ENZYMATIC VARIATION IN THE LAND SNAIL *EUGLANDINA TEXASIANA* (GASTROPODA: PULMONATA) FROM SOUTH TEXAS AND NORTHEASTERN MEXICO

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Abstract.—Enzymatic variation in four specimens of the land snail *Euglandina texasiana* (Pfeiffer) from south Texas and northeastern México (150 km distant) was examined using cellulose acetate gel electrophoresis. Ten of the 15 loci examined were found to be monomorphic for all specimens. Considerable variation was observed to occur in the remaining five loci. A computer analysis of the resulting enzymatic variation revealed that specimens from these two locations were 94.5% genetically similar. A single specimen of *Euglandina singleyana* (Binney) from New Braunfels in central Texas was found to be 47.6% similar to specimens of *Euglandina texasiana*.

Resumen.—La variación enzimática en cuatro especímenes del caracol terrestre *Euglandina texasiana* (Pfeiffer) del sur de Texas y del nordeste de México (a 150 km distante) fue examinada usando electroforesis de gel de acetato celuloso. Se encontró que diez de los 15 lugares examinados son monomórficos para todos los especímenes. Un análisis de computadora de la variación enzimática resultante reveló que los especímenes de estas dos localidades fueron el 94.5% genéticamente similares. Se encontró que un solo espécimen de *Euglandina singleyana* (Binney) de New Braunfels en Texas central es el 47.6% parecido a especímenes de *Euglandina texasiana*.

Two naturally occurring widespread species of the predaceous land snail *Euglandina* are currently recognized from Texas. *Euglandina singleyana* is reported from a large area of central Texas. It ranges from Terrell County in the west to Fayette County in the east, and south to Refugio County (Strecker 1935; Pilsbry & Ferriss 1906; Pilsbry 1946; Fullington & Pratt 1974; Neck 1980; Hubricht 1985: Map 342). *Euglandina texasiana* inhabits areas of Hidalgo, Cameron and Willacy counties in the Rio Grande Valley of south Texas (Pilsbry 1946; Fullington & Pratt 1974; Harry 1983; Neck 1984; Hubricht 1985: Map 341). These two species of *Euglandina* are allopatric and separated by a zone of over 200 km.

Euglandina texasiana also inhabits a region of coastal lowlands in México which extends from the Rio Grande Valley south through Tamaulipas to eastern San Luis Potosí and northern Veracruz (Pilsbry 1907-08; 1946; Pilsbry & Vanatta 1936; Correa 1999; 2000; Correa et al. 1998). It also ranges westward to Nuevo León (Correa 1999). While northeastern México in general is characterized by the presence of numerous conspecifics (Pilsbry 1907-08; Pilsbry & Vanatta 1936; Correa 1993; 1996-97; 1999; 2000; Correa et al. 1998), these coastal lowlands of northern Tamaulipas appear to lack additional species and subspecies of *Euglandina*. Collections made during this study at both San Fernando and Soto la Marina yielded only specimens of *E. texasiana*.

This study was undertaken to examine and determine the level of enzymatic variation among specimens of *Euglandina texasiana* from two distant collection localities in south Texas and northern Tamaulipas. The collection site of San Fernando represents a location near the center of the distributional range of *Euglandina texasiana* in México. The selection of this collection location also appears to minimize any possible influence of the numerous additional species and subspecies which are present in areas to both the south and west of this region of northeastern México. In addition, these electrophoretic results are compared with those from a single specimen of *E. singleyana* from near its type-locality in central Texas.

It should be noted that the habitat of *E. texasiana* in south Texas is rapidly being eliminated due to agricultural clearing (Fullington & Pratt 1974; Neck 1984; 1988) and that *Euglandina* specimens in Texas are generally considered to be uncommon (Singley 1893; Neck 1984; 1988). As a result of their very specialized feeding habits, rarity, habitat preferences, as well as the results of human activities, the five specimens examined during this study represent a significant collection of living specimens of *Euglandina*. Additionally, Gorman & Renzi (1979) support the validity of the use of small sample sizes in electrophoretic studies such as this one.

Table 1. Enzymes with buffer system used. The buffer system used for all enzyme systems was Tris-Glycine pH 8.5. Recipe from Hebert & Beaton (1993).

