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A PHYLOGENETIC PERSPECTIVE ON THE EVOLUTION OF SIMULTANEOUS HERMAPHRODITISM IN A FRESHWATER MUSSEL CLADE (BIVALVIA: UNIONIDAE: *UTTERBACKIA*)

Walter R. Hoeh, Kenneth S. Frazer, Edna Naranjo-García and Richard J. Trdan

ABSTRACT

The variability in reproductive mode (gonochorism vs. simultaneous hermaphroditism [SH]) in the freshwater mussel genus *Utterbackia* offers a significant opportunity for an increased understanding of the ecological, genetic, historical, and morphological correlates involved in the origin and maintenance of SH. Furthermore, since portions of the ranges of the species within *Utterbackia* lie in the panhandle and peninsula of Florida, an opportunity exists to evaluate historical biogeographic hypotheses for this region. Specimens of *Utterbackia* (577 individuals, 52 populations, representing the simultaneous hermaphroditic *U. imbecillis* and the gonochoric *U. peggyae*) were scored for allozyme variation at 25 presumptive genetic loci. The results indicate that *Utterbackia* comprises four distinct species: *U. imbecillis, U. "imbecillis"* (southern Atlantic Slope drainages), *U. peggyae* (panhandle of Florida drainages), and *U. "peggyae"* (northwestern peninsula of Florida drainages). Phylogenetic analyses of the allozyme data set produced the following topology: ((*U. imbecillis sensu lato, U. "peggyae"*) *U. peggyae*). The monophyly and uniform SH of *U. imbecillis s.l.* is consistent with a hypothesis of a single transition from gonochorism to SH in the ancestor of *U. imbecillis s.l.* Based on an analysis of homoplasy distribution and a historical reconstruction of *Utterbackia* evolution, it is inferred that 1) two vicariance events (mid-Miocene and early Pliocene) in the southeastern USA may be causally related to the phylogenetic hypothesis for *Utterbackia* and 2) the evolution of SH in the *U. imbecillis s.l.* lineage may have been potentiated by introgressive hybridization.

Key words: phylogenetics, simultaneous hermaphroditism, gonochorism, allozymes, homoplasy, vicariance, introgression, hybridization, Unionidae, *Utterbackia*.

INTRODUCTION

Reproductively diverse taxa have provided biologists with fertile grounds for testing mechanisms of evolutionary change (*e.g.*, see Ghiselin, 1969, 1974; Williams, 1975, 1988; Maynard Smith, 1978, 1988; Bell, 1982; Charnov, 1982; Bull, 1983; Raven, 1988). Among animals, the Mollusca (especially gastropod and bivalve mollusks) offer much in the way of comparative material for studies of reproductive system evolution. Scattered about in this predominantly gonochoric (= dioecious) phylum are cases of sequential and simultaneous hermaphroditism (SH) and even reports of parthenogenesis (*e.g.*, see Tompa *et al.*, 1984). North American freshwater mussels (Bivalvia: Unionidae) are a particularly useful study group for addressing the evolution of SH from gonochorism for a number of reasons:

1) From an array of approximately 300 species of unionids north of Mexico (Burch, 1975; Turgeon *et al.*, 1988), SH is known in only seven species (*Lasmigona compressa* [Lea 1829], *L. subviridis* [Conrad 1835], *Margaritifera falcata* [Gould 1850], *Toxolasma parvum* [Barnes 1823], *T. pullum* [Conrad 1838], *Uniomerus tetralasmus* [Say 1831], and *Utterbackia imbecillis* [Say 1829]; Sterki, 1898a, 1898b; Heard, 1970; van der Schalie, 1970; Kat, 1983a). Its rarity together with outgroup comparisons suggest that SH is derived with respect to gonochorism.

2) SH occurs in each of the three unionid subfamilies (*sequens* Davis & Fuller, 1981; Davis, 1984) suggesting multiple origins of the trait.

3) SH is not the predominant reproductive mode of any higher taxon within the Unionidae. This finding is consistent with a hypothesis of relatively recent transitions to SH.

4) The morphological simplicity of the unionid reproductive tract (*e.g.*, see Mackie, 1984) and the lack of sex-specific modifications of the gonad, or sexual accessory structures, may allow simultaneous hermaphroditic individuals to arise from a gonochoric population without the

fixed-cost penalty of diverting energy to dual-sexual specializations prior to gaining a fitness return, should SH be favored by selection (Heath, 1977). Without sex-specific specializations, the reproductive system should more easily track the selective regime presented by the environment (Charnov, 1982).

5) The fixation of the brooding habit within the Unionidae (Lefevre & Curtis, 1912; Coker *et al.*, 1921) "controls" for the possible correlation between SH and the brooding habit predicted by theory (Heath, 1979), and empirically observed in diverse animal groups (Strathmann & Strathmann, 1982; Strathmann *et al.*, 1984). That is, something besides lack of brooding must be invoked to explain why SH is not more widespread within the Unionidae.

Given the above, studies of unionid clades containing both gonochoric and simultaneous hermaphroditic species offer significant opportunities for an increased understanding of the ecological, genetic, historical, and morphological correlates involved in the origin and maintenance of SH. However, the generation of a phylogenetic hypothesis for the taxa in question is fundamental to any explication of reproductive system evolution. A hypothesis of relationships will allow 1) an estimate of the number of times SH arose within a higher taxon and, combined with knowledge of the geographic distributions of the constituent species, 2) a historical reconstruction of the events in time and space that may have contributed to the reproductive system transition.

The freshwater mussel genus *Utterbackia* F. C. Baker 1927 (Unionidae: Anodontini, see Hoeh, 1990) contains two recognized species, *U. imbecillis* and *U. peggyae* (Johnson 1965). The geographic distribution of *U. imbecillis* includes much of the eastern half of the U.S.A. while *U. peggyae* is restricted to panhandle and peninsular Florida Gulf coast drainages (Fig. 1). Recent comparative analyses on *Utterbackia* have been limited to a relatively small number of works (*e.g.*, see Johnson, 1965, 1970, 1972; Heard, 1975; Kat, 1983a, 1983b; Hoeh, 1990). A significant difference exists between *U. imbecillis* and *U. peggyae* with respect to reproductive mode. Like the great majority of freshwater mussels, *U. peggyae* is predominantly gonochoric while *U. imbecillis* is a simultaneous hermaphrodite (*i.e.*, produces mature oocytes and spermatozoa concurrently; *e.g.*, see Sterki, 1898a, 1898b; Ortmann, 1910, 1911; Allen, 1924; Baker, 1927, 1928; van der Schalie, 1970). Histological analyses on individuals from geographically widespread populations suggest that *U. imbecillis* is uniformly hermaphroditic (Hoeh, unpublished data; but see Heard, 1975; Kat, 1983a). The presence of low testicular to ovarian tissue volume ratios and extreme heterozygote deficiencies is consistent with a hypothesis of self-fertilization for some populations of *U. imbecillis* (Kat, 1983a; Hoeh *et al.*, 1986). Self-fertilization, which is correlated with enhanced dispersal capacity (*e.g.*, see McMahon, 1991), may, in part, be responsible for the broader geographic distribution of *U. imbecillis* with respect to *U. peggyae*. Alternatively, since unionid bivalve larvae are obligate parasites on freshwater fishes (*e.g.*, see Fuller, 1974), a greater number and/or vagility of the fish hosts for *U. imbecillis* may enhance its dispersal capacity with respect to that of *U. peggyae*.

Given that gonochorism represents the ancestral condition for *Utterbackia* (*e.g.*,*Pyganodon* Crosse & Fischer 1893, the sister taxon of *Utterbackia* [see Hoeh, 1990], contains only gonochoric species) and that relatively low levels of genetic and morphological divergence exist between *U. imbecillis* and *U. peggyae* (Hoeh, 1990; Kat, 1983b), it appears that the transition from gonochorism to SH in *Utterbackia* occurred relatively recently. This recency of origin for SH offers an opportunity to investigate possibly still-operative factors involved in the transition (*e.g.*, see Williams, 1975). Basic to this end is the generation of a population-level phylogenetic hypothesis that allows for the discrimination among evolutionary scenarios (Fink, 1982). For example, are *U. imbecillis* and *U. peggyae* each monophyletic taxa? If the transition from gonochorism to simultaneous hermaphroditism is selectively advantageous, given particular environmental conditions and the presence of genetic variation for reproductive mode, SH may have evolved multiple times within *Utterbackia*.

