GENETIC DIVERGENCE DURING SPECIATION IN FRESHWATER
SNAILS OF THE GENUS Goniobasis

By

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Genetic divergence was compared with taxonomic divergence using 18 Florida populations of the freshwater snail genus Goniobasis. Genetic divergence was determined by using starch gel electrophoresis to study 18 loci in each population, and then computing genetic distances between all of the populations studied. The average genetic identities (Nei statistics) of populations at each level of taxonomic divergence are as follows: Local populations, 0.860; subspecies, 0.808; semispecies, 0.795; sibling species, 0.366; non-sibling species, 0.404. These results confirm the findings of recent studies on Drosophila. Considerable genetic divergence can take place without reproductive isolation, and very little, if any, additional change accompanies the completion of reproductive isolation. The use of these data in taxonomic and historical interpretations is discussed.

INTRODUCTION

One of the most persistent unsolved problems in evolutionary biology is how species originate in sexually reproducing animals. Mayr (1970) has championed the geographic speciation theory, which has become the most widely accepted model of speciation (Lewontin, 1974; Bush, 1975). According to this theory, the crucial event is the division of the coadapted gene pool of a species into two or more coadapted gene pools that are different enough to be reproductively isolated from each other; and an essential requirement for the divergence of these gene pools is a geographic barrier. Mayr believes that only a geographic barrier can restrict gene flow between separate divisions of the original gene pool sufficiently to permit genetic divergence under differing influences of sampling, drift, mutation, and selection. If at some future time the isolates should reestablish contact, and genetic divergence has been great enough, they will be reproductively isolated from one another; or hybrids between them will have greatly lowered fitness so that natural selection will soon complete reproductive isolation. The result is that the former isolates have become distinct species. If genetic divergence has not been great enough, and interbreeding takes place with little or no reduction in fitness of hybrids, the former isolates would still be conspecific. Mayr has termed the divergence and reconstruction of the genome that is necessary to form different coadapted gene pools a "genetic revolution." Analysis of

how and at what stage of speciation such a genetic revolution occurs is the object of this study.

The most extensive attempt to quantify the genetic changes occurring during the speciation process is the study of Ayala et al. (1974) on the Drosophila willistoni group. Since long periods of time are required for speciation in the geographic speciation model, evolutionary biologists have been forced to make inferences about the process using contemporary populations existing in varying degrees of evolutionary divergence. This has been the approach of the Ayala group. They have compared evolutionary divergence, as measured by taxonomic divergence, to genetic divergence, as measured by electrophoretically detected divergence in structural gene loci. Genetic distances between populations were calculated using the genetic similarity index (I) of Nei (1972), which is the normalized probability of identity of alleles. Comparisons were made between samples at the different taxonomic levels of local populations, subspecies, semispecies, sibling species, and non-sibling species. Local populations were found to be very similar, with I averaging 0.970. Subspecies showed an average I of 0.795, indicating that a significant amount of divergence had occurred during the time required for the development of these taxa. Semispecies, which show substantial but not complete reproductive isolation, had an average I of 0.798, not significantly different from the value for subspecies. Sibling species and non-sibling species showed average I values of 0.563 and 0.352, respectively. These data are interpreted as indicating that (1) major genetic changes can take place prior to speciation, (2) very little more, if any, takes place as reproductive isolation is developed, and (3) the greatest amount of divergence takes place

after reproductive isolation is complete and the separate gene pools are free to diverge independently. This implies that relatively little genetic change is required for the evolution of reproductive isolation. Zouros (1973) came to a similar conclusion based on his work with an unrelated group of Drosophila. Ayala (1975) and Avise (1976) summarize similar data from this and other studies on vertebrates. Although the general patterns of divergence are similar to that found in the Drosophila willistoni group, information on the crucial step, that of semispecies, is lacking.

The techniques of gel electrophoresis of proteins have become the most widely used method of measuring genetic variability and divergence. The methods are relatively simple and inexpensive in comparison to other biochemical genetic techniques. The number of individual gene loci that can be identified and studied with electrophoresis is larger than that of loci controlling morphological characters in most animals. A major assumption in the use of isozyme data is that the sample of genes studied is representative of the entire genome. It is becoming clear that in at least some cases isozyme evolution is slow or conservative relative to evolution of morphology or behavior. King and Wilson (1975) and Wilson (1976) have provided examples of such discrepancies, and have suggested that it is the evolution of regulatory genes, and not the structural genes studied by electrophoresis, that are more relevant to organismal evolution. Although the work of Wilson's group is based on some faulty taxonomic comparisons, such as the assumed equivalency of genera of frogs and mammals and the use of overly split hominoid taxa, there is still some question as to how directly changes in structural genes are correlated with changes in morphology. Use of

electrophoretic data must be viewed with the possibility in mind that electrophoretically detected structural genes are conservative in comparison to other measures of evolutionary change.

Electrophoresis separates molecules on the basis of charge and conformational differences. Different molecules having the same net charge may be electrophoretically indistinguishable. Electrophoresis will therefore underestimate the amount of divergence between samples (Coyne, 1976; Singh et al., 1976). Differences rather than similarities between samples should be stressed in the interpretation of isozyme data. However, there is no reason to suspect that the electrophoretically detected variation is not representative of variation in structural genes if enough loci are studied.

Goniobasis in Florida

Freshwater snails of the genus Goniobasis (Family Pleuroceridae) are abundant in most of the springs and spring-fed rivers and streams of Florida. These animals provide an ideal subject for the analysis of the genetics of speciation.

The distribution and taxonomic affinities of Florida Goniobasis were comprehensively described by Clench and Turner (1956). The classification of Goniobasis recognized and extended by Clench and Turner was based largely on shell sculpture characteristics (Fig. 1). Goniobasis floridensis is the most widespread of the seven recognized Florida species, extending from the Chipola River to the central Florida peninsula. The shell sculpture pattern of this species consists of axial costae and spiral cords that intersect to form nodules. Clench

and Turner considered G. athearni of the Chipola River and G. vanhyningiana of the mid-St. Johns River basin to be closely related to G. floridensis. These species have shell sculpture patterns similar to that of G. floridensis, except that the costae are weak or absent in G. athearni, and spiral cords in the adult whorls are lacking in G. vanhyningiana.

The remaining Florida Goniobasis were thought by Clench and Turner to be related in varying degrees to G. curvicostata. These species are all characterized by arcuate axial costae as the most conspicuous aspect of the shell sculpture. Goniobasis curvicostata is the most widely distributed of these species, being found from the Flint River west to the Escambia River. Goniobasis albanyensis is found in the Apalachicola River. Goniobasis clenchi displays spiral sculpture in addition to well-developed costae, and is found in the Choctawhatchee River system. Clench and Turner thought that G. dickinsoni, which they described in their 1956 study, had no close affinities in the area, but was closest to G. curvicostata.

