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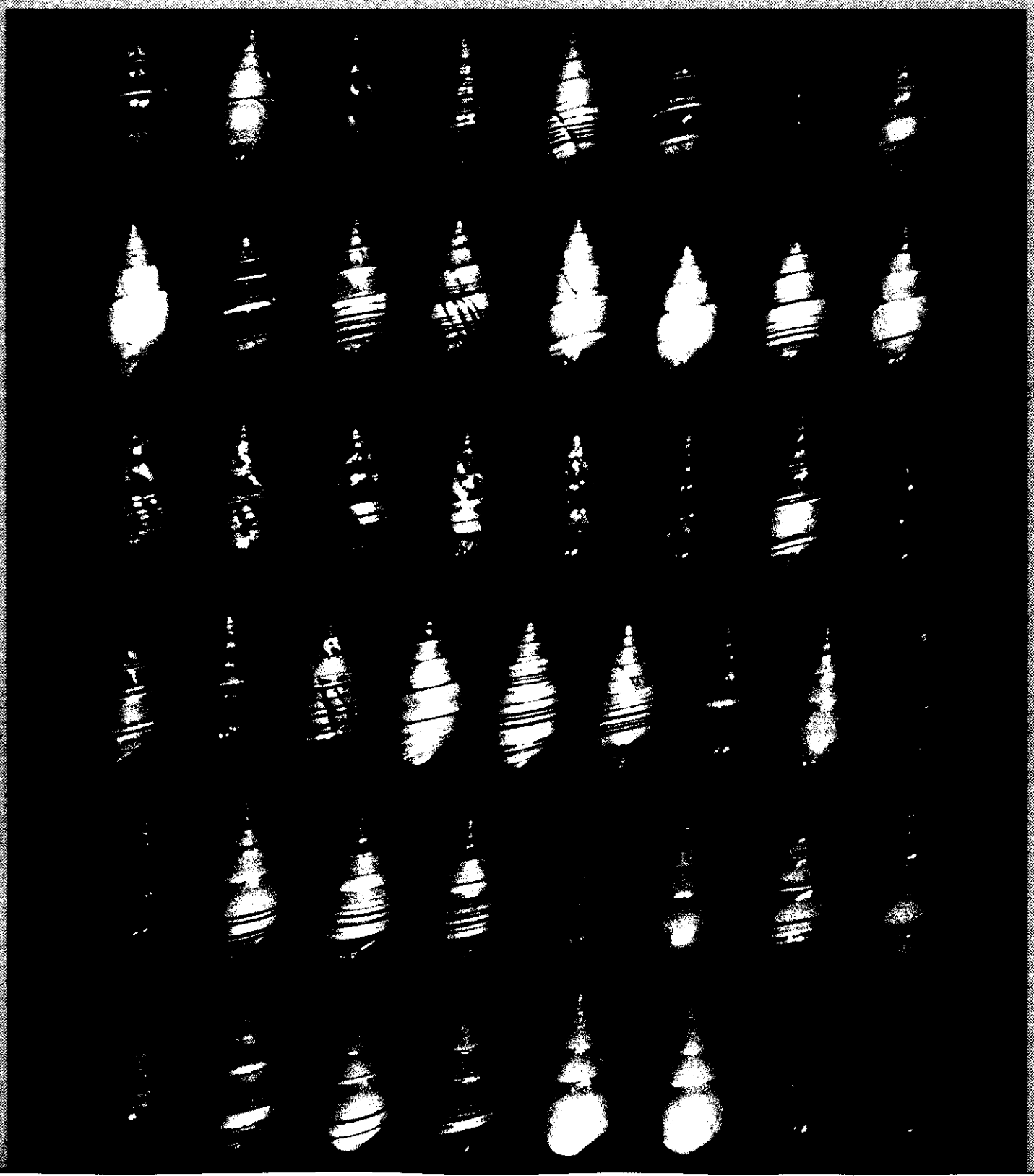

Minimal Genetic Variation in a Morphologically Diverse Species (Florida Tree Snail, *Liguus fasciatus*)

