

Reeves, R. E., and J. M. Bischoff. 1968. Classification of Entamoeba species by means of electrophoretic properties of amebal enzymes. J. Parasitology. 54: 594-600.

Reid, Robert G. B., and Robert M. Dunnill. 1969. Specific and individual differences in the esterases of members of the genus Macoma (Mollusca:Bivalvia). Comp. Biochem. Physiol. 29:601-610.

Schwabl, Gerda, and James Murray. 1970. Electrophoresis of proteins in natural populations of Partula (Gastropoda). Evolution 24(2):424-430.

Sibley, C. G. 1960. The electrophoretic patterns of avian egg-white proteins as taxonomic characters. Ibis 102:215-284.

Smith, Allan Goodwin. 1966. Land snails of the Galapagos. Pages 240-251 in R. I. Bowman, ed., The Galapagos: Proceedings of the symposia of the Galapagos International Scientific Project. University of California Press, Berkeley.

Ukoli, F. M. A. 1974. Electrophoretic studies on foot muscle esterases of some African Biomphaloria species. Malc. Rev. 7(1):15-24.

Wahren, H., and H. Tegelström. 1973. Polymorphism of esterases and tetrazolium oxidases in the Roman snail. Helix pomatia: A Study of populations in Sweden and Germany. Biochem. Genet. 9:169-174.

Wright, C. A., and Sharon K. File. 1968. Digestive gland esterases in the genus Bulininus (Mollusca, Planorbidae). Comp. Biochem. Physiol. 27:871-874.

Wu, Shi-Kuei. 1972. Comparative studies on a polyploid series of the African snail genus Bulinus (Basomatophora: Planorbidae). Malac. Rev. 5:95-164.