In addition to its explicatory relevance regarding the question of reproductive system transitions, a phylogenetic hypothesis for the genus *Utterbackia* will be useful in a historical biogeographic sense. There is some consensus among biologists and historical geographers that parts

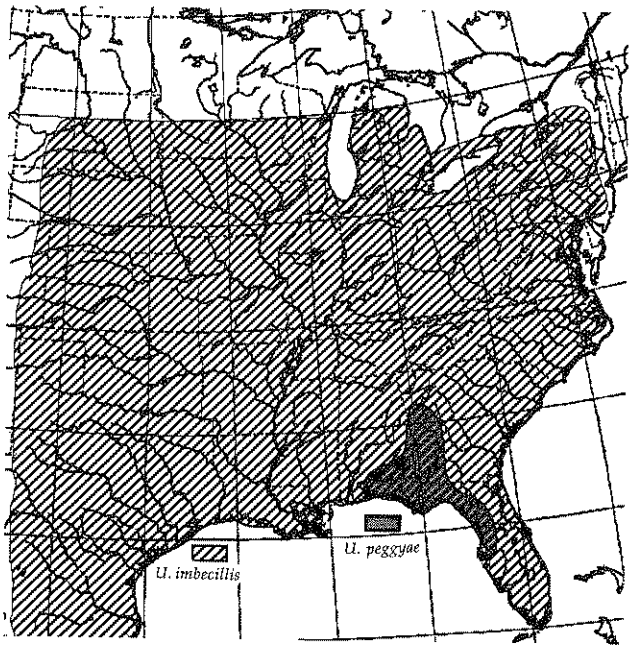

FIG. 1. The general geographic ranges of *Utterbackia imbecillis* (cross-hatching) and *U. peggyae* (gray stippling) in the USA. (Northern-most extension of *U. peggyae* range based on recent collections by J. Brim Box and J. D. Williams, unpublished data).

of the Florida peninsula have been isolated from the southeastern USA mainland multiple times during the Cenozoic; these vicariance events may have played a major role in the diversification of the biota of the southeastern US (*e.g.*, see Dall, 1903; Hubbell, 1932; Carr, 1940; Cooke, 1945; Olson *et al.*, 1954; Clench & Turner, 1956; Neill, 1957; Johnson, 1970, 1972; Burgess & Franz, 1978; Riggs, 1984; Bert, 1986; Bermingham & Avise, 1986; Swift *et al.*, 1986; Gilbert, 1987; Dillon & Popenoe, 1988; Moler & Kezer, 1993). Inferences from freshwater mollusk distributional patterns have been used to both support and discount the hypothesis that an isolated Florida peninsula has had a significant effect on the freshwater molluscan fauna of the area (*e.g.*, see Dall, 1903; Clench & Turner, 1956; Thompson, 1968; Johnson, 1972, 1973). Given the distribution of *U. imbecillis* and *U. peggyae* in both the Florida panhandle and peninsula, a population-level estimate of phylogeny for *Utterbackia* will enable, at least with respect to freshwater mussels, an evaluation of this hypothesis.

In this paper, a phylogenetic analysis using allozyme data will 1) test the hypothesized monophyly of *Utterbackia* (Hoeh, 1990), 2) estimate the relationships among multiple populations of *U. imbecillis* and *U. peggyae* in order to test the hypothesis of monophyly for each species, 3) enable an estimate of the minimum number of gonochorism-to-simultaneous hermaphroditism transitions that occurred within the *Utterbackia* lineage, and, together with species distributional data, 4) enable a historical reconstruction of the evolution, in space and time, of the *Utterbackia* lineage that is consistent with geological, genetic, and zoogeographic data sets. Generating an estimate of the phylogenetic relationships among multiple populations of *U. imbecillis* and *U. peggyae* is a necessary first step toward gaining an increased understanding of the events that led to the origin of simultaneous hermaphroditism in *Utterbackia*.

MATERIALS AND METHODS

In an attempt to secure adequate sampling of the genetic variability within *Utterbackia*, 37 populations (399 individuals) of *U. imbecillis* and 15 populations (178 individuals) of *U. peggyae* were collected (Appendix I). Four species of *Pyganodon*, namely *P. cataracta* (Say 1817) *P. fragilis* (Lamarck

1819), *P. lacustris* (Lea 1857) and *P. grandis* (Say 1829) were used as the outgroup. Soon after specimen collection, gill tissues were excised and cleaned of macroscopic parasites and debris, frozen in liquid nitrogen, and subsequently stored at -70°C. Gill tissues were homogenized with a glass pestle in conical-bottomed 1.5 ml microcentrifuge tubes. The gill tissues contained sufficient water to eliminate the need for homogenization buffer. The resultant homogenate was centrifuged at 13,605 x g for 10 minutes at 4°C.

Horizontal starch gel electrophoresis (12% starch gels; 51 g Connaught starch in 425 ml of gel buffer) was used to detect electromorphs at 25 putative genetic loci using five buffer systems (Appendix II). Stain recipes followed Shaw & Prasad (1970), Siciliano & Shaw (1976), Wurzinger (1980), and Murphy *et al.* (1990). Electromorphs were designated with lower case letters (the least mobile electromorph at a locus was designated "a").

For each population (= terminal taxon), the allelic arrays at each locus (see Appendix III) were coded as multi-state characters (locus as the character with qualitative coding, *e.g.*, see Buth, 1984). The resulting matrix (Table 1) was analyzed using PAUP 3.0r (Swofford, 1991). This particular character coding and analytical scheme is desirable since it allows for the explicit assignment of evidential support to each cladogram node (*e.g.*, see Murphy *et al.*, 1990). The characters were run unordered with equal weighting. The PAUP tree search parameters were the following: ROOT=OUTGROUP, SEARCH=HEURISTIC, MAX TREE=10,000, BRANCH SWAPPING=TBR. Random addition sequence runs were performed as an aid in finding minimal length trees (*e.g.*, see Swofford & Olsen, 1990; Maddison, 1991; Templeton, 1991; Swofford & Begle, 1993). Equally parsimonious trees were combined into a strict consensus tree (Sokal & Rohlf, 1981) using the algorithm contained in PAUP. In order to generate estimates of allozymic divergence, Nei's (1978) genetic distance estimates were calculated using BIOSYS-1 (Swofford & Selander, 1981).

RESULTS

Ninety-five electromorphs were detected at 25 presumptive loci from 56 populations (see Table 1, Appendix III). Allelic-compositional identity was observed among a number of the 37 *Utterbackia imbecillis* populations. This phenomenon resulted in 16 non-unique populations. All genetically identical populations were combined into a composite terminal taxon. The six composite terminal taxa are designated by bold-faced type in the data matrix (Table 1) and by quotation marks in the cladograms to follow. Therefore, the phylogenetic analysis was conducted on 40 terminal taxa.

The strict consensus tree (Fig. 2; from 10,000 equally parsimonious trees of 107 steps, each with a consistency index of 0.823, retention index of 0.874, and rescaled consistency index of 0.735) corroborates the hypothesized monophyly of *Utterbackia* (Hoeh, 1990) and suggests that 1) *U. imbecillis* is monophyletic, 2) *U. peggyae* is a paraphyletic assemblage, 3) both *U. peggyae* and *U. imbecillis* comprise at least two species, and 4) *U. imbecillis* is most closely related to the peninsular populations of *U. peggyae*.

Utterbackia peggyae is made up of two distinct clades. One clade is restricted to the drainages of the panhandle of Florida area from the Escambia River to the Ochlockonee River (Fig. 3; hereupon, this clade is referred to as *U. peggyae* since the type locality for *U. peggyae* is in the Ochlockonee River) while the other is restricted to the drainages of northwestern peninsular Florida from the Suwannee River to the Hillsborough River (hereupon, this clade is referred to as *Utterbackia "peggyae"*). Each of these two clades was supported as a monophyletic assemblage in each of the 10,000 equally parsimonious trees.