Field and museum examination of the species discussed by Clench and Turner revealed a large amount of intraspecific variation, with complex patterns of distribution and evident interbreeding between forms and even some recognized species. This variation is indicative of varying degrees of evolutionary divergence. Great amounts of divergence within a small geographic area make these animals a favorable group for the elucidation of the genetics of speciation. The object of this study is to compare taxonomic divergence with genetic divergence using starch gel electrophoresis, in order to determine the level of the speciation process at which the greatest divergence of structural genes takes place.

MATERIALS AND METHODS

Collection Sites

Collection sites were chosen on the basis of the morphological form or species which was present in the area, the area's accessibility, and the relative abundance of Goniobasis. Each site is briefly described below, together with the name of the species sampled, an estimate of its abundance, the abbreviation for each sample that will be used in the tables, and the locality code number which appears in Figure 2. More detailed information on the spring sites is in Ferguson et al. (1947). All collection sites are in Florida.

1. Rock Spring, Kelly Park, Orange Co. This spring flows from a vertical limestone wall 60 m to a wide pool and thence as a spring run into the Wekiva River. Goniobasis occurs here in relatively low densities as compared to other Florida springs. Algae are very abundant, an unusual condition for a Florida spring. Goniobasis vanhyningiana (RSP).

2. Juniper Spring, Ocala National Forest, Marion Co. Although this spring has been greatly modified and heavily used as a swimming pool, it supports a moderate sized population of Goniobasis floridensis (JSP).

3. Juniper Creek at Fla. 19, Ocala National Forest, Marion Co. This is the outlet of Juniper Spring to Lake George. The site is 6 km down the creek from Juniper Spring. Goniobasis floridensis (JCR).

4. Rainbow River, Marion Co., at K. P. Hole County Recreation Center. The water is very clear, being 1 km from the river source at Rainbow Springs. Goniobasis floridensis (RR).

5. Wekiva River at Fla. 343, Levy Co. This is a clear spring run fed by Wekiva Spring. It should not be confused with the Wekiva River in Orange Co. Goniobasis floridensis (WEK).

6. Waccasassa River at US 19, Levi Co. This small river drains a low area including cypress swamp. The water is therefore often clear but colored brown from tannic acid. Goniobasis floridensis (WAC).

7. Ichetucknee River at the Florida Department of Transportation roadside park on US 27, Ichetucknee Springs State Park, Columbia Co. This large clear spring run is fed by Ichetucknee Springs. Two species were sampled here: Goniobasis sp., which will be referred to as the reference population (REF), and G. floridensis (ICH).

8. Blue Spring, Withlacoochee River at Fla. 6, Madison Co. The water is very clear as this is a large spring that flows to the Withlacoochee River, about 30 m from the spring. This is a tributary of the Suwannee and has no connection with the river of the same name that receives the Rainbow River. On a recent visit to this spring (12 March 1977), high river levels were flooding the area of the spring so that there was no evidence of the presence of the spring. No Goniobasis could be seen at this time, although when a collection was made of G. floridensis (BSP) the previous year, they were very abundant.

9. Apalachicola River, 0.5 km south of the US 90 bridge. This site on the wide and muddy Apalachicola River is 1 km below the Jim Woodruff Dam. Goniobasis albanyensis (Gal).

10. Chipola River at Fla. 167, Jackson Co. This river is spring-

fed and is quite clear except when receiving runoff from local rains. Goniobasis floridensis (CHP), G. curvicostata (Gcu), and G. athearni (Gat).

11. Blue Hole Spring, Florida Caverns State Park, Jackson Co. This is a small spring with a large, shallow pool that is used as a public swimming area. Goniobasis floridensis (BHS).

12. Spring Creek at Fla. 75, Jackson Co. This creek was flooding during visits in January and March 1977. It appeared clouded, even though fed by springs in southern Alabama. Goniobasis dickinsoni (GdS).

13. Holmes Creek at Fla. 2, Jackson Co. This creek is slow-moving and appears clouded. It drains an area extending into southern Alabama that is dominated by hog farms. Goniobasis dickinsoni (GdH).

14. Wrights Creek at Fla. 179, Holmes Co. This creek was flooding during March 1977, when a collection of Goniobasis dickinsoni (GdW) was made. Goniobasis curvicostata was observed here and G. clenchi has also been reported from this creek.

15. Choctawhatchee River at US 90, Washington Co. This site was flooded when the collection of G. clenchi (Gcl) was made in January 1977.

Collection and Handling of Animals

Goniobasis were collected in field by hand or, especially in the case of sites that were at high water levels, by use of a bottom sampling net. The snails were sampled without regard to phenotype even in highly variable populations. They were then brought to Gainesville on the day of collection in unaerated jars or plastic boxes and placed

in aquaria. Whenever possible, these 10 gallon aquaria were prepared weeks in advance of their occupation. The bottoms of the aquaria were prepared by spreading 2-3 cm of fine sand. The water was inoculated with an algae and diatom culture. The aquaria were aerated and supplied with individual outside filters. Survival was virtually complete if the aquaria were not overcrowded (typically about 60 snails in a 10 gallon aquarium), and occasional dead snails were removed daily.

Electrophoresis

Snails to be electrophoretically examined were removed from their holding aquaria the night before electrophoresis and kept in a clean bowl of water so that they could not feed. Only adult snails were used. The following day, each animal was extracted from its shell and placed in an individual centrifuge tube with an equal volume of the Tris-EDTA grinding buffer of Selander et al. (1971). They were then homogenized by sonication with a Branson Model W185 sonicator equipped with a micro tip. The samples were centrifuged at 4300 g for 5 minutes. The supernatant was drawn off and the pellet discarded.

Horizontal starch gel electrophoresis was carried out with the samples applied to gels as Whatman No. 1 chromatography paper tabs soaked in the homogenate supernatant of a single snail and then lightly blotted. Following electrophoresis, gels were sliced and placed individually in stains for 14 different enzymes. These enzymes, with their abbreviations, are Acid phosphatase (Acph), aldolase (Aldo), alkaline phosphatase (Aph), esterase (Est), glutamate oxalacetate transaminase (Got), glyceraldehyde-3-phosphate dehydrogenase (G3pd),

