# GENETIC CONSEQUENCES OF PARTIAL SELF-FERTILIZATION ON POPULATIONS OF *LIGUUS FASCIATUS* (MOLLUSCA: PULMONATA: BULIMULIDAE)

**DAVID M. HILLIS**
DEPARTMENT OF ZOOLOGY
THE UNIVERSITY OF TEXAS
AUSTIN, TEXAS 78712, U.S.A.

## ABSTRACT

Reproductive modes of the highly polymorphic Florida tree snail, *Liguus fasciatus* (Müller), were investigated by laboratory breeding experiments and field study. Variation of glucose-phosphate isomerase and shell phenotypes was assessed. The laboratory crossings demonstrated that partial self-fertilization does occur in this species, but too few informative crosses were performed to estimate the frequency of self-fertilization. A transect study through two populations that have recently come into contact demonstrated high population substructure ($F_{ST}$ = 0.437) across short distances. Levels of heterozygosity in subpopulations along 20 m sections of the transect were used to estimate levels of self-fertilization. Estimates ranged from 46% to 94% self-fertilization, with a mean of 69%. The genotypic frequencies of subpopulations did not differ significantly from expected frequencies assuming the mean estimate of 69% self-fertilization, but did differ significantly from expected frequencies assuming Hardy-Weinberg equilibrium with no self-fertilization. Partial self-fertilization appears to be largely responsible for the low within-population variation compared to the high among-population variation of this species.

Tree snails of the genus *Liguus* are noted for their morphological diversity among populations (Clench, 1946, 1954, 1965; Pilsbry, 1946). In Florida, approximately 58 named varieties of *Liguus fasciatus* (Müller) occur (Roth and Bogan, 1984), many of which are restricted to single tropical hardwood hammocks in the Everglades and Florida Keys (Deisler, 1982). In spite of this high morphological diversity in *L. fasciatus*, allozymic variation is very low among and within populations of this species (Hillis *et al.*, 1987). Furthermore, populations of *L. fasciatus* deviate significantly from Hardy-Weinberg expectations at variable loci because of marked heterozygote deficiencies (Hillis *et al.*, 1987).

Although geographic patterns of phenotypic shell variation have been studied extensively in *Liguus fasciatus* (Pilsbry, 1899, 1912, 1946; Deisler, 1982; Roth and Bogan, 1984), very little is known about the inheritance of these traits or reproduction in this species. Roth and Bogan (1984) proposed a system for describing morphological variation in *L. fasciatus* that consisted of twelve characters, each with two to four states. They stated that they chose characters "...in which the alternate states can be seen to segregate in randomly selected material." However, Hillis *et al.* (1987) suggested that these characters are not independent, and that many fewer than twelve loci are probably responsible for the observed phenotypic variation of shells. Furthermore, although most past authors (e.g. Brown, 1978; Young, 1960) have assumed that *L. fasciatus* is an obligate outcrosser, Hillis *et al.* (1987) suggested that partial self-fertilization might account for some of the patterns of genetic variation seen among populations of this hermaphroditic species. Self-fertilization and outcrossing are both common modes of reproduction in gastropods, and a few species contain some populations that are self-fertilizing and others that are outcrossing (McKracken and Selander, 1980). Other species are facultatively self-fertilizing and self-fertilize when mates are unavailable, and in at least one species reproduction following copulation is either by self-fertilization or outcrossing (McKracken and Selander, 1980). However, partial self-fertilization (a single clutch containing both self-fertilized and outcrossed eggs), as suggested for *L. fasciatus* (Hillis *et al.*, 1987), has not been demonstrated among gastropods.

This study was undertaken to determine the mode of reproduction and its consequences on genetic variation in *Liguus fasciatus*. Laboratory and field studies were designed to determine if partial self-fertilization occurs, and if so, at what frequency. In addition, a population was examined to

American Malacological Bulletin, No. 7(1) (1989):7-12

determine the extent of genetic substructure as well as the effects of possible self-fertilization on heterozygosity, allozymic variation, and phenotypic variation of shells.