Analogous panhandle/peninsula partitioning for *Utterbackia imbecillis* was not observed in the strict consensus tree (Fig. 2). However, four *U. imbecillis* populations, CRHi, FPCi, ORTi, and SRGi, displayed geographic contiguity as well as phylogenetic cohesion (*cf.* Figs. 2 & 3). These populations were collected from the Combahee, Peedee, Altamaha, and Santee River drainages, respectively, of the southern Atlantic Slope region. This group of four populations (represented by three terminal taxa in the phylogenetic analysis) was supported as a monophyletic assemblage in each of the 10,000 equally parsimonious trees. Hereupon, this clade of four populations will be referred to as *U. "imbecillis"*. The *U. imbecillis/U. "imbecillis"* clade will be, herein, referred to as *U. imbecillis sensu lato*. Phenetic analyses (not shown) suggest that the

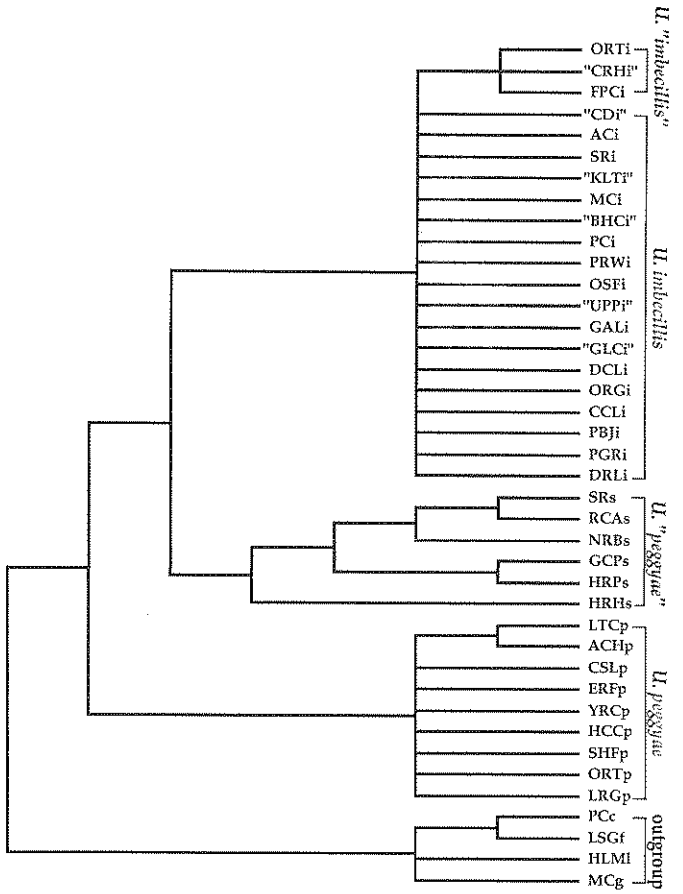

FIG. 2. Strict consensus tree for *Utterbackia* produced from 10,000 equally parsimonious trees of 107 steps, each with C.I. = 0.823, R.I. = 0.874, and R.C. = 0.735. Four species of *Pyganodon* form the outgroup. Terminal taxa with quotation marks are composites of multiple, genetically identical populations (see Table 1 and Appendix I for the specific populations contained within these composite terminal taxa).

relatively differentiated *U. "imbecillis"* clade may be the result of an early cladogenic event within *U. imbecillis s.l.*

Based on 25 loci, the average estimate of among-population divergence (Nei's [1978] D) within *Utterbackia imbecillis s.l.* was 0.089 with a range of 0.000 to 0.274. Those estimates within *U. peggyae* and *U. "peggyae"* were 0.075 (range: 0.000 to 0.152) and 0.098 (range: 0.000 to 0.190), respectively. The average estimate of divergence between *U. imbecillis s.l.* and *U. peggyae* populations was 0.289 (range: 0.173 to 0.483) while that between *U. imbecillis s.l.* and *U. "peggyae"* populations was 0.381 (range: 0.224 to 0.570). The average estimate of divergence between *U. peggyae* and *U. "peggyae"* populations was 0.354 (range: 0.301 to 0.445).

In addition to the overall level of genetic divergence, the fact that *Utterbackia "peggyae"* is diagnosed by five alleles at four loci (AAT-b, ACOH1-b, FUMH-d, GPI-b, GPI-e) further accentuates the distinction between it and the *U. peggyae* lineage. A preliminary estimate of mitochondrial DNA (mtDNA) sequence divergence between *U. peggyae* and *U. "peggyae"* is 12% (Hoeh, unpublished data). This estimate was based on restriction endonuclease fragment comparisons using 10, six-base-recognizing restriction endonucleases. Although these two clades are conchologically very similar, the molecular data are consistent with species-level distinction.

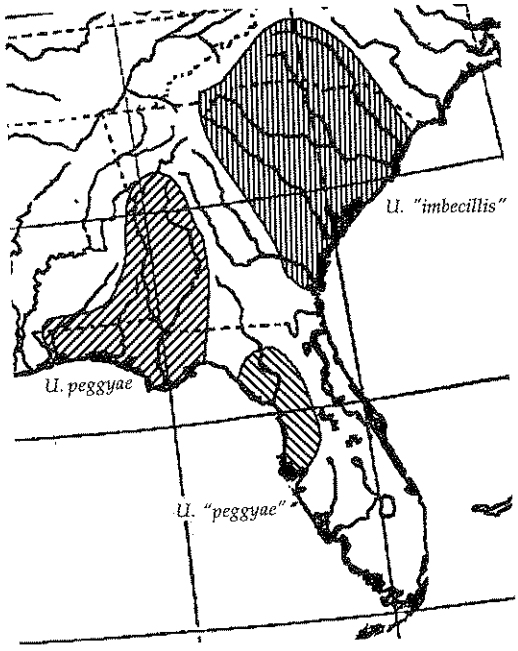

FIG. 3. Geographic ranges of *Utterbackia peggyae* and *U. "peggyae"* with an estimate of the range of *U. "imbecillis"*. *U. imbecillis* is sympatric with *U. peggyae* and *U. "peggyae"*.

The *Utterbackia "imbecillis"* clade is diagnosed by four electromorphs at three loci (AO-d, AO-e, CAT1-b, GAPDH-c). The average pair-wise Nei D (1978) between these four populations and the remaining 33 *U. imbecillis* populations was 0.183 while the average pair-wise Nei D among these four populations was 0.016. The Altamaha River drainage was sampled at two sites during the course of this study: the Ocmulgee River (BHCi) and the Ohoopee River (ORTi). The Ocmulgee River site contained *U. imbecillis* while the Ohoopee River site represented one of the four *U. "imbecillis"* populations. No evidence for gene flow between these two Altamaha River drainage populations was noted (*i.e.*, no heterozygotes at diagnostic loci). This observation, combined with the level of genetic differentiation between *U. "imbecillis"* and *U. imbecillis*, and the geographic contiguity of the four populations of the former, is consistent with species-level status for this southern Atlantic Slope clade. The *U. "peggyae"* and *U. "imbecillis"* clades will be formally recognized as distinct species in subsequent papers (Bogan & Hoeh, submitted). Voucher specimens, representing *U. peggyae*, *U. "peggyae"*, *U. imbecillis*, and *U. "imbecillis"*, have been deposited in the Museum of Zoology, University of Michigan (Mollusk Division #s 253578, 253579, 253580, and 253581, respectively).

As depicted by the strict consensus tree (Fig. 2), the degree of intraspecific phylogenetic resolution observed within *Utterbackia* varied greatly. The populations representing *U. "peggyae"* were fully resolved while those representing *U. imbecillis s.l.* and *U. peggyae* were much less resolved. The intraspecific resolution depicted in a majority-rule consensus tree (not shown) is largely concordant with geography for both *U. peggyae* and *U. "peggyae"*. This is not generally the case for *U. imbecillis*. On the strict consensus tree, only one clade (representing *U. "imbecillis"*) of three terminal taxa was resolved out of the 21 terminal taxa of *U. imbecillis s.l.* entered into the analysis.

In order to evaluate the robustness of the ((*Utterbackia imbecillis s.l.*, *U. "peggyae"*) *U. peggyae*) topology, the constraint function of PAUP was used to specify, *a priori*, the two other topological outcomes for a three taxon statement. Both the ((*U. imbecillis s.l.*, *U. peggyae*) *U. "peggyae"*) and ((*U. peggyae*, *U. "peggyae"*) *U. imbecillis s.l.*) topologies required a single additional step (tree length=108) above that of the most parsimonious tree (tree length=107).