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The Florida tree snail, *Liguus fasciatus*, is morphologically extremely variable, and many of the phenotypes occur in geographically isolated populations. Although only a single species of Floridian *Liguus* has been recognized by most recent authors, confirming evidence of the conspecific nature of the morphologically diverse populations from throughout Florida is lacking. This study involved a genetic survey of *Liguus* from throughout Florida, including most of the named varieties, using the technique of allozyme electrophoresis. Genetic divergence of the populations was very low. Of 34 loci examined, only one (glucose-phosphate isomerase, *Gpi*) was variable, and only two alleles were present at this locus. The maximum genetic distance among any two populations was 0.03. Observed individual heterozygosity was minimal (0.0 – 0.029; $\bar{x} = 0.0015$); among mollusks, only some self-fertilizing species have similar levels of heterozygosity. Although most populations are fixed for one or the other *Gpi* allele, both alleles are widely distributed. The distribution of alleles and low genetic diversity support the view of a single, partially self-fertilizing species that diversified within Florida after a single founder event.

Although there is broad correspondence between evolutionary histories that are inferred from morphology with those inferred from molecular data (Hillis 1987), morphological and molecular divergence sometimes occur at very different rates (Wayne and O'Brien 1986; Wilson et al. 1977). In some cases, morphologically similar organisms are genetically diverse (Hillis 1988; Wayne et al. 1989). In other cases, major morphological radiations are not accompanied by much molecular change (e.g., Meyer et al. 1990). Therefore, the potential exists to seriously overestimate or underestimate biological diversity if analyses are limited to a single kind of comparison.

The Florida tree snail, *Liguus fasciatus*, is an extreme example of a polytypic species (see cover photograph). Trivial names are available for at least 58 morphologically distinct forms in Florida, and many more forms are known from Cuba (Clench 1946, 1954, 1965; Jones 1979; Roth and Bogan 1984). The Floridian populations of this species occur in tropical hardwood hammocks throughout the southern Everglades, the Atlantic Coastal Ridge of southern Florida, and the Florida Keys. Most populations are isolated from other populations by intervening barriers of water, sawgrass, buttonwood, cypress, or pine forest. Many of these isolated populations are morphologically distinguishable from all other populations, although some phenotypes are relatively widespread throughout the range of the species in Florida (Deisler 1982; Roth and Bogan 1984). Transplantation and crossing experiments have demonstrated that shell color pattern is genetically determined in *Liguus*, although the number of loci responsible for shell patterns is as yet undetermined (Hillis et al. 1987; Roth and Bogan 1984).

Several taxonomic systems have been used to classify Floridian *Liguus*. For instance, Pilsbry (1912) recognized 15 subspecies of three species: *L. crenatus*, *L. fasciatus*, and *L. solidus*. Simpson (1929) recognized the same three species, but expanded the number of subspecific names to 35. Clench and Fairchild (1939) recognized only a single species, *L. fasciatus*, with three geographically parapatric subspecies (*lignumvitae*, *roseatus*, and *solidus*). Subsequently, Pilsbry (1946) adopted a system he attributed to T. L. and P. L. McGinty, in which varieties were grouped within forms, which were grouped within eight subspecies, which were grouped within three subspecies groups, which were contained within the single species *L. fasciatus*. However, Pilsbry (1946:

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50) noted that his "subspecies" were not geographical races and suggested that races that had been originally allopatric had become sympatric through the expansion of hammocks. If differentiated taxa of *Liguus* now occur sympatrically and are reproductively isolated, then these taxa would be regarded as distinct species under virtually any modern species concept [e.g., the Biological Species Concept (Mayr 1969), the Evolutionary Species Concept (Wiley 1978), or the Phylogenetic Species Concept (Cracraft 1983)].

Hillis et al. (1987) tested for the possibility of reproductive isolation between morphological forms of *Liguus* within some of the most polymorphic populations—those in the Pinecrest region of the Everglades—and concluded that there was no evidence to support this hypothesis. Furthermore, the genetic variation as revealed by allozyme electrophoresis was minimal in these populations: only a single locus was variable among 24 loci screened, and the two alleles at this locus were distributed across forms (Hillis et al. 1987). However, a deficiency of heterozygous individuals (compared to the expectations of Hardy-Weinberg equilibrium) led Hillis et al. (1987) to suggest the possibility of partial self-fertilization in *Liguus*. This possibility was confirmed (Hillis 1989) through laboratory crosses and study of the fusion of two formerly isolated hammocks that contained morphologically distinct populations of *Liguus*. Hillis (1989) concluded that the pattern of many morphologically distinct populations of *Liguus* in Florida could be explained by a high mutation rate at the locus or loci responsible for shell patterns, combined with rapid local fixation of color pattern mutations through self-fertilization.