ENZYME (E.C. NO.)	ABBREVIATION
Adenylate kinase (2.7.4.3)	ADK
Aspartate aminotransferase (2.6.1.1)	AAT-1 AAT-2
Catalase (1.11.1.6)	CAT
Glucose-6-phosphate Dehydrogenase (1.1.1.49)	G6PDH
Glucose-6-phosphate Isomerase (5.3.1.9)	GPI
Glutamate Dehydrogenase (1.4.1.2)	GTDH
Hexokinase (2.7.1.1)	HK
Isocitrate Dehydrogenase (1.1.1.42)	IDH
Malate Dehydrogenase (1.1.1.37)	MDH-1 MDH-2
Malate Dehydrogenase (NADP+) (1.1.1.40)	MDHP-1 MDHP-2
Mannose Phosphate Isomerase (5.3.1.8)	MPI
Phosphoglucomutase (5.4.2.2)	PGM

MATERIALS AND METHODS

Two specimens each of *Euglandina texasiana* were collected from Mission in Hidalgo County of south Texas and San Fernando (150 km distant to the south) in Tamaulipas, México. One specimen of *Euglandina singleyana* was collected from New Braunfels in Comal County of central Texas. A single specimen of *Rabdotus alternatus* from Nacimiento de Río Frío in Tamaulipas was selected as an outgroup.

Following collection, individual specimens were held without feeding for 7-10 days. They were then removed from their shells and the tissue was frozen in cryotubes in liquid nitrogen and stored at -80°C in an ultracold freezer until analysis. Samples of muscular foot tissue were homogenized in two volumes of distilled water using a glass rod and centrifuged to obtain an aqueous extract. Procedures for cellulose acetate electrophoresis followed those of Hebert & Beaton (1993). Gels were purchased from Helena Laboratories Inc. (Beaumont, Texas). The stain and buffer recipes used follow those of Shaw & Prasad (1970) and Hebert & Beaton (1993). The buffer used was Tris-Glycine pH 8.5. Scorable data for fifteen loci (Table 1) were obtained and analyzed using

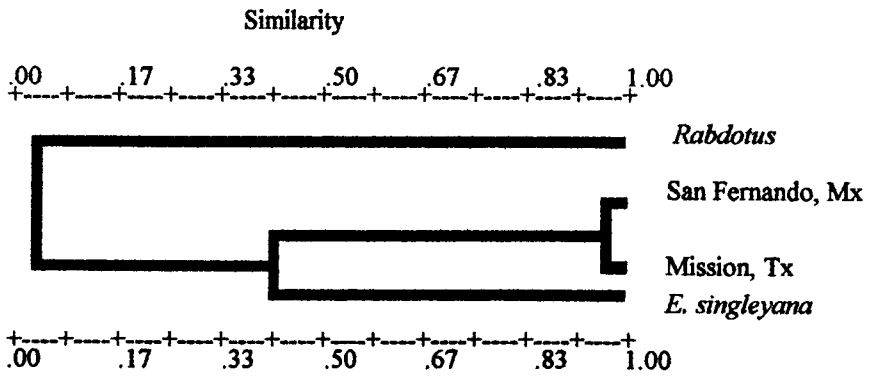

Figure 1. Phenogram of genetic similarity based upon cellulose acetate gel electrophoresis of specimens of *Euglandina texasiana* from Texas (Mission) and Tamaulipas (San Fernando), *Euglandina singleyana* from central Texas (New Braunfels) and *Rabdotus alternatus* (outgroup) from Tamaulipas, México.

the BIOSYS-1 computer program (Swofford & Selander 1981). To determine genetic similarity, Rogers' genetic similarity (1972) was calculated. An unweighted pair group method using arithmetic averages (UPGMA) cluster analysis was then performed using Rogers' genetic similarity matrix. The shells of all specimens examined during this study are deposited with the holdings of the Strecker Museum (SM) of the Mayborn Museum Complex of Baylor University.

Material Examined

Euglandina texasiana.—Two specimens (SM 32449, 32450), Mission, Hidalgo County, Texas, 1 July 1992; two specimens (SM 32447, 32448), 5 km S of San Fernando, Tamaulipas, México, 20 May 1992.