DISCUSSION

Phylogenetic Considerations

The strict consensus tree topology (Fig. 2) obtained from 10,000 equally parsimonious trees of 107 steps is, herein, regarded as the best supported hypothesis of the relationships within *Utterbackia* until further evidence is brought to bear on the question. Comparative anatomical and mitochondrial DNA studies are in progress. Preliminary analyses of both mtDNA restriction fragments and cytochrome c oxidase I sequences support the ((*U. imbecillis s.l.*, *U. "peggyae"*) *U. peggyae*) topology. Notwithstanding, the observed topological instability (from the topological constraint analysis; above) is quite unexpected given the level of allozymic divergence among these taxa. Levels of interspecific divergence between ingroup terminal taxa range from a Nei's D (1978) of 0.173 to 0.570 while interspecific comparisons between ingroup and outgroup terminal taxa have a maximum Nei's D (1978) of 0.919. Based on theoretical and empirical considerations (Nei, 1987; Murphy *et al.*, 1990), these levels of divergence should be within the range of variability where allozymic analyses are phylogenetically robust.

A high level of character conflict (homoplasy) in the data set may be responsible for a relatively unstable branching topology. The overall level of homoplasy (for a most parsimonious tree of 107 steps, Homoplasy Index=0.177) is relatively low given the number of terminal taxa in the analysis (*e.g.*, see Sanderson & Donoghue, 1989). However, *Utterbackia* seems to have a very high level of homoplasy at certain loci. To illustrate this phenomenon, ACCTRAN character optimization (which should minimize parallel gains in the terminal branches; see Swofford & Maddison, 1987) was carried out on one of the most parsimonious trees (Fig. 4, tree length of 107 steps; the general results of this optimization procedure are not dependent on choice of shortest tree). Under this particular optimization, the PGM-f and AAT-a electromorphs are hypothesized to have "evolved" four and two times, respectively, within *U. imbecillis s.l.* (note that PGM-f "evolved" once within *U. peggyae* and is not present in *U. "peggyae"*; AAT-a is fixed in *U. peggyae* and is not present in *U. "peggyae"*). Both of these electromorphs are geographically widespread in *U. imbecillis*.

There are two other loci at which *Utterbackia imbecillis s.l.* and *U. peggyae* share apomorphic electromorphs not found in *U. "peggyae"*: AO and DDH (Fig. 4). AO-d was detected in three populations of *U. "imbecillis"* that are geographically adjacent in three Atlantic Slope drainages in Georgia and South Carolina. In *U. peggyae*, AO-d was found in two populations; one from the Escambia River (CSL) and the other from the nearby Yellow River (YRC). It is interesting to note that PGM-f, which is widely distributed in *U. imbecillis*, was also detected in two populations of *U. peggyae* (CSL and ERF), both of which are located in the Escambia River drainage. DDH-c was detected in one population of *U. imbecillis* (PGR) from the Pascagoula River of Mississippi and three populations of *U. peggyae* (ACH, LTC, and ORT) from the Ochlockonee River drainage of Florida and Georgia.

The sharing of electromorphs between these non-sister taxa (assuming that [[*Utterbackia imbecillis s.l.*, *U. "peggyae"*] *U. peggyae*] is the correct topology), *i.e.*, between *U. imbecillis s.l.* and *U. peggyae*, may be due to one (or more) of five different causes: 1) the alleles are distinct at the DNA level but the proteins migrate in an identical fashion during starch gel electrophoresis (*e.g.*, see Singh *et al.*, 1976), 2) the alleles evolved convergently in the *U. imbecillis s.l.* and *U. peggyae* lineages, 3) stochastic sorting of ancestral polymorphism (*e.g.*, see Tajima, 1983; Neigel & Avise, 1986; Nei, 1987), 4) relatively recent introgression between various populations of *U. imbecillis s.l.* and *U. peggyae*, or 5) *U. imbecillis s.l.* (and / or *U. peggyae*) is a taxon with a relatively ancient reticulation event in its history. This may have involved either a) a hybridization event between individuals from the lineages of *U. peggyae* and *U. "peggyae"* producing *U. imbecillis s.l.* (reticulate speciation) or b) a *U. imbecillis s.l.* origin via cladogenesis with subsequent hybridization with *U. peggyae*. These possibilities will be discussed in turn.

The likelihood that the PGM-f, AAT-a, AO-d, and DDH-c electromorphs are gel convergent or evolved independently a hypothesized minimum of eight times in *Utterbackia imbecillis s.l.* (four, two, one, and one times, respectively), four times in *U. peggyae*, and zero times in *U.*

"peggyae" seems relatively low but cannot be excluded from consideration.

Since the AAT-a electromorph is found in the outgroup, its distribution in *Utterbackia* (present in *U. imbecillis* and *U. peggyae* but not in *U. "peggyae"*) could possibly be explained by the retention of the ancestral allele in *U. imbecillis* and *U. peggyae* together with its loss in the ancestor of *U. "peggyae"*. However, this is not the most parsimonious hypothesis according to ACCTRAN character optimization on a minimum length tree (Fig. 4). Even assuming that AAT-a is a plesiomorphic retention in *U. imbecillis* and *U. peggyae*, that still leaves at least ten independent origins, within *U. imbecillis s.l.* and *U. peggyae*, for the apomorphic electromorphs PGM-f, AO-d, and DDH-c to be explained.

If relatively recent introgression between *Utterbackia imbecillis s.l.* and *U. peggyae* is invoked to explain the distribution of these homoplastic electromorphs, then one might predict that within *U. imbecillis s.l.* the electromorphs would be restricted to areas of current sympatry with *U. peggyae*. This is not the case. For example, within *U. imbecillis* PGM-f is found in widely separated populations (*e.g.*, populations from Arkansas, Florida, Illinois, Indiana, North Carolina, Tennessee, and Texas) many of which are relatively distant from populations of *U. peggyae*.

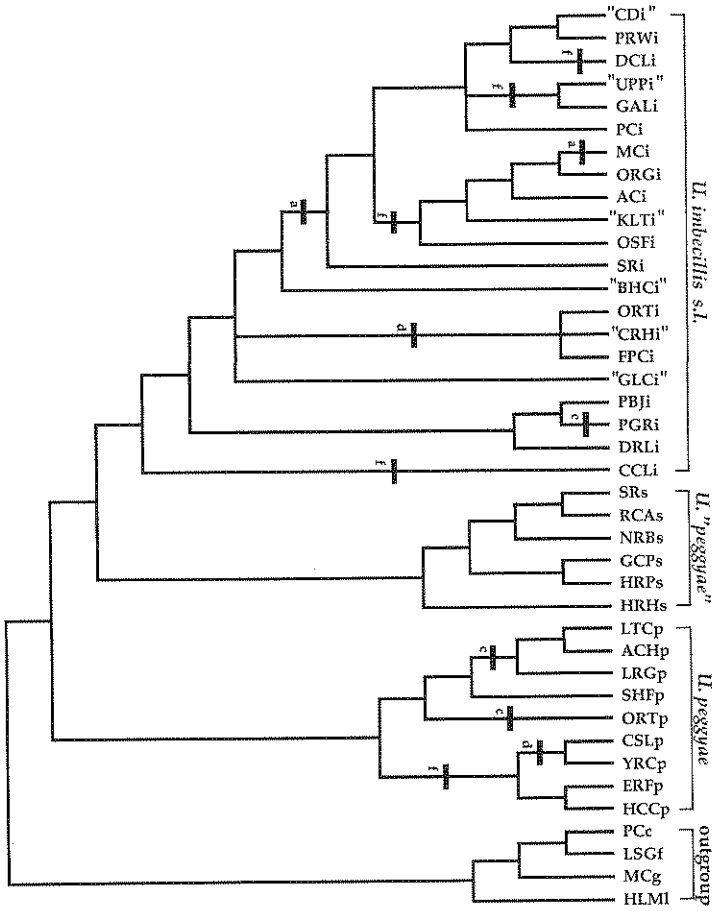

FIG. 4. ACCTRAN character optimization of hypothesized gains of the AAT-a, AO-d, DDH-c, and PGM-f electromorphs on a most parsimonious tree of 107 steps. Four species of *Pyganodon* form the outgroup. Terminal taxa with quotation marks are composites of multiple, genetically identical populations (see Table 1 and Appendix I for the specific populations contained within these composite terminal taxa).