The populations studied by Hillis et al. (1987) and Hillis (1989) were all in the northwestern portion of the range of *Liguus*—the Pinecrest region (see Figure 1). Although many of the populations in this region are highly polymorphic in color pattern, all the *Liguus* from the Pinecrest area fall into just three of Pilsbry's (1946) eight subspecies (*castaneozonatus, testudineus,* and *lossmanicus*), all three of which fall into just one of his three main groups (the *castaneozonatus–testudineus* group). Pilsbry's other subspecies are found further south in the Everglades, in the Florida Keys, or along the Atlantic Coastal Ridge. The other two major groups recognized by Pilsbry (1946) are limited to the Middle and Lower Florida Keys (the *solidus* group) and the extreme northeastern end of the range of *Liguus*, along the Atlantic Coastal Ridge in the vicinity of Fort Lauderdale (the *septentrionalis* group; Figure 1). The purpose of this study was to assess the genetic diversity and systematic status of distinct populations of *Liguus* from throughout the range of the genus in Florida.

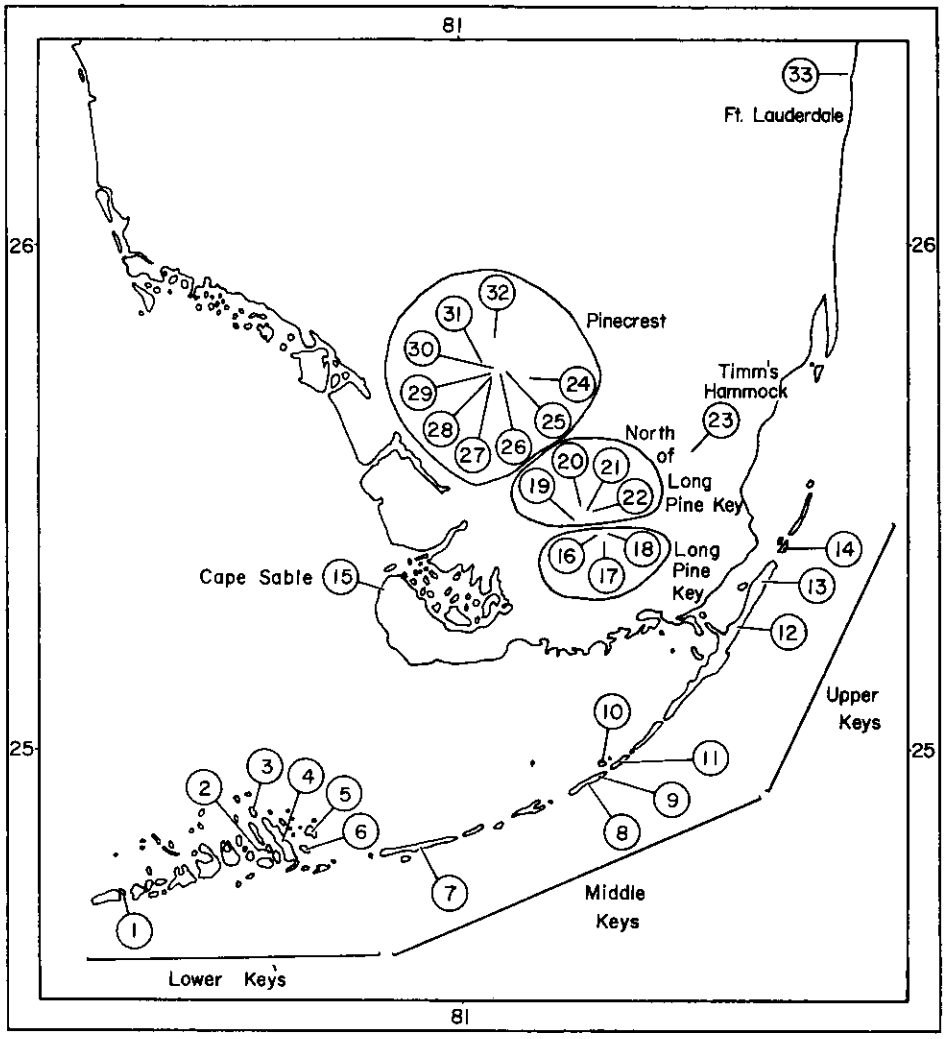

Figure 1. Populations of *Liguus fasciatus* sampled in this study. Numbers correspond to the list of localities in Table 1. Names shown are regions mentioned in the text.