## MATERIALS AND METHODS

### ELECTROPHORETIC METHODS

Standard procedures of horizontal starch gel electrophoresis were followed (Selander *et al.*, 1971; Hillis, 1985). Digestive glands of *Liguus fasciatus* were ground and diluted 1:1 in 0.01 M tris-0.001 M EDTA-.001 M 2-mercaptoethanol, pH 7.5. Homogenates were centrifuged at 7,000 g for 5 min, after which the supernatants were refrozen at -85°C. A buffer system of 175 mM tris-17.5 mM boric acid-2.75 mM EDTA, pH 9.1 was used. Gels were prepared from 50% Sigma starch (lot 85F-0010) and 50% Otto Hiller electrostarch (lot 392). Gels were electrophoresed for 12 hr at 12.5 V/cm. Histochemical staining for glucose-phosphate isomerase (E.C. 5.3.1.9; GPI) followed Harris and Hopkinson (1976). This enzyme was the only variable locus of the 24 allozyme loci surveyed in *L. fasciatus* by Hillis *et al.* (1987).

### BREEDING STUDY

Between 18 January and 5 July 1986, 60 specimens of *Liguus fasciatus* were collected from hammocks in the Pinecrest region, Big Cypress National Preserve, and near Long Pine Key, Everglades National Park, Florida, for captive breeding experiments. Mating in this species begins in late July or early August in these regions (Jones, 1954). Pairs of *L. fasciatus* remain together for several days after mating, so the beginning of the breeding season can be easily ascertained. In summer 1986, the study populations were observed at least twice weekly, and the first mated pairs were found during the first week of August. Therefore, all of the specimens used in the captive breeding study were collected at least one month prior to the breeding season. Specimens from single populations were paired at random and kept in isolation in plastic boxes (10 cm x 20 cm x 30 cm) with 3-4 cm of decayed leaves and hammock soil. Snails were fed with lichen-covered branches supplemented with a mixture of cornstarch, oatmeal, spinach, vitamins, and calcium carbonate. Snails were maintained at approximately 25°C, and were sprayed with water 5 times per week until eggs were deposited (24 Sept - 5 Oct). During egg deposition, the egg-producing individuals were marked. After eggs had been deposited, cages were sprayed with water at approximately two week intervals until hatching occurred (Jan - Feb 1987). Parental snails and offspring were then examined for variation at the glucose-phosphate isomerase locus as described above.

### FIELD STUDY

The study site was located near Pinecrest, Big Cypress National Preserve, Monroe County, Florida. Pinecrest hammocks (PC) 16 and 16a (numbering system follows Pilsbry, 1946) were separated by a narrow channel of water until the 1960's or 1970's (Hillis *et al.*, 1987; Fig. 1). Prior to connection of these hammocks, *Liguus fasciatus* in PC 16 were of