hexanol dehydrogenase (Hexdh), leucine aminopeptidase (Lap), malic enzyme (Me), 6-phosphogluconate dehydrogenase (6-Pgd), phosphoglucose isomerase (Pgi), phosphoglucomutase (Pgm), sorbitol dehydrogenase (Sdh), and tetrazolium oxidase (To). Throughout the remainder of this paper, these enzymes will be referred to by their abbreviations. Staining solutions were prepared according to Bush and Huettel (1972) for Acph, Aph, Est, Hexdh, Lap, 6Pgd, and Pgi; according to Ayala et al. (1973) for Aldo, G3pd, Sdh, Me and Pgm; and according to Selander et al. (1971) for Got. Five different gel buffers were used to resolved these enaymes. Gels using the Poulik (1957) system and 20 g of Connaught starch, 20 g of Electrostarch, and 20 g of sucrose per 440 ml of gel buffer were used for Est and Aph. The same starch and sucrose mixture was used with the lithium hydroxide buffer system of Selander et al. (1971) for Pgi and Got. The remaining systems used 44 g of electrostarch per 440 ml of gel buffer. The Poulik (1957) gel system was used for Sdh, Lap, Me, and Pgm. Buffer B of Ayala et al. (1973) with the pH adjusted to 9.1 was used for G3pd, Aldo, Hexdh, and To. The histidine buffer system of Brewer (1970, p. 90) was used for Aph and 6Pgd with the following modifications: The concentration of the bridge buffer was 0.2 M and the pH of both bridge and gel buffers was set at 8.0.

Other enzymes were stained for use during initial screening but were not used in this study due either to lack of activity or to inconsistent results. Enzymes that gave no staining activity were glycerophosphate dehydrogenase, galactose dehydrogenase, glutamate dehydrogenase, glycerol dehydrogenase, malate dehydrogenase, octanol dehydrogenase, lactate dehydrogenase, xanthine dehydrogenase, and succinate dehydrogenase. Enzymes that were detected on gels but could not be consistently

resolved were alcohol dehydrogenase, peroxidase, glucose-6-phosphate dehydrogenase, adenylyl kinase, aldehyde oxidase, general proteins, isocitrate dehydrogenase, hexokinase, fumarase, and hydroxybutyrate dehydrogenase.

Breeding Experiments

Breeding experiments were attempted in order to verify genetic interpretations of isozyme patterns and to determine interbreeding ability of different populations. Young Goniobasis about 2 mm in shell length were placed in previously prepared 5 gallon aquaria either singly or in pairs. Aquaria were prepared by spreading about 3 mm of fine sand on the bottom and adding a 10 cm square glass plate that had previously been kept in an algae and diatom culture. This culture eventually spread to the walls of the aquaria. Aquaria were aerated and kept under Gro-lux fluorescent light bulbs. Initially, the light-dark cycle was 12 hours-12 hours. When snails failed to breed under these conditions, they were changed to 11 hours light-12 hours dark, and this cycle produced good results.

RESULTS

Electrophoresis

The results of the electrophoretic analysis are given in Table 1. Scoring of gels was according to the criteria of Ayala et al. (1973). Gels were scored as autosomal loci with codominant alleles. The most common allele, or electromorph, in the reference population for each locus was designated with the superscript ¹⁰⁰, with other alleles at the locus designated by adding to 100 the distance in millimeters a band migrates anodal to the standard, or by subtracting from 100 the number of millimeters a band runs cathodal to the standard. Loci were designated when the genotype frequencies were found to be in Hardy-Weinberg equilibrium. Genetic interpretations were further confirmed for Aph, Got, G3pd, 6Pgd, and Pgi by the presence of heterodimers in the heterozygotes. Offspring from breeding experiments have yet to reach adult size (April 1977) for further confirmation of Mendelian inheritance of isozyme patterns. All bands migrated anodal from the origin.

The Ichetucknee River site was chosen as the site of the reference population because it was close to Gainesville (where the laboratory work was performed), it had a very large population of Coniobasis, and these snails were thought to be typical G. floridensis. As discussed below, this last assumption turned out to be incorrect.

Using the data in Table 1, the genetic identity (I) and distance

(D) between all samples were computed according to the method of Nei (1972). These values are given in Table 2. As derived by Nei, I represents the normalized probability of identity of alleles over all loci and is computed by the formula:

$$I = \frac{\frac{\sum x_i y_i}{n}}{\sqrt{\frac{\sum x_i^2}{n} \cdot \frac{\sum y_i^2}{n}}}$$

where x_i and y_i are the frequencies of the i th allele in populations X and Y, respectively; and n is the number of loci. The genetic distance (D) is the accumulated number of gene substitutions per locus and is computed by the formula:

$$D = -\log_e I$$

Breeding Experiments

Five successful crosses have been made as of this writing. One was between an individual from Rainbow River and one from Blue Spring, another between two snails from the Waccasassa River site, and the rest between pairs of snails from Rainbow River. Goniobasis are dioecious, and since it is not possible to determine the sex of these snails while they are alive, some crosses were probably set up with individuals of the same sex. Twelve other crosses are currently in progress.

The generation time of snails in the successful crosses is about six months, although there is a large variance even among snails from the same population. Goniobasis seem to grow especially slowly in aquaria where blue-green algae have become established. All laboratory-reared snails exhibit the shell sculpture characteristics of their

parental population, the only exception being the retention of nuclear whorls in the laboratory-reared animals. In natural populations, these whorls are usually broken off or eroded away by the time adult size is attained.

It is possible that some or all of the progeny described above are actually produced by parthenogenetic females. Parthenogenesis is unknown in the family Pleuroceridae, although it is the normal mode of reproduction in the Thiaridae, a related family. Eight individuals from the Rainbow Run and Blue Spring populations have been maintained in isolation for over a year without producing offspring. Further, males have been identified in all three populations from which successful crosses have been made. Therefore, the possible role of parthenogenesis is minimized but cannot be ruled out altogether.

DISCUSSION

The principal points to be considered are as follows: patterns of divergence in different taxa and geographical areas; historical interpretations; and the taxonomic implications of the electrophoretic data. The section will close with a discussion of the relevance of these data to genetic divergence during speciation.

Patterns of Divergence

The Ichetucknee River Reference Population and its Relationships

As mentioned earlier, the rationale for choosing the Ichetucknee River site for a reference population assumed that this was a typical Goniobasis floridensis population. After using this population as a reference for comparison with several G. floridensis populations, the wide divergence between the reference and these populations became obvious. Both the reference population and most G. floridensis populations have what shall be referred to as the "standard" G. floridensis shell sculpture pattern. This pattern is comprised of axial costae and spiral cords that intersect to form nodules. The peripheral cord is generally the largest (Fig. 3). The members of the Ichetucknee reference population were noted to differ in having the shell spire more eroded and less sharply sculptured, but this condition could be attributed to abrasion against the bare limestone substrate of this

rapidly flowing river. The shell morphology seemed well within the range of variation observed statewide in G. floridensis.