Materials and Methods

We collected samples of *Liguus* that represented most of the described varieties from Florida (Table 1). Many populations of *Liguus* from outside the Everglades region (i.e., those from the Atlantic Coastal Ridge and the Florida Keys) are threatened or extinct in their native habitat. However, in the 1950s and 1960s, A. Jones, R. Humes, C. C. Von Paulsen and E. Winte transplanted individuals from many of these populations to isolated hammocks within Everglades National Park that lacked native populations of *Liguus*. When we collected samples for this study, these transplanted populations were thriving. The localities given in Table 1 are the original sources of these transplanted populations (transplanted populations are marked with an asterisk). The transplanted populations exist as pure colonies, as evidenced by the uniform morphology of the respective individuals. Except for these transplanted populations, several of the named varieties are believed to be extinct.

The digestive gland was removed from each snail, diluted 1:1 in 0.01 M Tris–0.001 M EDTA–0.001 M 2-mercaptoethanol, pH 7.5, and homogenized. We centrifuged homogenates at 10,000 *g* for 5 min, and froze the supernatant at −85°C for up to several months prior to use. We followed standard methods of starch gel electrophoresis and histochemical staining (Murphy et al.

Table 1. Collection localities, morphological varieties, and *Gpi* genotypes

Population no.	Locality[a]	Varieties present	*Gpi* genotype[b] FF	FS	SS
1	Stock Island*	*solidulus*	0	0	10
2	Middle Torch Key*	*vonpaulseni*	0	0	10
3	Howe Key*	*osmenti*	0	0	10
4	Big Pine Key*	*pictus*	0	0	10
5	Little Pine Key*	*dryas*	0	0	10
6	No Name Key*	*graphicus*	0	0	10
7	Key Vaca*	*marmoratus*	10	0	0
8	Middle Hammock, Lower Matecumbe Key*	*pseudopictus*	0	0	10
		splendidus	0	0	10
9	Upper Hammock, Lower Matecumbe Key*	*delicatus*	0	0	10
		simpsoni	0	0	50
		subcrenatus	0	0	10
10	Lignumvitae Key*	*lignumvitae*	0	0	10
11	Upper Matecumbe Key*	*matecumbensis*	0	0	10
12	7 km N US 1, Key Largo*	*ornatus*	3	5	0
13	Garden Cove, Key Largo*	*castaneozonatus*	9	1	0
14	Totten Key*	*lineolatus*	4	5	0
15	NW Cape Sable*	*capensis*	10	0	0
16	Osteen Hammock, LPK 23	*versicolor*	7	3	0
17	Palma Vista Hammock #2, LPK 27	*castaneus*	10	0	0
		cingulatus	10	0	0
		luteus	10	0	0
18	Pfleuger Hammock, LPK 55	*deckerti*	10	0	0
19	NLPK 26	*eburneus*	0	0	10
20	NLPK 36	*wintei*	0	0	10
21	NLPK 81	*humesi*	0	0	10
22	NLPK 80	*framtoni*	0	0	10
23	Timm's Hammock*	*alternatus*	10	0	0
		fuscoflamellus	10	0	0
24	Pinecrest 55*	*margaretae*	10	0	0
25	Pinecrest 11	*lossmanicus*	12	0	0
		lucidovarius	1	0	0
26	Pinecrest 10	*aurantius*	1	0	0
		barbouri	1	1	15
		livingstoni	0	0	5
		miamiensis	0	1	4
		roseatus	0	0	4
		walkeri	5	0	15
27	Pinecrest 16a	*aurantius*	4	1	1
		barbouri	156	4	6
		walkeri	6	2	7
28	Pinecrest 16	*aurantius*	1	2	8
		barbouri	16	6	14
		roseatus	0	1	4
		walkeri	45	59	154
29	Pinecrest 14	*elegans*	1	0	0
		walkeri	14	6	20
30	Pinecrest 1a	*roseatus*	3	0	0
		walkeri	27	0	0
31	Pinecrest 13*	*kennethi*	10	0	0
32	Pinecrest 88	*aurantius*	1	2	3
		clenchi	1	1	1
		floridanus	0	5	0
		livingstoni	2	6	14
		lossmanicus	6	17	24
		mosieri	2	5	2
		ornatus	1	4	3
		roseatus	0	1	0
		testudineus	0	0	1
33	Fort Lauderdale*	*septentrionalis*	0	0	10

Population numbers correspond to the numbers in Figure 1.