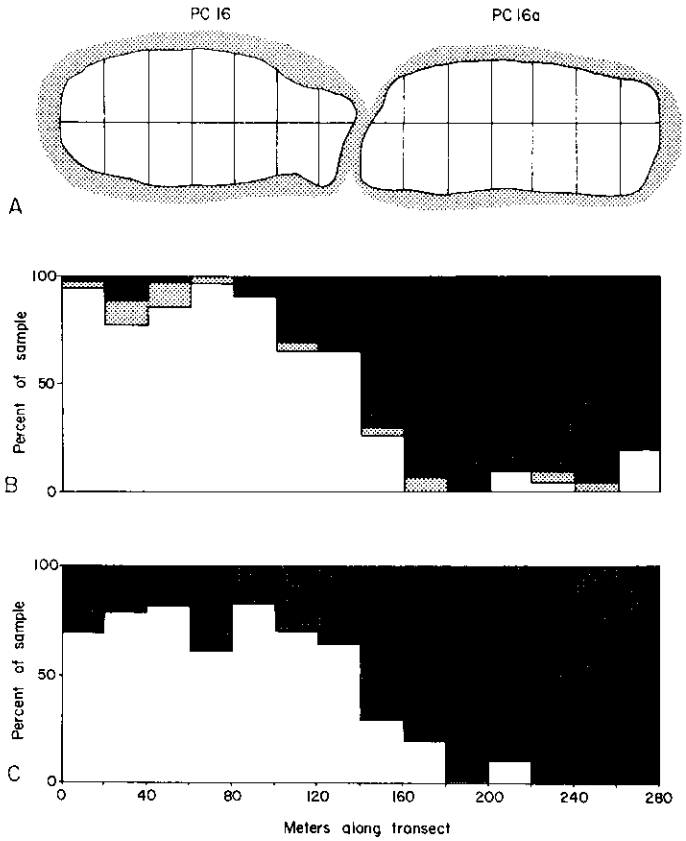


**Fig. 1. A.** Map of Pinecrest hammocks 16 and 16a, showing location of transect. The shading around the hammocks represents the approximate extent of recent woody growth that is seasonally flooded. This growth provides a connection between the hammocks for movement of *Liguus fasciatus*. **B.** Shell phenotypes of *L. fasciatus* collected in corresponding sections of the transect shown in A. The darkly shaded portion of the histogram represents the percentage of the *barbouri* phenotype, the lightly shaded portion the *aurantius* phenotype, and the white portion the *walkeri* phenotype. **C.** GPI allelic frequencies of *L. fasciatus* collected in corresponding sections of the transect shown in A. The darkly shaded portion of the histogram represents the percentage of the F allele in the sample, and the white portion the percentage of the S allele.

the *walkeri* phenotype (banded shells with pink tips), whereas *L. fasciatus* in PC 16a were of the *barbouri* phenotype (dark snails with white tips); a third phenotype, *aurantius* (orange snails), was uncommon in both hammocks (Hillis *et al.*, 1987). Fire prevention in the Pinecrest area over the past several decades has resulted in increased woody growth around many hammocks, and by the 1960's or early 1970's tree growth (primary willows) had joined the two hammocks sufficiently for movement of *Liguus* between PC 16 and PC 16a (Fig. 1A). Because the two populations are also strongly differentiated at the glucose-phosphate isomerase locus, this site provided an opportunity to study the effects of self-fertilization on the interaction of differentiated populations of *L. fasciastus*.

A transect was constructed perpendicular to the axis of the contact through the two hammocks (Fig. 1). Fourteen

20 m intervals were marked along the transect, and snails were collected from sections perpendicular to these 20 m intervals. Between 20 and 44 snails were collected from each section. For each snail, section number and morphological phenotype were recorded; snails were then transferred to the laboratory where each was assessed for genotype at the GPI locus.

### ANALYSIS

F-statistics were calculated using the formulae of Weir and Cockerham (1984), which do not make assumptions concerning numbers of populations, sample sizes, or heterozygote frequencies. Indirect estimates of self-fertilization were calculated using the method described by Hedrick (1983). Statistical tests for goodness-of-fit and correlation were conducted as described by Sokal and Rohlf (1981).

## RESULTS

Of the 30 pairs of *Liguus* used in the captive breeding experiments, 11 pairs produced clutches of eggs. In one of these pairs, both individuals produced clutches. However, it was determined after the pairings had been made that many pairs came from populations that were fixed for one or the other of the GPI alleles, so only one of the crosses was informative about self-fertilization (Table 1). In this cross, a snail heterozygous for the two GPI alleles (FS) was mated with a snail homozygous for the fast GPI allele (FF). The FS individual produced eggs, and the offspring expressed FF, FS, and SS genotypes (Table 1).

In the field study, both GPI allelic frequencies and shell phenotypic frequencies changed markedly along the transect between PC 16 and PC 16a. The F allele of GPI increased and the S allele decreased along this transect (from PC 16 to PC 16a), and there was a corresponding shift in frequencies from mostly *walkeri* phenotype to mostly *barbouri* phenotype (Fig. 1 and Table 2). Frequencies of heterozygotes at GPI were considerably below Hardy-Weinberg expectations (Table 3).

## DISCUSSION

Both population substructuring and self-fertilization appear to have major effects on reduction of heterozygosity in populations of *Liguus fasciatus*. Even though the transect was divided into subpopulations just 20 m wide, variation among subpopulations is very high ($F_{ST}$ = 0.479). This value is even higher than the average fixation index for self-fertilizing plants ($F_{ST}$ = 0.437; Hamrick, 1983). In addition, the inbreeding coefficient is also very high ($F_{IS}$ = 0.478), indicating substantial self-fertilization. The reduction in individual heterozygosity in the study population due to both of these factors (substructuring and inbreeding) is quite substantial ($F_{IT}$ = 0.728).

Except for potential sperm-storage from the previous breeding season, a possibility unsupported by data, the captive breeding data demonstrate that self-fertilization does occur in *Liguus fasciatus*, because only through self-fertilization could the SS offspring result from a mating of FS x FF individuals (Table 1). However, a direct estimation of self-fertilization frequency is not possible from the captive breeding data because of the paucity of appropriate crosses. On the other hand, it is possible to estimate self-fertilization frequency from the transect study.