Using the Got locus, which was fixed for a different allele in the Ichetucknee reference population not found in any of the G. floridensis samples, Goniobasis were sampled from other nearby sites in the Suwannee River system to indicate the extent of the divergent genotype of the Ichetucknee reference population. Samples were obtained at Ginnie Spring on the Santa Fe River, Ichetucknee Spring, and the Suwannee River near Branford. All of these samples showed the typical G. floridensis band Got⁹³. Careful study of the morphology of new collections from the Ichetucknee River locality indicated that there were two species at the site of the reference population, one of which had not been collected earlier. This finding was further confirmed by the electrophoretic data in Table 1 for the Ichetucknee reference sample and typical G. floridensis specimens from the same site. The lack of common alleles in these two samples at the Aph, Est-2, Got, Lap-2, Me, 6PgD, Pgm, and Sdh loci is strong evidence that there is no gene flow between these sympatric samples. No visible taxonomic character will consistently separate the two species. The most useful characters include the presence of freshwater sponges, the worn shell spire, and habitat preference for rock rather than vegetation on the part of the reference species. The overall aspect of the shell is shorter and costae are not as strongly developed in most individuals of the reference species, and the outer lip of the aperture is straighter than the sigmoid shaped outer lip of the G. floridensis aperture. Even so, some individuals are impossible to place in one or the other species, especially if they are abraded, without electrophoretic

determination. For these reasons, the two Ichetucknee River species of Goniobasis can be considered sibling species.

Table 3 gives values for I between the reference species from the Ichetucknee River, G. floridensis from the Ichetucknee River and Rainbow River, and G. albanensis and G. atearni from the Florida Panhandle. It can be seen that the identities are very high between the reference sample and G. atearni and G. albanensis, the Florida Panhandle forms (Fig. 4). Genetic identities in this range (greater than 0.8) are within the range of conspecific populations of Drosophila. The Ichetucknee reference species is closest to G. atearni, which it resembles in overall form, differing only in more-developed costae and a less-developed peripheral cord.

Goniobasis vanhyningiana and Goniobasis floridensis in the Mid-St. Johns River Basin

Goniobasis vanhyningiana was described by Goodrich (1921) from a creek below Seminole Springs in Lake County, Florida. Clench and Turner (1956) listed localities for this species as the type locality, Alexander Spring, and Rock Spring (Fig.5). This species is characterized by its lack of shell sculpture on the adult whorls and brown color. Clench and Turner had no record of sympatry between G. floridensis and G. vanhyningiana. The Goniobasis from Juniper Spring are very similar to G. vanhyningiana (Fig. 6), but were included with G. floridensis by Clench and Turner. About 6 km down Juniper Creek from the spring, the Goniobasis have the standard G. floridensis sculpture pattern (Fig. 7). Between this site and the spring, the two

forms can be found together, with a full range of intermediate forms (Fig. 8). The extremely high I values for Rock Spring, Juniper Spring, and Juniper Creek samples (Table 2) indicate insignificant divergence between them. All common alleles are shared by all three samples (Table 1). The Juniper Spring and Juniper Creek samples, which differ so greatly in the sculptural characteristics which have been so important in the classification of this group, are essentially identical from a genetic standpoint. This morphological differentiation has occurred with essentially no detected divergence in structural genes.

Goniobasis floridensis in the Waccasassa and Wekiva Rivers

A smooth-shelled form of G. floridensis is found along the Waccasassa River in Levy Co., Florida. This form has well-developed costae, but lacks the spiral cords. A spring-fed branch of the Waccasassa River, the Wekiva River, contains a standard sculptured form (Fig. 9). At the confluence of these rivers (Fig. 10), these forms hybridize, with a full range of intermediate forms (Fig. 11). The I values for the samples taken of these two forms is 0.904, well within the range of conspecific populations of Drosophila. This morphological differentiation has occurred without dramatic isozyme differentiation. Also, the Wekiva River sample is more divergent from other standard G. floridensis than is the Waccasassa River sample.

Genetic Variation in "Standard" Goniobasis floridensis

The I values between standard sculptured G. floridensis populations,

including G. clenchi, (Fig. 12) are given in Table 4. Most of the values in the table range from 0.8 to 0.9, which is similar to the values for Drosophila subspecies. Values of 0.7 to 0.8 are found between the Ichetucknee River and Juniper Creek samples and the other samples. These two populations are divergent from each other and from the rest of the G. floridensis samples. With the exception of these two samples, the identities between samples are generally inversely correlated with their geographic distance from each other.

Goniobasis floridensis and Goniobasis dickinsoni in the
Florida Panhandle

Three species of Goniobasis are found at the collection site on the Chipola River. The identities between these species along with G. dickinsoni from Holmes Creek are in Table 6. Goniobasis dickinsoni and G. floridensis are not greatly divergent ($I = 0.888$). Goniobasis curvicostata and G. athearni are divergent from G. floridensis and G. dickinsoni and from each other. The remainder of this section will be a discussion of the relationships between G. dickinsoni and G. floridensis.

Collection sites for the Blue Hole Spring and Chipola River samples of G. floridensis, the sample of G. clenchi, and three samples of G. dickinsoni are indicated in Fig. 13. Individuals from Blue Hole Spring in Florida Caverns State Park tend to be large with heavy costae and weak cords. Some individuals have moderate spiral cords. The typical G. floridensis sculpturing increases with increasing distance down the spring run. The distinct form in Blue Hole Spring evidently

intergrades with the standard G. floridensis population in the Chipola River.

Goniobasis clenchi from the Choctawhatchee River system is a form of G. floridensis, as will be demonstrated in the section on taxonomic interpretations. Goniobasis dickinsoni is found in the upper tributaries of the Choctawhatchee and Chipola river systems. As pointed out by Clench and Turner (1956), the adjacent tributaries of these two systems can be found in an area of low relief in southern Alabama. Goniobasis dickinsoni is sympatric with G. clenchi in Wrights Creek, with no indication of hybridization between these morphologically distinct species (Fig. 14). In the Chipola River, on the other hand, G. dickinsoni appears to be interbreeding with G. floridensis. The frequency of standard shell sculpturing increases going down the Chipola River. This was traced as far south as Spring Branch, high river levels preventing further tracing of this trend to the collection site of G. floridensis on the Chipola River on visits made in January and March 1977. The G. dickinsoni-G. floridensis intergrades appear similar to G. floridensis in Blue Hole Spring (Fig. 15).

Genetic identities between G. clenchi, the two samples of G. floridensis from this area, and three samples of G. dickinsoni are presented in Table 6. The lowest of these values is 0.863. The three G. dickinsoni samples have very high identities (0.934 to 0.991), verifying their conspecific status. The Blue Hole Spring sample is intermediate between G. dickinsoni and G. floridensis, agreeing with morphological similarities. Goniobasis dickinsoni has therefore achieved reproductive isolation from G. floridensis in one part of

its range, but has not in another. Goniobasis dickinsoni can then be considered a semispecies, incompletely isolated from G. floridensis.