[a] Abbreviations: LPK: Long Pine Key; NLPK: North of Long Pine Key. Numbered hammocks refer to numbering system given in Pilsbry (1946), except for NLPK localities, which are based on a report by A. Jones on file with Everglades National Park. The populations marked with an asterisk were obtained from transplanted populations now located in Everglades National Park.

[b] FF = fast homozygote, FS = fast/slow heterozygote, SS = slow homozygote.

1990). We prepared gels from 50% Otto Hiller Electrostarch (lot 392) and 50% Sigma starch (lot 85F-0010) at a concentration of 12%. The following buffer systems were used: TBE 9.1 (175 mM Tris–17.5 mM boric acid–2.75 mM EDTA, pH 9.1), TCB 8.7 (gel: 76 mM Tris–5 mM citric acid, pH 8.7; electrode: 300 mM boric acid, pH 8.2), TC 8.0 (gel: 23 mM Tris–5 mM citric acid, pH 8.0; electrode: 687 mM Tris–157 mM citric acid, pH 8.0), and LiOH 8.3 (gel: 45 mM Tris–7 mM citric acid–19 mM boric acid–3 mM lithium hydroxide, pH 8.3; electrode: 190 mM boric acid–30 mM lithium hydroxide, pH 8.1).

We examined 34 enzyme loci (Enzyme Commission numbers from Bielka et al. 1984 are given in parentheses): alkaline phosphatase (3.1.3.1), aspartate aminotransferase (2 loci; 2.6.1.1), esterase (10 loci; 3.1.1.-), glucose-phosphate isomerase (5.3.1.9), β-glucosidase (2 loci; 3.2.1.201), isocitrate dehydrogenase (1.1.1.42), lactate dehydrogenase (2 loci; 1.1.1.27), malate dehydrogenase (2 loci; 1.1.1.37), malic enzyme (1.1.1.40), mannose-6-phosphate isomerase (5.3.1.8), peptidase (A, B, C, and S loci; 3.4.-.-), peptidase-D (3.4.13.9), peroxidase (1.11.1.7), phosphoglucomutase (5.4.2.2), superoxide dismutase (2 loci; 1.15.1.1), and triosephosphate isomerase (5.3.1.1). All loci were examined in 60 individuals representing all populations and named varieties, and all 1,092 individuals were examined for the only variable locus discovered (glucose-phosphate isomerase). Details of histochemical staining for these enzymes are given by Murphy et al. (1990).

Results

Of the 34 loci examined, only the glucose-phosphate isomerase (*Gpi*) locus was variable (Table 1). The same two *Gpi* alleles reported from the Pinecrest populations (Hillis 1989; Hillis et al. 1987) were the only alleles observed from any Florida *Liguus* populations. Most populations were fixed for one or the other allele (Figure 2); the only populations that were polymorphic for the two alleles were from the Upper Keys (all three populations), Long Pine Key (one of three populations), and the Pinecrest region (five of nine populations). The Fort Lauderdale (*septentrionalis*) population and most of the populations from the Middle and Lower Keys were fixed for the slow *Gpi* allele, although the Key Vaca population (*marmoratus*) was fixed for the fast *Gpi* allele. The Long Pine Key populations are fixed (or nearly so) for the fast *Gpi* allele, whereas the native populations from hammocks just north of Long Pine Key (populations 19–22) are all fixed for the slow *Gpi* allele. The only populations examined from the Redlands region (Timm's Hammock: *alternatus, fuscoflamellus*) and Cape Sable (*capensis*) were fixed for the fast *Gpi* allele (Figure 2). The greatest Nei's genetic distance (Hillis 1984; Nei 1972) between any two populations or varieties was 0.03. Observed individual heterozygosity ranged from 0 to 0.029.