The same type of argument could be made for the other homoplastic electromorphs. Furthermore, at four localities where *U. imbecillis* and *U. peggyae* exist syntopically (ACH, LRG, LTC, ORT10), there is no allozymic evidence of ongoing hybridization.

If *Utterbackia imbecillis s.l.* is a taxon with a relatively ancient reticulation event in its past, this could account for the seemingly anomalous distribution of the PGM-f, AAT-a, AO-d, and DDH-c electromorphs found in both *U. imbecillis s.l.* and *U. peggyae* but not in *U. "peggyae"*. However, the reticulate speciation hypothesis has two major problems: 1) out of a total of 38 electromorphs, *U. imbecillis s.l.* has seven that are unique, and 2) at loci which have very different electromorphic arrays between *U. peggyae* and *U. "peggyae"*, *U. imbecillis s.l.* does not generally demonstrate the allelic additivity expected of a hybrid (*e.g.*, see McDade, 1990, 1992). The hypothesis of a relatively ancient *U. imbecillis s.l.* hybridization with *U. peggyae* is more plausible. Under this hypothesis, the anomalous distribution of electromorphs is attributed to an ancient introgression event between *U. imbecillis s.l.* and *U. peggyae* with subsequent loss of some of the introgressed electromorphs in certain *U. imbecillis s.l.* (and *U. peggyae*?) populations.

Biogeographic Considerations

Utterbackia imbecillis s.l.

Utterbackia imbecillis is one of the most broadly distributed North American freshwater mussels (*e.g.*, see Burch, 1975). However, previous to the present study, reports of this species from the peninsular Florida region were infrequent (*e.g.*, see Johnson, 1970, 1972; Heard, 1979; Kat, 1983a, 1983b). Johnson (1970, 1972), based on an extensive 1962 survey, concluded that *U. imbecillis* (as *Anodonta imbecilis* [sic]) was absent from peninsular Florida. The present study reported on 13 Floridian populations of *U. imbecillis*, eight of which were from the peninsular region. These peninsular localities were distributed the full length of the peninsula from the Suwannee River to the C100A canal in Miami, FL. The size of each of the eight collections ranged from one to 34 individuals per site with an average of 17.25 individuals per site. These numbers, based on non-exhaustive sampling, suggest that *U. imbecillis* is very broadly distributed in peninsular Florida and that these populations are relatively large in size. This finding is in stark contrast to that reported by Johnson (1970, 1972). At least two hypotheses can account for this discrepancy: 1) *U. imbecillis* was overlooked during the 1962 Johnson survey or 2) this species has successfully colonized many of the freshwater drainages of the Florida peninsula within the past 30 years. This latter hypothesis has some support from both theoretical and empirical perspectives. If the simultaneous hermaphroditic *U. imbecillis* is capable of self-fertilization, this breeding strategy would likely enhance its colonizing ability (*e.g.*, see McMahon, 1991). Furthermore, *U. imbecillis*' colonization of recently glaciated terrain north of the Ohio River (Fig. 1) and the suggestions of historical range expansions (*e.g.*, see Kat, 1983a) are consistent with a hypothesis of rapid colonization of the Florida peninsula. The general lack of concordance between geography and the "clades" within *U. imbecillis*, in a majority-rule consensus tree (not shown), is consonant with the hypothesis that this species is very mobile (*e.g.*, see Thorpe, 1984).

The southern Atlantic Slope has a high degree of endemicity in its freshwater fauna. This is exemplified by the ichthyofauna (*e.g.*, see Lee *et al.*, 1980; Shute *et al.*, 1981; Wiley & Mayden, 1985; Hocutt *et al.*, 1986; Swift *et al.*, 1986; Wood & Mayden, 1992) as well as the freshwater mussel fauna (*e.g.*, see Johnson, 1970; Kat, 1983c; Clarke, 1985). It seems reasonable to assume that the processes that played a role in the general diversification of this highly endemic freshwater fauna may also be responsible for the origin of *Utterbackia "imbecillis"*. It is interesting to note that the location of the distributional boundary between *U. imbecillis* and *U. "imbecillis"* in Georgia is very similar to that between *Gambusia holbrooki* Type I and *G. holbrooki* Type II (Wooten *et al.*, 1988). Since *G. affinis* has been reported as a host for *U. imbecillis* larvae (Stern & Felder, 1978), the similarity of location for the distributional boundaries in *Utterbackia* and *Gambusia* is suggestive of host/parasite co-differentiation.

Further sampling will be necessary to determine the exact geographic range of *Utterbackia "imbecillis"*. For example, its presence in the Altamaha, Combahee, Santee, and Peedee River drainages suggests that it may also inhabit the geographically interposed Ogeechee, Savannah, and Edisto River drainages. Furthermore, the proposed stream capture of the upper Waccamaw/Peedee rivers by the Cape Fear River (*e.g.*, see Shute *et al.*, 1981) should prompt the sampling of the latter drainage. Additional investigation is also warranted to elucidate the causal factors underlying the unusual, with respect to *U. imbecillis*, geographic localization of this clade. Specialization of host use to one or more of the ichthyofauna endemics of that region or lack of ability to self-fertilize may be involved.

The Peninsular Florida Refugium Hypothesis

The geographic placement of the range discontinuity between *Utterbackia peggyae* and *U. "peggyae"* is not unprecedented. The eastern-panhandle-of-Florida region constitutes the major freshwater ichthyofaunal species distributional boundary along the Gulf coastal plain (Swift *et al.*, 1986). Similarly-placed intraspecific genetic discontinuities in mtDNA have been detected in four species of freshwater fish from the region (Bermingham & Avise, 1986). Bermingham and Avise (1986) hypothesized that a marine transgression during the late Cenozoic inundated smaller Coastal Plain rivers and created freshwater refugia in the headwaters of the major southeastern drainages (*e.g.*, Alabama/Tombigbee, Apalachicola and Savannah Rivers) and/or in the Florida peninsula. Subsequent to marine regression, dispersal from these refugia produced the present-day distribution of mtDNA genotypes. From their phylogenetic analyses, Bermingham and Avise (1986) suggested that each species comprised two, relatively differentiated, clades of mtDNA genotypes that were geographically localized, *i.e.*, distinct western and eastern clades were observed in each species. However, the geographic placement of the discontinuity between western and eastern mtDNA genotypes varied considerably. Differential dispersal rates and/or multiple refugia were offered as explanations for this lack of superposition.

Additional inference as to the location of the refugium for the eastern clades comes from the topologies obtained in the parsimony analyses of Bermingham and Avise (1986). For each of the four fish species examined, the basal-most lineage in the eastern clade contained at least one mtDNA genotype from peninsular Florida. This finding is consistent with a peninsular Florida refugium for the eastern clades (*sequens* Maddison, 1991; Maddison *et al.*, 1992). This interpretation suggests that, subsequent to saltwater regression, dispersal of the four fish species out of the Floridian refugium proceeded northward and, for two species, westward as well. The distributional and genetic discontinuities seen in both freshwater fishes (Swift *et al.*, 1986; Bermingham & Avise, 1986) and *Utterbackia* are readily explained by inferring the late Cenozoic existence of a refugium on the peninsula of Florida.

The late Cenozoic peninsular Florida refugium hypothesis has a relatively long history (*e.g.*, see Dall, 1903; Hubbell, 1932; Carr, 1940; Olson *et al.*, 1954; Neill, 1957) and was initially based on 1) the large number of taxonomically diverse northern peninsular Florida endemics that are believed to either have geographically nearby close relatives or be very distinct relictual taxa with no geographically nearby close relatives, and 2) the multiple marine terraces of the SE USA Coastal Plain that are interpreted to be the result of multiple oscillations of sea level during the late Cenozoic which repeatedly altered the coastline of, insularized or inundated various portions of the Florida peninsula for significant lengths of time (*e.g.*, see Cooke, 1945; Alt & Brooks, 1965; Alt, 1968; Vail *et al.*, 1977; Vail & Hardenbol, 1979). Gilbert (1987) presented a scenario of two insularization events for the northern Florida peninsula during the late Cenozoic; one *ca.* 15 Mybp (mid-Miocene) and the other *ca.* 5 Mybp (early Pliocene). He hypothesized that the duration of peninsular isolation from the mainland was 5 My and 3My, respectively. Gilbert (1987) went on to attribute the differences in degree of morphological differentiation observed in various endemic freshwater fish taxa to be the result of these two isolation events that were separated in time by *ca.* 10 My. Subsequent Pleistocene interglacial high sea stands likely produced similar, if not as pronounced, effects (*e.g.*, see Neill, 1957).