The proportion of progeny produced by self-fertilization ($S$) can be estimated from the proportion of heterozygous individuals ($H$) in each of the suppopulations in the transect by solving the equation

$$H = \frac{4pq\,(1\text{-}S)}{2\text{-}S}$$

where $p$ and $q$ are the allelic frequencies (Hedrick, 1983). This requires the assumption that the subpopulation divisions are small enough to account for population substructuring. Given that individual seasonal movements of *Liguus fasciatus* are typically greater than the 20 m widths of the transect sections (Brown, 1978), this assumption is probably valid. The above calculations were made for each of the subpopulations in which allozymic variation was observed (Table 2). These estimates range from 46% to 94% self-fertilization ($\bar{S}$ = 0.69, SD = 0.154). If population substructuring is not fully accounted for by the 20 m transect divisions, then these estimates of self-fertilization are somewhat inflated. An alternative to self-fertilization that could explain the deficiency of heterozygotes is assortative mating. However, in polymorphic populations of *L. fasciatus* mating appears to be random with

**Table 1.** GPI genotypes of offspring resulting from 12 crosses of *Liguus fasciatus*.

| Number clutches | Maternal genotype | Paternal genotype | Offspring FF | FS | SS |
|---|---|---|---|---|---|
| 5 | FF | FF | 76 | 0 | 0 |
| 6 | SS | SS | 0 | 0 | 106 |
| 1 | FS | FF | 7 | 6 | 1 |

**Table 2.** Observed GPI genotypes of *Liguus fasciatus* from sections along a transect through Pinecrest hammocks 16 and 16a, and estimates of frequency of self-fertilization (S) in each section.

| Section | GPI genotype SS | SF | FF | $S$ |
|---|---|---|---|---|
| 1-20 m | 24 | 9 | 11 | .71 |
| 21-40 m | 29 | 8 | 5 | .58 |
| 41-60 m | 26 | 8 | 3 | .46 |
| 61-80 m | 17 | 4 | 10 | .84 |
| 81-100 m | 25 | 4 | 4 | .75 |
| 101-120 m | 20 | 7 | 6 | .65 |
| 121-140 m | 10 | 11 | 12 | .50 |
| 141-160 m | 5 | 3 | 18 | .82 |
| 161-180 m | 5 | 1 | 20 | .94 |
| 181-200 m | 0 | 0 | 20 | — |
| 201-220 m | 1 | 2 | 17 | .62 |
| 221-240 m | 0 | 0 | 20 | — |
| 241-260 m | 0 | 0 | 20 | — |
| 261-280 m | 0 | 0 | 20 | — |

**Table 3.** Expected genotypic frequencies of no self-fertilizing and 69% self-fertilizing models for sections of the PC 16-16a transect, and probabilities of the observed data fitting the expected frequencies. "n.s." designates expected frequencies that do not differ significantly ($p > 0.05$) from the observed values.

| | No self-fertilization | | | | 69% self-fertilization | | | |
|---|---|---|---|---|---|---|---|---|
| Section | SS | SF | FF | p | SS | SF | FF | p |
| 1 | 18.5 | 20.1 | 5.5 | <.001 | 23.9 | 9.5 | 10.7 | n.s. |
| 2 | 26.0 | 14.1 | 1.9 | <.01 | 29.6 | 6.8 | 5.6 | n.s. |
| 3 | 24.3 | 11.3 | 1.3 | n.s. | 27.3 | 5.3 | 4.3 | n.s. |
| 4 | 11.7 | 14.7 | 4.6 | <.001 | 15.5 | 6.9 | 8.6 | n.s. |
| 5 | 22.1 | 9.8 | 1.1 | <.005 | 24.6 | 4.7 | 3.7 | n.s. |
| 6 | 16.7 | 13.5 | 2.7 | <.01 | 20.3 | 6.4 | 6.3 | n.s. |
| 7 | 7.2 | 16.4 | 9.3 | <.05 | 11.6 | 7.8 | 13.6 | n.s. |
| 8 | 1.6 | 9.8 | 14.6 | <.01 | 4.2 | 4.6 | 17.2 | n.s. |
| 9 | 1.2 | 8.7 | 16.1 | <.001 | 3.4 | 4.1 | 18.4 | <.05 |
| 10 | 0 | 0 | 20.0 | n.s. | 0 | 0 | 20.0 | n.s. |
| 11 | 0.2 | 3.7 | 16.1 | <.01 | 1.2 | 1.8 | 17.0 | n.s. |
| 12 | 0 | 0 | 20.0 | n.s. | 0 | 0 | 20.0 | n.s. |
| 13 | 0 | 0 | 20.0 | n.s. | 0 | 0 | 20.0 | n.s. |
| 14 | 0 | 0 | 20.0 | n.s. | 0 | 0 | 20.0 | n.s. |

respect to shell phenotype (Brown, 1978).