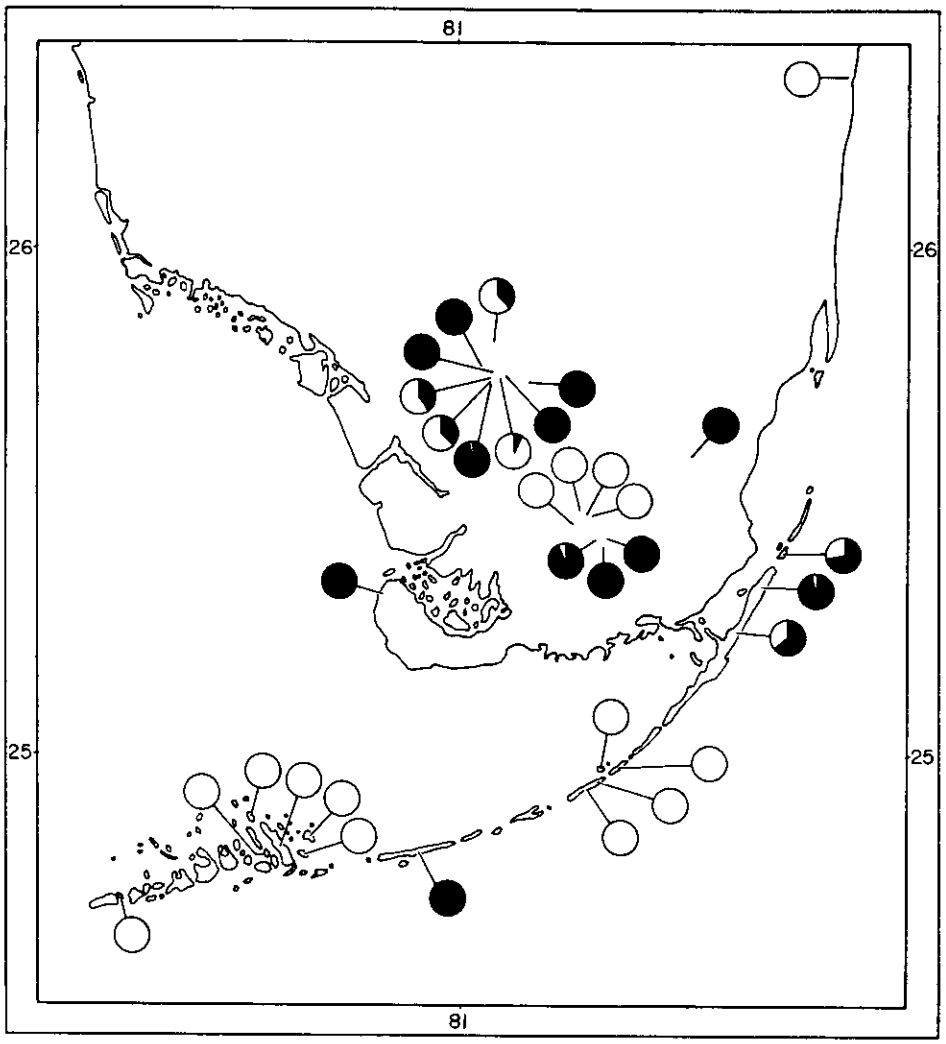

Figure 2. Frequencies of the slow (white) and fast (black) alleles of glucose-phosphate isomerase for each of the study populations.

Among the 60 individuals examined for all loci, average heterozygosity was 0.0015. If the 33 loci other than *Gpi* are truly monomorphic (as suggested by the individuals examined at all loci), the average individual heterozygosity for all 1,092 *L. fasciatus* was 0.0037.

Discussion

The very low genetic diversity among the many varieties of Florida *Liguus* supports the view that they are all members of a single species. No evidence was found for genetic barriers within or among differentiated populations or phenotypes. The maximum level of genetic divergence between any two populations of Florida *Liguus* is well within the levels expected for intraspecific comparisons (Avise 1974, 1976; Janczewski et al. 1990). The low genetic variation is even more notable considering that we included even the usually highly variable esterases and peptidases in our survey (Sarich 1977).

Individual heterozygosity for *L. fasciatus* is far below the average heterozygosity of sexual species (Nevo 1978). Among mollusks, the only lower values of individual heterozygosity observed to date are for self-fertilizing species, especially in introduced populations (Hillis and Patton 1982; McCracken and Selander 1980; Selander and Kaufman 1973a,b). This finding, together with the low genetic divergence of the populations (maximum genetic distance of 0.03), suggests that *Liguus* may have colonized Florida relatively recently.