While the details of the timing, extent, and duration of insularization (or inundation), and, subsequently, effects on the biota are subjects of some continuing debate (*e.g., cf.* Burgess & Franz, 1978; Opdyke *et al.*, 1984; Gilbert, 1987), the relatively recent origin of peninsular Florida (*ca.* mid-Oligocene, Gilbert, 1987), the presence of many, taxonomically diverse endemics in the peninsular Florida region, and extensive evidence for drastic changes in sea level during the late Cenozoic strongly support the notion of repeated vicariance/dispersal events for the region. Cyclical transgressions of the sea could allow for alternating episodes of cladogenesis and dispersal in relatively low-vagility organisms. Certainly, populations of obligate freshwater organisms, such as unionid mussels and primary-division freshwater fishes, would be reproductively isolated if separated by salt water. Persistence of the salt water barriers for the lengths of time hypothesized by Gilbert (1987) could have led to the evolution of intrinsic reproductive barriers in allopatry. Subsequent to sea regression, the generally low topographical relief of the area would have potentiated dispersal as freshwater drainages (from previously isolated areas) became confluent as they re-established routes to the sea. With these processes in mind, Swift *et al.*, (1986) hypothesized that there were two or three major lowland vicariant events in the southeastern U.S.A. during the late Oligocene-Pleistocene time interval.

Despite the dearth of freshwater mussel fossils (*e.g.*, see Henderson, 1935; Bogan & Grady, 1991), the phylogenetic hypothesis presented in Figure 2 in conjunction with the hypothesis of at least two major vicariant events in the southeastern USA during the late Cenozoic can facilitate a reconstruction of *Utterbackia* evolution and dispersal during this time period. During the late Oligocene, sea level lowering provided access to the northern peninsula of Florida for the ancestral *Utterbackia* lineage. The ultimate source of the colonization may have been Mexico (Hoeh, unpublished data). The mid-Miocene high sea stand (*ca.* 15 Mybp) would have isolated part of this ancestor in the Florida panhandle drainages (*U. peggyae* lineage) and part in drainages of the insularized Florida peninsula (*U. imbecillis s.l./U. "peggyae"* lineage). Dispersal of the *U. imbecillis s.l./U. "peggyae"* lineage north from the peninsula of Florida into the Altamaha (and Savannah?) River drainage ensued during the late Miocene sea level lowering. This event may have been the result of a confluence of the then northward flowing Suwannee River (Swift *et al.*, 1986) and the Altamaha (and Savannah?) River in the low lying Savannah Basin area. The directionality of this dispersal event is consistent with that inferred above for the Floridian refugium fishes (Bermingham & Avise, 1986). A subsequent vicariant event during the early Pliocene high sea stand (*ca.* 5 Mybp), again isolating the Florida peninsula from the mainland, initiated divergence between the "peninsular" *U. "peggyae"* lineage and the mainland *U. imbecillis s.l.* lineage. Thus the lowland vicariant events in the southeastern USA during the late Oligocene-Pleistocene postulated by Swift *et al.* (1986) and Gilbert (1987) may be causally related to the hypothesized phylogeny of *Utterbackia* (*i.e.*, [*U. imbecillis s.l.*, *U. "peggyae"*] *U. peggyae*]) reported in this work.

While speculative, this particular reconstruction of *Utterbackia* evolution and dispersal can help to reconcile 1) the paradoxical homoplasy shared by *U. imbecillis s.l.* and *U. peggyae*, 2) the location of the geographically restricted *U. "imbecillis"* populations, 3) the hypothesized historical range expansion of *U. imbecillis* into peninsular Florida (see above discussion), and 4) the lack of panhandle/peninsular Florida genetic differentiation in *U. imbecillis*. Under the reconstruction proposed here, *U. peggyae* and *U. imbecillis s.l.* could have been parapatrically or sympatrically distributed on the southeastern USA mainland during or subsequent to the early Pliocene high sea stand that isolated the ancestral *U. "peggyae"* lineage on "peninsular" Florida. This would have provided the opportunity for hybridization and subsequent introgression of electromorphs between the *U. peggyae* lineage and the then recently diverged *U. imbecillis s.l.* lineage. An increased susceptibility of genetic systems to invasion for recently diverged animal lineages has been postulated (Grant & Grant, 1992). This hypothesized ancient introgression could account for the unusual distribution of homoplasy observed in *Utterbackia* (Fig. 4). Furthermore, if one allows that 1) *U. "imbecillis"* represents a lineage derived from an early cladogenic event in the *U. imbecillis s.l.* lineage and 2) *U. "imbecillis"* is less vagile than *U. imbecillis*, then the current range of *U. "imbecillis"* may delineate part of the original (= pre-*U. imbecillis* dispersal) range of the *U. imbecillis s.l.* lineage. This hypothesized retention of ancestral range

for *U. "imbecillis"* may be due to a relatively recent (or no) transition to self-fertilization or to parasitic larval specialization on one or more of the ichthyofaunal endemics of the region. Either of these possibilities could account for a perceived decrease in vagility for *U. "imbecillis"*. Notwithstanding, the current range of *U. "imbecillis"* is consistent with the reconstruction presented above. This same reconstruction is consonant with the hypothesis of a relatively recent colonization of the Florida peninsula by *U. imbecillis*. If the *U. imbecillis s.l.* lineage originally evolved on the mainland during the early Pliocene, then *U. imbecillis* must have dispersed to the Florida peninsula at some later time. This same line of reasoning explains the lack of panhandle/peninsula genetic differentiation observed in *U. imbecillis*.

Overall, the ease with which the above historical reconstruction accounts for the previously anomalous and seemingly independent aspects of both homoplasy distribution and taxa distributions over time and space of the species within *Utterbackia* suggests that the Late Cenozoic peninsular Florida refugium hypothesis should be seriously evaluated in subsequent studies of southeastern USA biogeography. Rigorous estimates of phylogeny for other southeastern USA freshwater and terrestrial taxa with peninsular Florida members are necessary to judge the generality of this biogeographic hypothesis (*e.g.*, see Rosen, 1978). The construction of population-level phylogenetic hypotheses for additional freshwater mussel taxa with panhandle and peninsular Florida (herein, including the Suwannee River drainage) representatives, such as the genera *Anodonta (sequens* Hoeh, 1990), *Elliptio, Lampsilis, Medionidus, Pleurobema, Quincuncina, Toxolasma, Uniomerus,* and *Villosa,* is central to this evaluation.

The Origin of Simultaneous Hermaphroditism in *Utterbackia*

The simultaneous hermaphroditic *Utterbackia imbecillis s.l.* clade is more successful than either the *U. peggyae* or *U. "peggyae"* lineage when comparisons are based on the number of extant individuals/populations or the extent of current geographic/climatic ranges. The relative success of simultaneous hermaphrodites in *Utterbackia* (Anodontini) is paralleled in two distantly related genera of unionids: *Toxolasma* (*T. parvum*; see Tepe, 1943) in the Lampsilini and *Uniomerus* (*U. tetralasmus*; see Morrison, 1976) in the Pleurobemini. This suggests a fitness advantage for SH over gonochorism in these genera. This inference leads one to speculate as to the possible reasons for the relative rarity of SH in freshwater mussels.