Taxonomic Implications

Isozyme data provide useful information for determining if sympatric populations are reproductively isolated. Fixation of alternative alleles at a number of loci is strong evidence of lack of gene flow between sympatric populations. The use of isozyme data for making taxonomic decisions about allopatric populations is far more subjective. The data previously presented on G. dickinsoni indicate that reproductive isolation and taxonomic change do not always occur concomitantly. However, as the final section of this paper will indicate, there is a general agreement between isozyme and taxonomic divergence. Isozyme data can therefore be used to make general predictions for further taxonomic study. It is important that the isozyme data be derived from a good sample from a population. Lewontin (1974: p. 115) estimates that a sample size of 50 individuals is probably adequate to detect all common alleles in a population. In addition to an adequate sample size of individuals, as large a sample of loci as possible should be used in order to maximize confidence in determinations of divergence and variability.

The isozyme data presented in this paper support the contention that the populations designated as G. floridensis are all one species. All I values are in the range found for conspecific populations of Drosophila. The Ichetucknee River and Juniper Creek populations are the most divergent, but not outside of this range. Goniobasis

vanhyningiana, including the Juniper Spring population, is extremely similar to the Juniper Creek sample of G. floridensis. Hybridization between these forms and high genetic identities warrant the reduction of G. vanhyningiana to synonymy with G. floridensis. Goniobasis clenchi may also be reduced to synonymy with G. floridensis. It has I values greater than 0.8 for all populations of G. floridensis except the slightly divergent Ichetucknee and G. vanhyningiana forms. As further evidence, R. Franz and F. Thompson (personal communication) have discovered a population of Goniobasis in the Econfinia River (geographically between the Chipola and Choctawhatchee systems) that is intermediate between G. floridensis and G. clenchi in shell sculpture. Clench and Turner (1956) suggested G. clenchi was related to G. curvicostata. Its low identity with that species (0.562) renders this unlikely.

Clench and Turner (1956) thought G. athearni and G. floridensis were so similar that they were surprised to find both at the same sites on the Chipola River. The isozyme data indicates that these species are fully separated, and perhaps have been so for a very long time. The highest identity with any G. floridensis population is 0.547 with the Ichetucknee sample. Other identities between the two species are around 0.4 or less. The reference population has a very high identity with G. athearni (0.911), suggesting that they may be conspecific. Morphological differences are not distinct, further supporting this interpretation. The high identity of G. albanyensis and G. athearni (0.836) and the reference population (0.862) are in the range of conspecifics, but the different shell patterns, color, and habitats argue against taxonomic revision without further study.

Goniobasis dickinsoni was thought by Clench and Turner (1956) to be related to G. curvicastrata on the basis of the presence of axial costae. Genetic identity and hybridization indicates that G. dickinsoni has much closer affinities to G. floridensis.

Goniobasis curvicastrata has a very low identity (0.258) to G. albanensis. This is in sharp contrast to Clench and Turner's suggestion that these species were related due to the similarity of the smooth costae of their shells.

The general relationships between these species can be summarized as follows. There are three species groups: The G. floridensis group includes G. clenchii and G. dickinsoni; the G. albanensis group includes the Ichetucknee reference population, G. atearni, and G. albanensis; and the G. curvicastrata group is distinct from both, but closer to the G. floridensis group. Conclusions about G. curvicastrata, and to a lesser extent the G. albanensis group, are very tentative since so few populations have been studied.

An overall conclusion about the classification of these snails is that the shell sculptural patterns are poor taxonomic characters. The relative strengths of costae and spiral cords, used extensively in this group, can give very misleading classifications. An outstanding example is the presence of spiral cords in the Juniper Creek population and their absence in Juniper Spring. The two forms are interbreeding and show no isozyme divergence, yet would be placed in different species based on published species descriptions.

In another case, the convergent sculpture patterns of G. floridensis and the Ichetucknee River reference population obscures very profound differences between the species. More reliable in the classification of these snails is the angle of the shell spire and the shape of the

outer lip of the shell aperture: the angle of the spire is much sharper in the G. floridensis group; and the shape of the outer lip of the aperture tends to be sigmoid in the G. floridensis group, flattened in the G. albanensis group. No obvious characters separate the G. curvicastrata and G. albanensis groups. Davis (1969) found spire angle to be useful in distinguishing species, but not species groups, in the Japanese pleurocerid genus Semisulcospira. The lack of reliability of shell sculpture, and the paucity of measurable shell characters underline the importance of studies of genetics and soft anatomy for reliable gastropod classification.

Historical Interpretations

Historical interpretations of aquatic animals in an area of porous limestone like Florida are extremely tenuous. The problem is only slightly simplified by the apparent inability of these snails to disperse through the aquifer. All are inhabitants of open areas of springs and rivers, with only G. curvicastrata showing significant burrowing behavior. One conclusion that can be drawn is that Goniobasis floridensis has lived in the Florida peninsula throughout the Pleistocene. Rough estimates of divergence times related to the genetic distance measure of Nei are $D \times 5$ million years (Nei, 1975) and $D \times 18$ million years (Gorman et al., 1976). Nei's estimate was based on mutation rates, while that of Gorman et al. was based on the genetic distance-immunological distance correlation reported by Maxon and Wilson (1974). This means that the populations of G. floridensis from Juniper Creek and the Wekiva River have been separated from 1.4 to

5.1 million years, depending on the estimate used. It appears that these forms have been separate since at least early Pleistocene, perhaps much longer. It is possible that both of these forms are from different ancestral stocks and are recent colonists to the peninsula, but that situation would require some unlikely distributions and extinction of the hypothetical ancestral populations.

The seemingly disjunct distribution of G. athearni in the Chipola River and the reference population 280 km distant in the Ichetucknee River has interesting parallels in other groups. Jackson (1975) has described the presence of a fossil aquatic turtle of the genus Graptemys in the Santa Fe River. Graptemys barbouri, to which Jackson has referred these fossils, is today found only in the Apalachicola River system, including the Chipola River. He offers the explanation that the Apalachicola and Suwannee (including the Ichetucknee River) river systems are in close proximity where they drain adjacent river basins in southern Georgia. Jackson discusses similar patterns of distribution in other aquatic organisms.

Genetic Divergence During Speciation

Identity values between populations of Goniobasis at different levels of taxonomic divergence are given in Table 7. Also in the table are the values determined by Ayala et al. (1974) for the Drosophila willistoni group. Goniobasis were assigned to taxonomic levels as follows:

Local populations: These are allopatric but very similar populations within the same subspecies. In Goniobasis, these are

morphologically very similar in sculpture patterns and shell color.