Euglandina singleyana.—One specimen (SM 32451), New Braunfels, Comal County, Texas, 12 March 1991.

Rabdotus alternatus.—One specimen, Nacimiento de Río Frío (22 km NNW of Ciudad Mante), Tamaulipas, México, 24 May 1991.

Results and Conclusions

The results of this study (Figure 1, Table 2) reveal the presence of a moderately high degree of genetic similarity in all four specimens of *Euglandina texasiana* examined from both Texas and Tamaulipas. Ten

Table 2. Allele frequencies in specimens of *Euglandina texasiana* (San Fernando and Mission), *E. singleyana* and *Rabdotus alternatus* from Texas and México.

	R. alter. (outgrp)	S. Fern., Tamp.	Mission, Texas	*E. sing.*
n	1	2	2	1
Locus				
PGM				
A	.000	.000	.000	.000
B	.000	.000	.000	.000
C	.500	1.000	.500	.000
D	.500	.000	.000	.000
E	.000	.000	.000	.500
F	.000	.000	.500	.500
GTDH				
A	1.000	.000	.000	.000
B	.000	1.000	.750	1.000
C	.000	.000	.250	.000
MDHP-1				
A	.000	1.000	1.000	1.000
B	1.000	.000	.000	.000
MDHP-2				
A	1.000	.000	.000	.000
B	.000	1.000	1.000	1.000
HK				
A	.000	.750	.000	.000
B	1.000	.000	.500	.000
C	.000	.250	.000	1.000
D	.000	.000	.500	.000
IDH				
A	.000	.000	.000	.000
B	.000	.000	.000	1.000
C	.000	1.000	1.000	.000
D	1.000	.000	.000	.000
ADK				
A	.000	.500	.750	.000
B	.000	.500	.250	1.000
C	1.000	.000	.000	.000
AAT-1				
A	.000	1.000	.500	.000
B	1.000	.000	.000	.000
C	.000	.000	.500	1.000

	R. alter. (outgrp)	S. Fern., Tamp.	Mission, Texas	*E. sing.*
n	1	2	2	1
Locus				
AAT-2				
A	.000	1.000	1.000	.000
B	.000	.000	.000	1.000
C	1.000	.000	.000	.000
CAT				
A	1.000	.000	.000	.000
B	.000	1.000	1.000	1.000
MDH-1				
A	.000	1.000	1.000	1.000
B	1.000	.000	.000	.000
MDH-2				
A	.000	.000	.000	.000
B	.000	.000	.000	.500
C	.000	1.000	1.000	.000
D	1.000	.000	.000	.500
GPI				
A	.000	1.000	1.000	.000
B	.000	.000	.000	1.000
C	1.000	.000	.000	.000
G6PD				
A	.000	1.000	1.000	.000
B	.000	.000	.000	.000
C	.000	.000	.000	1.000
D	.000	.000	.000	.000
E	.500	.000	.000	.000
F	.500	.000	.000	.000
MPI				
A	1.000	.000	.000	.000
B	.000	1.000	1.000	.000
C	.000	.000	.000	1.000

of the 15 loci examined were monomorphic for all specimens; variation was observed in five loci (PGM, GTDH, HK, ADK, AAT-1). The two specimens from San Fernando were identical at 14 of the 15 loci and differed only when stained for hexokinase (Table 2). The two specimens from Mission exhibited a greater degree of variation than the San Fernando specimens with variation observed at four loci (GTDH, HK, ADK, AAT-1). An analysis of the resulting enzymatic variation (Figure 1) revealed that specimens from the two collection localities were 94.5% genetically similar. This overall genetic similarity of 94.5% for all specimens from both locations is well within the range expected for genetic variation within a single species (Quicke 1993) and compares with similar results in populations of *Helix aspersa* by Selander & Kaufman (1975) and *Helicina orbiculata* by Strenth & Littleton (2000).

The single specimen of *Euglandina singleyana* from central Texas was found to be only 47.6% genetically similar (Figure 1) to specimens of *E. texasiana* from south Texas and northern Tamaulipas. This low level of genetic similarity supports the validity of the results of earlier workers in maintaining the distinction and separation of these two species of land snails based upon differences in shell morphology and geographical distribution.