At any given point in time, the number of simultaneous hermaphroditic lineages is dependent on the rate of origination and the rate of extinction of these lineages. The relative rarity of SH in freshwater mussels may be due to a low rate of origination, high rate of extinction, or both processes in concert. The observation that no higher taxon within the Unionidae is composed predominantly of simultaneous hermaphroditic species is consistent with a hypothesis of relatively high extinction rates for these lineages. This possibility has been offered to explain the dearth of predominantly asexual clades in many animal taxa (*e.g.*, see Maynard Smith, 1978, 1988; Bell, 1982; but see Williams, 1975). If self-fertilization proves to be the predominant breeding system in simultaneous hermaphroditic unionids, then the loss of genetic variability due to a combination of severe inbreeding and periodic population bottlenecks may render these lineages more vulnerable to attack by pathogens and parasites (*e.g.*, see Lively *et al.*, 1990) and, therefore, to extinction. The current lack of knowledge regarding breeding systems in simultaneous hermaphroditic unionid lineages prevents an evaluation of the high extinction rate hypothesis.

A relatively low origination rate may also be responsible for the paucity of simultaneous hermaphroditic unionid species. Lack of either selective advantage for SH or genetic variation for reproductive mode would preclude the transition from gonochorism to SH (discounting drift as a potential factor). As mentioned above, the success of simultaneous hermaphrodites relative to gonochores in three different unionid tribes suggests a fitness advantage for SH (at least in these tribes under the prevailing environmental conditions). Despite the general implicit or explicit assumptions of abundant genetic variability in theoretical treatments of evolution (*e.g.*, see Maynard Smith, 1978; Bell, 1982; Charnov, 1982; Bull, 1983), the lack of genetic variation for reproductive mode (*e.g.*, see Williams, 1975, 1988; Brooks, 1988) may be the prin-

ciple factor limiting transitions from gonochorism to functional SH in freshwater mussels. At the present time, the basis for sex determination in freshwater mussels is unknown. However, gonochorism is apparently under strong stabilizing selection in many animal taxa (*e.g.*, see Maynard Smith, 1988).

The hypothesis of monophyly for the *Utterbackia imbecillis s.l.* clade (Fig. 2) coupled with the lineage's uniform SH is consonant with the hypothesis that there was a single origin of SH in the common ancestor of this lineage. Furthermore, the uniform reproductive mode within this lineage suggests that the transition in breeding system took place before *U. imbecillis* and *U. "imbecillis"* diverged and also before *U. imbecillis* dispersed into its current range. The transition from gonochorism to SH in the *Utterbackia imbecillis s.l.* lineage may have depended upon a disruption of the normal sex determination system. This disruption could have produced the variation necessary for natural selection to act upon.

The hypothesis for *Utterbackia* evolution presented above offers unique insight to the nature of a potential disruptive agent. If the *U. peggyae* lineage did indeed hybridize with the *U. imbecillis s.l.* lineage shortly after the origin of the latter, then introgression of genetic elements from *U. peggyae* into *U. imbecillis s.l.* could have disrupted the latter's sex determination system. This saltational event could have potentiated the transition from gonochorism to SH. Hybridization between different species is strongly linked to the origin of asexual vertebrate taxa (*e.g.*, see Williams, 1988; Vrijenhoek, 1989; Vrijenhoek *et al.*, 1989). Furthermore, hybridization between different strains (Beamer *et al.*, 1978) and between different species (Rothbard *et al.*, 1982) has been implicated in the production of hermaphroditic individuals from normally gonochoric animal taxa. Thus, hybridization with introgression is a plausible mechanism that could have generated variation in the sex determination system in the ancestral *U. imbecillis s.l.* lineage such that natural selection could facilitate the gonochorism to SH transition.

While the specific introgression/origin of SH hypothesis in *Utterbackia* is quite tenuous, the multiple independent origins of SH in southeastern USA freshwater mussels provide a means to test the generality of the hypothesis. Evidence of introgression in other simultaneous hermaphroditic lineages such as *Toxolasma parvum* and *Uniomerus tetralasmus* (Sterki, 1898a; Tepe, 1943; Morrison, 1976) would corroborate the hypothesis that the presence of xenogeneic genetic elements is correlated with transitions in reproductive mode. Phylogenetic evaluations of both *Toxolasma* and *Uniomerus* are in progress. Corroboration of this hypothesis would further emphasize the evolutionary import of introgressive hybridization in animal evolution (*e.g.*, see Arnold, 1992; Grant & Grant, 1992; Dowling & DeMarais, 1993). Notwithstanding, further elucidation of the circumstances surrounding the origins of SH in freshwater mussels is dependent upon robust reconstructions, using all available data (*sequens* Kluge, 1989; Barrett *et al.*, 1991; Jones *et al.*, 1993), of the evolutionary histories of the pertinent lineages.

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APPENDIX 1 [1]

Locality information and sample sizes for the 56 populations examined in this study.

Ingroup

Utterbackia imbecillis (N = 399)

1) ACi-Apalachicola River, below Lake Seminole, Chattahoochee, Gadsden Co., FL (N = 33)
2) ACHi-Attapulgus Creek, at FL Route 159, Gadsden Co., FL (N = 3)
3) BHCi-Ocmulgee River, at the Ben Hill/Coffee Co. line boat ramp, GA (N = 11)
4) CACi-C100A canal, Miami, Dade Co., FL (N = 4)
5) CCLi-Lake Corpus Christi, at Lake Corpus Christi State Park, San Patricio Co., TX (N = 7)
6) CDi-Cedar River, below Wiggins Lake, Gladwin Co., MI (N = 32)
7) CRHi-Combahee River, at US Route 17A, Hampton Co., SC (N = 11)
8) CROi-Clinton River, at Orchard Lake Road, Pontiac, Oakland Co., MI (N = 1)
9) DCLi-Deep Creek, at US Route 258, Edgecombe Co., NC (N = 5)
10) DRLi-mouth of Dead River Lake, off of the Pascagoula River, Jackson Co., MS (N = 7)
11) FPCi-Fisher's Pond, Mt. Pleasant, Cabarrus Co., NC (N = 20)
12) GALi-Pond at Suntree Country Club, Brevard Co., FL (N = 33)
13) GLCi-Gantt Lake, off of US Route 29, Covington Co., AL (N = 14)
14) HRHi-Hillsborough River, at FL Route 579, Hillsborough Co., FL (N = 1)
15) KLTi-Kentucky Lake, at Paris Landing State Park, Henry Co., TN (N = 18)
16) KRK8i-Kokosing River, below Knox Lake, Knox Co., OH (N = 7)
17) KRK9i-Kankakee River, at US Route 45, Kankakee, Kankakee Co., IL (N = 6)
18) KRMi-Kankakee River, Momence, Kankakee Co., IL (N = 4)
19) LCHi-Lake Cass, Hillsborough Co., FL (N = 16)
20) LICi-Lake Istokpoga Canal, off of US Route 98, Highlands Co., FL (N = 2)
21) LRGi-Little River, at FL Route 12, Gadsden Co., FL (N = 5)
22) LTCi-Ochlockonee River, Lake Talquin, Coe's Landing, Leon Co., FL (N = 12)
23) MCi-Mill Creek, below Starve Hollow Lake, Jackson Co., IN (N = 12)
24) MCWi-Mantua Creek, at Lambs Road bridge, Pitman, Gloucester Co., NJ (N = 7)
25) MIi-Mosquito Creek Impoundment, Chattahoochee, Gadsden Co., FL (N = 1)
26) ORGi-south fork of the Ouachita River, Mount Ida, Montgomery Co., AR (N = 1)
27) ORTi-Ohoopee River, at GA Route 147, Tattnall Co., GA (N = 7)
28) ORT10i-Ochlockonee River, at US Route 84, Thomas Co., GA (N = 2)
29) OSFi-Oklawaha River, at FL Route 314, Marion Co., FL (N = 24)
30) PBJi-Lake Shelby, in Paul B. Johnson State Park, Forrest Co., MS (N = 3)
31) PCi-Pickering Creek, at PA Route 23, Chester Co., PA (N = 12)
32) PGRi-Pascagoula River, at the mouth of Black Creek, Jackson Co., MS (N = 1)
33) PRWi-Pearl River, at Walkiah Bluff, Pearl River Co., MS (N = 4)
34) SRi-Canal off of the Suwannee River, at Dilger's Campground, Dixie Co., FL (N = 34)
35) SRGi-Saluda River, at Saluda Dam Road, Pickens Co., SC (N = 13)
36) TRFi-Tippecanoe River, below Freeman Lake, Carrol Co., IN (N = 2)
37) UPPi-Pond off of Poinciana Blvd., Polk Co., FL (N = 24)

Utterbackia peggyae (N = 178)

1) ACHp-Attapulgus Creek, at FL Route 159, Gadsden Co., FL (N = 16)
2) CSLp-Chumuckla Springs Lake, Santa Rosa Co., FL (N = 15)
3) ERFp-Escambia River, at FL Route 4, Escambia Co., FL (N = 1)
4) GCPs-Gator Creek, at FL Route 471, Polk Co., FL (N = 14)
5) HCCp-Holmes Creek, at US Route 90, Holmes Co., FL (N = 8)
6) HRHs-Hillsborough River, at FL Route 579, Hillsborough Co., FL (N = 13)
7) HRPs-Hillsborough River, at FL Route 39, Pasco Co., FL (N = 5)
8) LRGp-Little River, at FL Route 12, Gadsden Co., FL (N = 16)
9) LTCp-Ochlockonee River, Lake Talquin, Coe's Landing, Leon Co., FL (N = 15)

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APPENDIX 1. (cont.)