Subspecies: Drosophila willistoni group subspecies were determined by Ayala et al. (1974) on the basis of their slight reproductive isolation from each other. This type of information is not yet available for most of the Goniobasis populations. Subspecies of Goniobasis were designated according to differences in shell sculpture and color. One subspecies was based on the standard shell sculpture of G. floridensis and included populations from Rainbow River, Blue Spring, Ichetucknee River, Wekiva River, and Chipola River. Another subspecies was characterized by light brown shell color, as opposed to the dark brown or black color of snails in the previous group, and included the populations from Rock Spring and Juniper Spring. Due to its genetic identity to the Juniper Spring population, the Juniper Creek population was excluded to avoid redundancy. Other forms designated subspecies for the purposes of this comparison include the smooth-shelled form from the Waccasassa River, the population at Blue Hole Spring, and the sample of G. clenchi.

Semispecies: Goniobasis dickinsoni and G. floridensis are the only semispecies recognized. Reproductive isolation has been completed in only part of their overlapping ranges.

Sibling species: The only sibling species identified are G. floridensis and the reference population from the Ichetucknee River. Sibling species are reproductively isolated species that are morphologically very similar. The average I value given in Table 7 was determined from the identities between the reference population and those G. floridensis populations with the standard sculpture pattern.

Non-sibling species: The species used in this comparison were

G. floridensis, G. athearni, G. albanyensis, and G. curvicostata. The data for this level of divergence are not necessarily equivalent to those of Ayala et al. (1974) because that study used only species within the same species group; whereas in Goniobasis, distinct species cannot be reliably placed in species groups at this time.

The average I values in Table 7 show the same general trend in both the D. willistoni group and Goniobasis, namely increasing genetic divergence with increasing taxonomic distinction. Interesting differences are seen between the two studies at some taxonomic levels.

The average identities for local populations are much lower for Goniobasis than for Drosophila. Some of the local populations of Goniobasis may actually show some reproductive isolation, but it has not been detected. Attempts at crossbreeding may reveal that some of these populations show some reproductive isolation and are therefore subspecies by the criterion used for Drosophila. The morphological similarity of these populations of Goniobasis is based on shell characters. A detailed study of soft anatomy might reveal further subdivisions of the standard G. floridensis forms. These populations may have been correctly assigned to the same subspecies, but isolation in different drainage systems has restricted gene flow. Allopatric populations of these aquatic snails might therefore be expected to be more isolated than more vagile animals such as flying insects.

The average identity of subspecies is not significantly different in Drosophila and Goniobasis. This is remarkable when one considers that different criteria were used in designating subspecies in the two groups.

Semispecies of Drosophila and Goniobasis show the same level of

genetic divergence. This is the crucial step in speciation, when reproductive isolation is being perfected. Goniobasis semispecies verify the findings in Drosophila that reproductive isolation can be attained with very little genetic change beyond what is observed in subspecies.

The much lower average identity of sibling species of Goniobasis may not be representative since only one sibling species pair is known. These species may not be true sibling species. Their similarity may be the result of convergent evolution in shell sculptural characters.

The average identities of non-sibling species are about the same for both groups. The standard error is much larger for Goniobasis, however. This may be the result of comparing species from more than one species group.

The data on Goniobasis supports the conclusion of Ayala et al. (1974), that a significant amount of genetic differentiation may occur without reproductive isolation, and that very little if any additional genetic divergence is found in fully reproductively isolated populations. Ayala's (1975) review of data from other groups of organisms, though lacking information on semispecies, is consistent with this conclusion. Reproductive isolation cannot always be predicted on the basis of structural gene divergence alone.

Ayala (1975) has discussed why structural gene divergence and reproductive isolation may not be strictly correlated. The genes detected by electrophoresis are either of unknown function or are enzymes in basic metabolic pathways. The genes involved in the evolution of reproductive isolation may be relatively few in number and therefore not detectable with the level of sensitivity of electrophoretic analysis. Knowledge of the nature of isolating mechanisms

and their genetic basis is crucial to understanding speciation in a group. To date, isolating mechanisms in Goniobasis are unknown. There is some isolation by habitat preference, but this alone is probably not an adequate mechanism.

King and Wilson (1975) argue that the structural gene loci are not the major factors in evolutionary change and have suggested that regulatory gene mutations or chromosomal rearrangements are more important in organismal evolution. Chromosomal changes may result in reproductive isolation or other rapid change without alteration of the structural genes contained on the chromosomes. For the reasons cited in the introduction to this paper, Wilson's (1976) conclusions on the importance of regulatory genes are questionable, and have not been tested directly.

This study has assumed that electrophoretic data give a representative estimate of divergence in structural genes. This assumption may not be completely valid. Coyne (1976) and Singh et al. (1976) have used more elaborate electrophoretic analyses to show that a locus that was thought to be very similar in different species or populations of Drosophila is actually quite different. Lewontin and Hubby (1966), in their original investigation of the amount of electrophoretically-detected variation in natural populations, pointed out that only about one-third of all variation is electrophoretically detectable. This is based on the fact that only about one-third of the amino acid substitutions that can be made in a protein will result in a difference in the charge of the molecule. As mentioned previously, lack of identity is therefore a more meaningful way of looking at electrophoretic data rather than drawing conclusions based on similarity. The work of

Coyne (1976) and Singh et al. (1976) is on a single locus in two species, and Coyne points out that the results may not be applicable to all loci. Yet these results demonstrate the danger that hidden variation presents when conclusions are based on a single or very few loci. If a sample of loci is large enough, the electrophoretically-detectable variation should be at least an approximation of the total.

To understand the crucial step in the completion of reproductive isolation between incipient species, a more detailed analysis of semispecies and the genetic basis of reproductive isolation will be necessary. The use of the techniques of Bernstein et al. (1973), Singh et al. (1976) and Coyne (1976) should reveal if the populations of semispecies that have achieved complete reproductive isolation are as similar as present electrophoretic data indicate. Such studies should be approached under the premise that there is probably no single model that will explain all speciation events. Rapid speciation events may take place by chromosomal rearrangement without divergence in structural genes. In other cases, such as where developmental disruption in hybrids is a stage leading to the evolution of isolating mechanisms, divergence in structural genes might still be a good estimator of the likelihood of reproductive isolation.

It is difficult to imagine two invertebrates more dissimilar than a freshwater snail and Drosophila, yet this study confirms recent work on Drosophila that indicates that the "genetic revolution" occurring during speciation may only be a "minor reform," as Lewontin (1974) has suggested. Reproductive isolation in Goniobasis, as previous studies have suggested for Drosophila, may be achieved without abrupt changes at structural gene loci.