The expected genotypic frequencies under assumptions of no self-fertilization and 69% self-fertilization (the mean of estimates from all subpopulations) are shown in Table 3 and graphically in figure 2. The observed frequencies were tested against the expected frequencies under these two models using a *G*-test (Sokal and Rohlf, 1981). All but one of the genetically variable subpopulations differ significantly from the expected genotypic frequencies under the assumption of no self-fertilization, whereas only one of the subpopulations differ significantly from the expected genotypic frequencies under the assumption of 69% self-fertilization (Table 3). The single subpopulation that differed from 69% self-fertilization expectations differed in having even fewer heterozygous individuals than expected. Given the number of comparisons (10 variable subpopulations), a single departure from expectations at $p = 0.05$ would be expected by chance 50% of the time, even if the model is correct. Therefore, the transect data are in close agreement with a self-fertilization frequency of approximately 69%.

The frequencies of GPI alleles are strongly correlated with shell phenotypes (Fig. 3), an observation that is probably a result of historical restriction of the *barbouri* phenotype and the F allele to PC 16a, and the *walkeri* phenotype and S allele to PC 16. These two correlations are nearly equally strong and significant: *barbouri*-F allele, $r = 0.95$, $p < .001$; *walkeri*-S allele, $r = 0.94$, $p < .001$. The third (uncommon) phenotype, *aurantius*, is not significantly correlated with either GPI allele, which is consistent with the distribution of this phenotype in both PC 16 and PC 16a before the contact of the two hammocks. However, the distribution of the two primary phenotypes is asymmetric with respect to the GPI allelic frequencies: the frequencies of *walkeri* are mostly higher than the corresponding S frequencies, whereas the frequencies of *barbouri* are generally lower than the corresponding F frequencies (Fig. 3). This discrepancy may indicate genetic dominance of the *walkeri* genotype over the *barbouri* genotype.

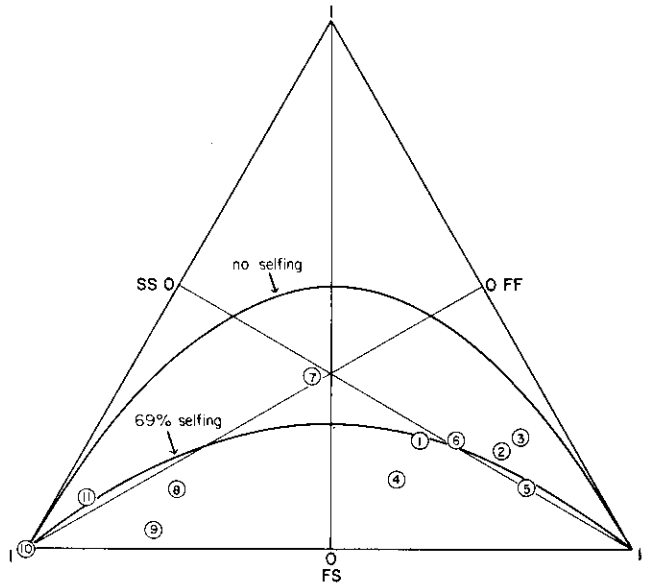


**Fig. 2.** Trivariate plot of the three GPI genotypes of *Liguus fasciatus* in sections along a transect through Pinecrest hammocks 16 and 16a. The upper curve represents the expected values of Hardy-Weinberg equilibrium without self-fertilization, and the lower curve represents the expected values with 69% self-fertilization. The numbered circles indicate the genotypic combinations of the sections of the transect. The location of section 10 (fixation of the FF genotype) is also the location of sections 12-14.