The fixation of alternative alleles in many populations is probably largely a result of the self-fertilizing abilities of this species (Hillis 1989). However, the geographic distribution of the glucose-phosphate isomerase alleles (Figure 2) is not random and, in fact, partly parallels the principal geographic pattern of shell coloration suggested by Pilsbry (1912, 1946). Pilsbry (1946) suggested that *Liguus* populations in Florida resulted from three separate dispersal events from Cuba (from snails sealed to floating trees swept by hurricanes), and his three unranked "groups" of varieties reflected this proposal. He grouped the *Liguus* from the Lower Keys together with snails from the Matecumbe and Lignumvitae Keys as the *solidus* group, which he and other authors (e.g., Pilsbry 1912; Simpson 1929) earlier had placed in the species *L. crenatus* and *L. solidus*. Pilsbry (1946) placed the mainland snails, as well as populations from the Upper Keys (Key Largo, Totten Key, and Elliott Key) and the lower Middle Keys (Key Vaca and Grassy Key) into his *castaneozonatus–testudineus* group. The third group was restricted to the northern populations of a single variety (the *septentrionalis* group).

In discussing the origin of the *solidus* group, Pilsbry (1946:46–47) suggested a single colonization of the Lower Keys and a subsequent dispersal of these founding populations to the upper Middle Keys (Figure 1). He suggested a separate colonization from Cuba for the mainland snails, which he suggested landed along the Atlantic Coastal Ridge and then spread down to Key Largo and across to Cape Sable and the Pinecrest region (Figure 1). However, Pilsbry (1912, 1946:49) also considered the *Liguus* from the lower Middle Keys (including Key Vaca, population 7 in Figure 1) to belong to this otherwise mainland group, and he suggested a colonization from the mainland to account for this pattern. Pilsbry's third hypothesized colonization event was of the northern *septentrionalis* populations.

There is some support for at least some of Pilsbry's (1912, 1946) colonization hypotheses. The fixation of the Key Vaca population for the fast glucose-phosphate isomerase allele, which is otherwise restricted to the Upper Keys and mainland, supports a historical relationship between populations in these two areas (Figure 2). The populations on Cape Sable and most of the populations on Long Pine Key immediately to the north of Key Vaca (across Florida Bay) are also fixed for the fast *Gpi* allele, whereas the populations on the keys to the east and west of Key Vaca are fixed for the slow *Gpi* allele (Figure 2). The Upper Keys populations are polymorphic, with a predominance of the fast *Gpi* allele, also supporting Pilsbry's connection of these populations with those of the mainland. The third colonization suggested by Pilsbry (1946) was that of the northern-

most *septentrionalis* group, which is like the *solidus* group in its fixation of the slow *Gpi* allele. Pilsbry (1946) noted that nothing like the *septentrionalis* variety is present in Cuba.

Although the geographic distribution of the *Gpi* alleles in Florida is consistent with Pilsbry's (1946) hypotheses of three separate colonizations of *Liguus* from Cuba, the extreme genetic similarity of all Florida *Liguus* populations is indicative of a single population bottleneck (unless Cuban *Liguus* populations are also genetically nearly uniform). Populations of introduced mollusks that are capable of self-fertilization typically are fixed for single alleles at most loci (e.g., Hillis and Patton 1982; Selander and Kaufman 1973a,b). Comparison between Cuban and Florida *Liguus* could falsify Pilsbry's (1946) multiple-colonization hypothesis if it is found that Cuban populations show more typical levels of genetic variation. The genetic similarity of all the Florida populations would then be evidence for a single population bottleneck, presumably from an initial colonization event. Either scenario requires rapid mutation within the colonized populations in the genes responsible for shell coloration, with local fixation in isolated populations due to self-fertilization and/or selection. The discordance between the near uniformity of allozymes and the extreme morphological polymorphism of *Liguus* suggests that rates of evolution of the genes responsible for color pattern are quite high compared to the rates of evolution of the allozyme loci. Of the alternative mechanisms for local fixation of color patterns (self-fertilization or strong selection), self-fertilization is likely to be the most important, because self-fertilization is known to occur in this species and because selection on shell patterns in polymorphic populations appears to be weak or absent (Brown 1978).

Although our data indicate high genetic similarity among Florida populations of *L. fasciatus,* efforts to protect the disappearing habitat of the many morphologically distinctive and/or geographically isolated populations of this species should nonetheless be encouraged. The geographic distribution of the morphological diversity (as well as the limited biochemical diversity) indicates that the majority of the (albeit low) genetic variation of *L. fasciatus* exists among, rather than within, populations. Indeed, the self-fertilization capabilities of this species result in populations that are genetically more like individuals of outcrossing species. Thus, the long-term survival of *L. fasciatus* probably depends on widespread preservation of the remaining populations.

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