Table 1 - continued

Locus	Allele	Population								
		RR	BSP	ICH	WEK	WAC	BHS	CHP	JCR	JSP
Lap-1	107	-	-	.038	.038	-	.673	-	1.00	1.00
	104	.826	.423	-	.923	1.00	.327	.288	-	-
	102	.058	-	.952	-	-	-	.587	-	-
	101	-	-	.010	-	-	-	-	-	-
	100	-	-	-	-	-	-	.125	-	-
	98	.019	-	-	-	-	-	-	-	-
	null	.096	.577	-	.038	-	-	-	-	-
Lap-2	103	.010	-	-	-	-	-	-	-	-
	102	.990	1.00	1.00	1.00	.990	1.00	1.00	1.00	1.00
	101	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	.010	-	-	-	-
Me	108	-	-	-	-	-	-	-	-	-
	102	.971	1.00	1.00	.990	.529	.873	.981	1.00	1.00
	100	.029	-	-	.010	.462	.127	.019	-	-
	98	-	-	-	-	.010	-	-	-	-
6Pgd	111	.029	-	-	1.00	1.00	-	-	-	-
	109	.942	.894	1.00	-	.990	-	-	.904	.971
	105	-	.106	-	-	-	-	-	-	-
	103	.029	-	-	-	-	.885	.817	.067	-
	100	-	-	-	-	-	.115	.183	-	-
	97	-	-	-	-	-	-	-	.029	.029
	109	-	-	.029	.058	-	.144	.019	.154	.087
Pgi	100	1.00	1.00	.971	.942	.990	.856	.981	.846	.904
	94	-	-	-	-	.010	-	-	-	-
	91	-	-	-	-	-	-	-	-	.010
Pgm	102	-	-	-	-	-	-	-	-	-
	101	.990	.990	1.00	1.00	.952	1.00	1.00	-	-
	100	.010	-	-	-	.048	-	-	-	-
	99	-	-	-	-	-	-	-	-	-
	98	-	-	-	-	-	-	-	-	-
	97	-	-	-	-	-	-	-	.971	1.00
	96	-	.010	-	-	-	-	-	-	-
Sdh	102	-	-	-	-	-	.019	-	-	-
	101	-	-	-	-	-	-	-	-	-
	100	-	-	-	-	-	.010	.288	-	-
	98	1.00	1.00	1.00	1.00	1.00	.962	.712	1.00	1.00
	96	1.00	1.00	1.00	1.00	1.00	.010	-	-	-
Loci heterozygous*		.167	.222	.000	.222	.056	.333	.333	.167	.111
Loci heterozygous**		.278	.278	.278	.333	.167	.444	.444	.222	.167
Ave. heterozygosity		.044	.073	.021	.077	.040	.118	.107	.036	.020

* A locus is considered polymorphic if no allele has a frequency greater than or equal to 0.99.

** A locus is considered polymorphic if no allele has a frequency greater than or equal to 0.95.

Table 1 - extended

<u>Rsp</u>	<u>Gcl</u>	<u>GdW</u>	<u>GdH</u>	<u>GdS</u>	<u>REF</u>	<u>Gat</u>	<u>Gal</u>	<u>Gcu</u>
.981	.500	.779	.413	.577	-	-	-	-
-	.442	.221	.558	.423	-	.154	.183	-
.019	.058	-	.029	-	.067	.769	.423	.538
-	-	-	-	-	-	-	-	-
-	-	-	-	-	.933	-	.240	.436
-	-	-	-	-	-	-	-	.026
-	-	-	.038	-	-	.077	.154	-
-	-	-	-	-	-	-	-	-
1.00	1.00	1.00	1.00	1.00	-	.058	-	-
-	-	-	-	-	-	-	-	1.00
-	-	-	-	-	1.00	.942	1.00	-
-	-	-	-	-	-	-	-	1.00
1.00	.510	1.00	1.00	.615	-	-	.096	-
-	.490	-	-	.385	1.00	1.00	.903	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
.923	.010	.077	-	-	-	.019	-	-
-	-	-	-	-	-	-	.010	-
.077	.990	.913	.923	.894	-	.029	.058	1.00
-	-	-	-	-	1.00	.923	.865	-
-	-	.010	.077	.106	-	.029	.067	-
.010	-	-	.010	.010	-	-	.019	-
.990	1.00	1.00	.990	.904	1.00	1.00	.981	1.00
-	-	-	-	-	-	-	-	-
-	-	-	-	.087	-	-	-	-
-	-	-	-	-	-	-	.192	-
-	1.00	.933	1.00	1.00	-	-	-	-
-	-	.029	-	-	.490	.010	.625	-
-	-	-	-	-	.298	.990	.163	1.00
-	-	-	-	-	-	-	-	-
1.00	-	.038	-	-	.212	-	-	-
-	-	-	-	-	-	-	-	-
-	-	-	-	-	.087	-	-	-
-	-	-	-	-	-	-	-	1.00
-	-	-	-	.260	.913	.760	.990	-
1.00	.625	1.00	1.00	.740	-	.240	.010	-
-	-	-	-	-	-	-	-	-
.111	.333	.222	.167	.278	.222	.333	.500	.167
.222	.333	.222	.278	.333	.222	.500	.611	.167
.037	.139	.051	.048	.099	.058	.182	.182	.078

Table 2. Genetic identities (above the diagonal) and distances (below the diagonal) for 18 samples of Goniobasis.

RR	BSP	ICH	WEK	WAC	BHS	CHP	JCR	JSP	RSP	Gc1	GdW	GdH	GdS	REF	Gat	Gal	Gcu
RR																	
BSP	.886	.784	.865	.929	.852	.815	.893	.893	.891	.809	.770	.773	.809	.296	.348	.347	.393
ICH	.121	.749	.854	.861	.810	.814	.792	.793	.816	.850	.769	.765	.804	.352	.398	.406	.441
WEK	.243	.288	.749	.818	.710	.702	.720	.721	.716	.670	.638	.627	.660	.468	.547	.420	.354
WAC	.145	.157	.290	.904	.823	.809	.751	.748	.763	.813	.760	.768	.804	.369	.415	.376	.392
BHS	.074	.150	.201	.101	.836	.788	.817	.818	.813	.822	.745	.750	.796	.380	.431	.385	.389
CHP	.160	.211	.342	.195	.179	.928	.814	.807	.810	.881	.938	.928	.929	.312	.358	.360	.422
JCR	.205	.205	.353	.212	.239	.075	.732	.727	.745	.863	.886	.888	.911	.345	.403	.403	.460
JSP	.113	.233	.329	.286	.202	.205	.312	.999	.996	.749	.747	.707	.758	.300	.327	.326	.384
RSP	.113	.233	.327	.290	.201	.214	.319	.001	.995	.743	.741	.701	.751	.299	.328	.325	.379
Gc1	.115	.204	.334	.270	.207	.210	.294	.006	.005	.761	.757	.717	.768	.322	.351	.350	.393
GdW	.212	.162	.400	.207	.196	.127	.147	.288	.297	.274	.893	.884	.932	.375	.419	.420	.563
GdH	.261	.262	.449	.274	.295	.064	.121	.292	.299	.278	.113	.991	.948	.297	.341	.346	.454
GdS	.257	.268	.468	.264	.287	.074	.119	.347	.355	.333	.123	.009	.934	.297	.344	.349	.444
REF	.212	.219	.416	.218	.229	.074	.093	.277	.286	.265	.070	.053	.934	.314	.357	.367	.512
Gat	1.22	1.04	.760	.997	.968	1.17	1.06	1.20	1.21	1.13	.981	1.21	1.16	.911	.862	.258	
Gal	1.06	.923	.603	.879	.842	1.03	.909	1.12	1.12	1.05	.870	1.08	1.07	1.03	.094	.836	.309
Gcu	1.06	.901	.867	.979	.956	1.02	.908	1.12	1.12	1.05	.867	1.06	1.05	1.00	.149	.179	.258
	.933	.818	1.04	.936	.945	.862	.777	.958	.970	.933	.575	.789	.811	.670	1.35	1.17	1.36