Roth and Bogan (1984) devised a system for describing phenotypic variation in *Liguus fasciatus* that incorporated twelve distinct characters, each with two to four states. They stated that they chose characters "...in which the alternate states can be seen to segregate in randomly selected material" (Roth and Bogan, 1984). Under the Roth and Bogan system, the three phenotypes present in PC 16 and PC 16a

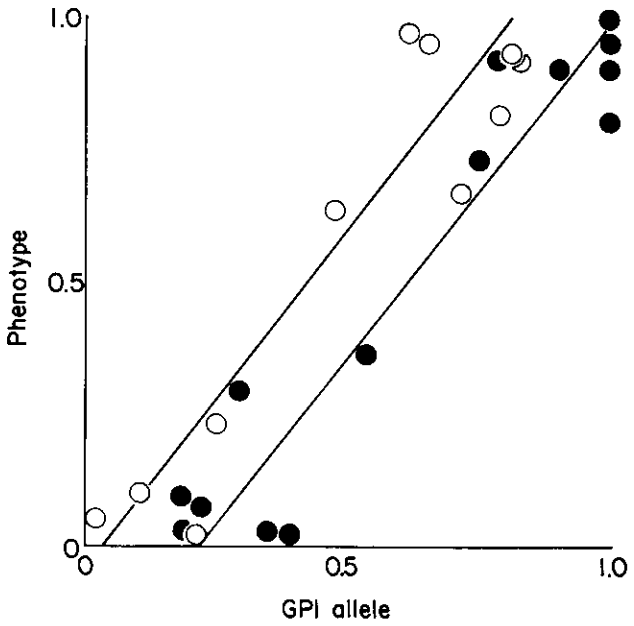


**Fig. 3.** Correlation of shell phenotypes and GPI alleles through Pinecrest hammocks 16 and 16a. The open circles represent frequencies of the *walkeri* phenotype and the S allele, and the closed circles represent frequencies of the *barbouri* phenotype and the F allele.

are designated as follows: *aurantius:* $C^{Y}B^{Y}S+E^{Y}U^{-}M^{-}L^{O}P^{O}A^{-}O^{-}W^{-}G^{+}$; *barbouri:* $C^{Y}B^{BY}S+E^{B}U^{-}M+L^{B}P^{B}A^{-}O^{-}W+G^{+}$; and *walkeri:* $C^{W}B^{BY}S^{-}E^{B}U^{-}M+L^{B}P^{B}A+O+W+G^{+}$. As only these three phenotypic combinations were observed among over 1,000 examined shells, the independence of the 12 characters seems highly doubtful. If the 12 characters were independent, one would expect 1024 phenotypic combinations of *L. fasciatus* in PC 16-16a, rather than the observed three combinations. Instead, these phenotypes seem to be inherited as single genes. This does not preclude the possibility of a few tightly linked loci, however. Some of the 1021 unobserved phenotypes do occur in other areas (Roth and Bogan, 1984), but probably represent distinct alleles rather than recombinations of the alleles present in PC 16-16a. Obviously, future attempts at understanding the genetics of *L. fasciatus* shell phenotypes must take into account self-fertilization.

Although partial self-fertilization of *Liguus fasciatus* is sufficient to account for the high among-population variation and low within-population variation observed throughout the range of this species, this phenomenon does not account for the overall low allozymic variability (Hillis *et al.*, 1987) compared to the high morphological variability (Pilsbry, 1912, 1946) found in Floridian *Liguus.* The low allozymic variaton could be a result of a relatively recent invasion of few individuals from Cuba, thus giving rise to fixation at most allozyme loci through the founder effect. Fixation at most allozyme loci has occurred in several introduced mollusks that are capable of self-fertilization (Selander and Kaufmanm, 1973; McKracken and Selander, 1980; Hillis and Patton, 1982). However, this does not account for the high morphological variation seen in Floridian populations of *L. fasciatus.* One possibility is that the shell phenotypes are adapted to different local conditions. However, adaptation is unnecessary to explain the distribution and variation of shell phenotypes. Instead, it is likely that the genes responsible for shell phenotype undergo much higher rates of mutation than do the allozyme loci, in which case the partial self-fertilization of *L. fasciatus* would explain the fixation of many of these phenotypes in the numerous isolated Floridian populations of this species.

## ACKNOWLEDGMENTS

I thank Oren Bass, Michael Dixon, Karen Ercolino, Archie Jones, Lourdes Rodriguez, Modesto Sanchez, and Eric Zimmerman for advise and assistance with this study, and Everglades National Park and Big Cypress National Preserve for collecting permits. This study was supported in part by a Nongame Wildlife Program Grant from the Florida Game and Freshwater Fish Commission and by National Science Foundation Grant BSR 8657640.

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Date of manuscript acceptance: 16 September 1988