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LITERATURE CITED

Correa-Sandoval, A. 1993. Caracoles terrestres (Mollusca: Gastropoda) de Santiago, Nuevo León, México. Revista de Biología Tropical, 41(3):683-687.

Correa-Sandoval, A. 1996-97. Caracoles terrestres (Mollusca: Gastropoda) de Iturbide, Nuevo León, México. Revista de Biología Tropical, 45(1):137-142.

Correa-Sandoval, A. 1999. Zoogeografía de los gastrópodos terrestres de la región oriental de San Luis Potosí, México. Revista de Biología Tropical, 47(3):493-502.

Correa-Sandoval, A. 2000. Gastrópodos terrestres del norte de Veracruz, México. Acta Zoologica Mexicana (nueva serie), 79:1-9.

Correa-Sandoval, A., A. García-Cubas & M. Reguero-Reza. 1998. Gastrópodos terrestres de la región oriental de San Luis Potosí, México. Acta Zoologica Mexicana (nueva serie), 73:1-17.

Fullington, R. W. & W. L. Pratt. 1974. The aquatic and land mollusca of Texas. Dallas Mus. Nat. Hist., Bull. 1 (part 3):1-48.

Gorman, G. C. & J. R. Renzi, Jr. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia, 2:242-249.

Harry, H. W. 1983. Notes on the flesh-eating land snail, *Euglandina rosea* in Texas, and its feeding habits. Texas Conchologist, 20(1):23-27.

Hebert, P. & M. J. Beaton. 1993. Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis. Helena Laboratories. Beaumont, Texas, pp. 1-32.

Hubricht, L. 1985. The distribution of the native land molluscs of the eastern United States. Fieldiana Zool., No. 24:1-191.

Neck, R. W. 1980. Two disjunct populations of *Euglandina singleyana* (W. G. Binney). The Veliger, 23:112.

Neck, R. W. 1984. Restricted and declining nonmarine molluscs of Texas. Texas Parks and Wildlife Department Technical Series No. 34: 17 pp.

Neck, R. W. 1988. Urban Refugia which support dense concentrations of *Euglandina singleyana*. Texas Conchologist, 24(3):78-82.

Nei, M. 1972. Genetic distance between populations. Amer. Nat., 106:283-292.

Pilsbry, H. A. 1907-08. Oleacinidae, Ferussacidae. Vol. XIX in G. W. Tryon's Manual of Conchology, Academy of Natural Sciences of Philadelphia, 366 pp. + 52 plates.

Pilsbry, H. A. 1946. Land mollusca of North America (north of México). Acad. Nat. Sci. Philadelphia. Monograph 3 (Vol. 2-part 1):188-199.

Pilsbry, H. A. & J. H. Ferriss. 1906. Mollusca of the southwestern states, II. Proc. Acad. Nat. Sci. Phila., 58:123-175.

Pilsbry, H. A. & E. G. Vanatta. 1936. Three Mexican Euglandinas. The Nautilus, 49(3):97-98 + plate 7.

Quicke, D. L. J. 1993. Principles and techniques of contemporary taxonomy. Blackie Academic & Professional, Glasgow, pp. 167-189.

Rogers, J. S. 1972. Measures of genetic similarity and genetic distance. Stud. Genet. VII, Univ. Texas, Pub., 7213:145-153.

Selander, R. K. & D. W. Kaufman. 1975. Genetic structure of populations of the brown snail (*Helix aspersa*). I. Microgeographic variation. Evolution, 29(3):385-401.

Shaw, C. R. & R. Prasad. 1970. Starch gel electrophoresis of enzymes - A compilation of recipes. Biochemical Genetics, 4:297-320.

Singley, J. A. 1893. Texas Molluscs. Geological Survey of Texas. Fourth Annual Report., Pt 2, 1892:301-302.

Strecker, J. K. 1935. Land and fresh-water snails of Texas. Trans. Texas Acad. Sci., 17:4-50.

Strenth, N. E. & T. G. Littleton. 2000. A revision of the land snail *Helicina orbiculata* (Gastropoda: Prosobranchia) from the southern United States. Texas Jour. Sci., 52(1):25-32.

Swofford, D. L. & R. B. Selander. 1981. BIOSYS-1: A Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered., 72:281-283.

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