Table 3. Genetic identities of five samples of Goniobasis.

	<u>Gat</u>	<u>Gal</u>	<u>ICH</u>	<u>RR</u>
REF	0.911	0.862	0.468	0.296
Gat		0.836	0.547	0.348
Gal			0.420	0.347
ICH				0.784

Table 4. Genetic identities of "standard" Goniobasis floridensis samples.

	<u>BSP</u>	<u>ICH</u>	<u>WEK</u>	<u>CHP</u>	<u>JCR</u>	<u>Gcl</u>
RR	0.886	0.784	0.865	0.815	0.893	0.809
BSP		0.750	0.854	0.814	0.792	0.850
ICH			0.749	0.702	0.720	0.670
WEK				0.809	0.751	0.813
CHP					0.732	0.863
JCR						0.749

Table 5. Genetic identities of three sympatric species of Goniobasis and G. dickinsoni from Holmes Creek.

	<u>GdH</u>	<u>Gcu</u>	<u>Gat</u>
CHP	0.888	0.460	0.403
GdH		0.444	0.344
Gcu			0.309

Table 6. Genetic identities of Florida Panhandle samples of the G. floridensis complex.

	<u>BHS</u>	<u>Gcl</u>	<u>GdW</u>	<u>GdH</u>	<u>GdS</u>
CHI	0.928	0.863	0.886	0.888	0.911
BHS		0.881	0.938	0.928	0.929
Gcl			0.893	0.884	0.932
GdW				0.991	0.948
GdH					0.934

Table 7. Average genetic identities, with standard deviations, of Drosophila and Goniobasis at different levels of taxonomic divergence.

	<u>Drosophila</u> *	<u>Goniobasis</u>
Local populations	0.970 $\pm$ 0.006	0.860 $\pm$ 0.090
Subspecies	0.795 $\pm$ 0.013	0.808 $\pm$ 0.064
Semispecies	0.798 $\pm$ 0.026	0.795 $\pm$ 0.088
Sibling species	0.563 $\pm$ 0.023	0.366 $\pm$ 0.063
Non-sibling species	0.352 $\pm$ 0.023	0.404 $\pm$ 0.122

* From Ayala et al., 1974. Evolution 28:576.

Figure 1. The seven recognized species of Florida Goniobasis.
 Top row (left to right): G. floridensis (site 4),
G. vanhuyningiana (site 1), and G. athearni (site 10).
 Bottom row: Goniobasis curvicostata (site 10), G. albanensis
 (site 9), G. clenchi (site 15), and G. dickinsoni (site 13).
 The scale is numbered in centimeters.

Figure 2. Localities where Goniobasis were sampled for electrophoretic analysis. The numbers correspond to those given in the text with the locality descriptions.

Figure 3. Goniobasis from the Ichetucknee River (site number 7). The two on the left are members of the reference population. The two on the right are G. floridensis.

Figure 4. Top row: Goniobasis sp. from the Ichetucknee River reference population (site 7).
Bottom row: The first two are G. athearni from the Chipola River (site 10), and the last two are G. albanyensis from the Apalachicola River (site 9).

Figure 5. Study area in the mid-St. Johns River basin. Closed circles indicate where samples were taken for the electrophoretic analysis.

Figure 6. The two on the left are Goniobasis from Juniper Spring (site 2), and the last two are G. vanhyningiana from Rock Spring (site 1).

Figure 7. The two on the left are G. floridensis from Juniper Creek (site 3), and the last two are Goniobasis from Juniper Spring (site 2).

Figure 8. Goniobasis from Juniper Creek, about midway between sites
2 and 3.

Figure 9. Top row: Goniobasis floridensis from the Waccasassa River
(site 6).
Bottom row: Goniobasis floridensis from the Wekiva River
(site 5).

Figure 10. Study area of Goniobasis floridensis in Levy Co., Florida. Closed circles indicate where samples were taken for electrophoretic analysis. The star indicates where extensive hybridization is found between different shell sculpture patterns.

Figure 11. Goniobasis floridensis from Wekiva River at US 19 and 98.

Figure 12. Goniobasis with the "standard" sculpture pattern. The two on the left are G. floridensis from Rainbow River (site 4), the next two are G. floridensis from Blue Spring (site 8), and the last two are G. clenchi from the Choctawhatchee River (site 15).

Figure 13. Study area for *G. floridensis*, *G. dickinsoni*, and *G. clenchi* in the Florida Panhandle. Closed circles indicate where samples were taken for electrophoretic analysis.

Figure 14. The two on the left are G. dickinsoni from Wrights Creek (site 14), and the remaining two are G. clenchi from the nearby Choctawhatchee River (site 15).

Figure 15. Top row: The first two are G. dickinsoni from Holmes Creek (site 13), the second two are G. dickinsoni from Spring Creek (site 12), and the last two are G. floridensis from the Chipola River (site 10).
Bottom row: Goniobasis from Blue Hole Springs, Florida Caverns State Park (site 11).

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BIOGRAPHICAL SKETCH

Steven Mark Chambers was born in Charles City, Iowa, on 22 September 1948. He attended public schools while growing up in La Mirada, California. After two years of study at the College of Agriculture, University of California, Davis, he transferred to the University of California, Riverside, where he received the degrees of B.A. in Biology in 1970 and M.A. in Biology in 1972. The next year was spent on research on diapause and evaluation of field releases in a biological control effort of the pink bollworm for the Division of Biological Control, Department of Entomology, University of California, Riverside. In 1973 he came to the University of Florida as a graduate student and began work on the evolutionary genetics of natural populations. He is married to Kristine Lofthus Chambers. He does not eat grits.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Thomas C. Emmel, Chairman
Professor and Chairman of Zoology

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Associate Professor of Zoology

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