10) NRBs-New River, at County Route 231, Bradford Co., FL (N = 5)
11) ORTp-Ochlockonee River, at US Route 84, Thomas Co., GA (N = 1)
12) RCAs-Rocky Creek, at FL Route 235, Alachua Co., FL (N = 20)
13) SHFp-sink hole, at Florida Caverns State Park, Jackson Co., FL (N = 19)
14) SRs-canal off of the Suwannee River, at Dilger's Campground, Dixie Co., FL (N = 20)
15) YRCp-Yellow River, at US Route 90, Okaloosa Co., FL (N = 10)

Outgroup

Pyganodon cataracta

PCc-Pickering Creek, at PA Route 23, Chester Co., PA (N = 9)

P. fragilis

LSGf-Lake St. George, at Lake St. George State Park, Waldo Co., ME (N = 8)

P. grandis

MCg-Mill Creek, below Starve Hollow Lake, Jackson Co., IN (N = 8)

P. lacustris

HLMl-Howe Lake, Marquette Co., MI (N = 8)

APPENDIX 2

Presumptive loci scored and buffer systems used in this study. References for the electrophoretic buffer systems are as follows: EDTA-Borate-Tris buffer pH8.6 (EBT8.6, Wurzinger 1980), Tris-Maleic Acid-Magnesium Chloride-EDTA pH7.4 (TMME7.4, Spencer *et al.*, 1964), Tris-Citrate pH8.0 (TC8.0, Selander *et al.* 1971), 3-Amino Propyl Morpholine-Citrate pH6.0 (MC6.0, Clayton and Tretiak 1972). The MC5.5 system was identical in composition to the MC6.0 system except that the gel buffer was pH 5.5. When NADP was added to a particular electrophoretic system, 20mg was added to the gel (425ml) and 10mg was added to the cathodal (-) electrode tray.

Enzyme	No. loci scored	Abbreviation	E.C. no.	Buffer system
Acid Phosphatase	1	ACP	3.1.3.2	MC 5.5
Aconitase Hydratase	2	ACOH	4.2.1.3	MC 6.0 NADP
Alcohol Dehydrogenase (octanol)	1	ADH	1.1.1.1	TC 8.0
Aldehyde Oxidase (benzaldehyde)	1	AO	1.2.3.1	TMME 7.4
Aspartate Aminotransferase	1	AAT	2.6.1.1	TMME 7.4
Catalase	2	CAT	1.11.1.6	EBT 8.6
Cytosol Aminopeptidase	2	CAP	3.4.11.1	MC 5.5
				TC 8.0
Dihydrolipoamide Dehydrogenase	1	DDH	1.8.1.4	MC 5.5
Esterase (alpha naphthyl acetate)	1	EST	3.1.1.-	MC 6.0
Fructose-bisphosphatase	1	FBP	3.1.3.11	MC 5.5
Fumarate Hydratase	1	FUMH	4.2.1.2	TMME 7.4
Glucose-6-phosphate Isomerase	1	GPI	5.3.1.9	MC 5.5
Glyceraldehyde-3-phosphate Dehydrogenase	1	GAPDH	1.2.1.12	TC 8.0
Glycerol-3-phosphate Dehydrogenase	1	G3PDH	1.1.1.8	TC 8.0
Isocitrate Dehydrogenase	2	IDH	1.1.1.42	MC 6.0 NADP
				TMME 7.4 NADP
Malate Dehydrogenase	2	MDH	1.1.1.37	MC 5.5
Phosphoglucomutase	1	PGM	5.4.2.2	MC 6.0
Phosphogluconate Dehydrogenase	1	PGDH	1.1.1.43	TMME 7.4 NADP
Superoxide Dismutase	1	SOD	1.15.1.1	TMME 7.4
Triose-phosphate Isomerase	1	TPI	5.3.1.1	MC 6.0
	Total = 25			

APPENDIX 3

The allelic arrays (lower case letters in parentheses) and corresponding character state designations (upper case letters) at each of 25 loci. Abbreviations for allozyme loci as per Appendix 2.

1 SOD	2 ADH	3 TPI	4 ACOH1	5 AAT	6 IDH1	7 FUMH
A(a)	A(a) B(b) C(b,c) D(c)	A(a)	A(a)	A(a) B(b) C(a,b)	A(b) B(a,b) C(c)	A(c) B(c,d) C(d) D(b) E(a,c)

8 CAT1	9 CAT2	10 PGM	11 G3PDH	12 GAPDH	13 CAP1	14 IDH2
A(a) B(b)	A(b) B(a) C(a,b) D(d) E(c,d)	A(d) B(d,f) C(f) D(a,d) E(c,d) F(d,g) G(b) H(b,d,e)	A(c) B(c,d) C(b,c) D(a,c)	A(b) B(c) C(a,b)	A(c) B(b,c) C(b,c,e) D(c,e) E(a,b) F(b) G(a,b,c) H(a,c) I(a,c,d) J(d) K(d,e,f,g)	A(e) B(d,e) C(e,f) D(b,e) E(a,c)

15 EST	16 ACOH2	17 GPI	18 PGDH	19 FBP	20 MDH2	21 MDH1
A(c) B(b,c) C(d) D(a,c)	A(c) B(a) C(a,c) D(b) E(b,c)	A(e) B(a,e) C(a) D(c) E(f,g) F(f) G(b) H(b,e) I(d)	A(a)	A(a)	A(a)	A(b) B(a) C(a,b) D(f) E(c,d,e)

22 DDH	23 CAP2	24 ACP	25 AO
A(f) B(c,f) C(c,e,f,g) D(e,f,g) E(d,g) F(d,f,g) G(d,f) H(a,d) I(b,d) J(a,b)	A(b) B(c) C(g) D(d,g) E(d,h) F(f,g) G(f) H(e) I(a,f) J(d,f) K(d)	A(a) B(c) C(d) D(c,d) E(b)	A(c) B(e) C(d,e) D(c,d) E(b,c) F(b) G(a,b)

TABLE 1. Character matrix from 25 presumptive loci. Characters (abbreviations for allozyme loci as per Appendix 2): 1, SOD; 2, ADH; 3, TPI; 4, ACOH1; 5, AAT; 6, IDH1; 7, FUMH; 8, CAT1; 9, CAT2; 10, PGM; 11, G3PDH; 12, GAPDH; 13, CAP1; 14, IDH2; 15, EST; 16, ACOH2; 17, GPI; 18, PGDH; 19, FBP; 20, MDH2; 21, MDH1; 22, DDH; 23, CAP2; 24, ACP; 25, AO. Taxa: The 40 OTUs represent populations of *Utterbackia imbecillis* (i), *U. peggyae* (p & s), *Pyganodon cataracta* (c), *P. fragilis* (f), *P. lacustris* (l), and *P. grandis* (g). Those OTUs in bold-faced type are composites of populations which were identical for electromorph arrays at all loci. **CDi** = CDi + KRK8i + CROi; **KLTi** = KLTi + TRFi; **BHCi** = BHCi + LRGi + MIi; **CRHi** = CRHi + SRGi; **GLCi** = GLCi + LTCi + MCWi + ACHi + ORT10i; **UPPi** = UPPi + LCHi + KRK9i + KRMi + HRHi + LICi + CACi (see Appendix 1 for locality information).

char. #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
CDi	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
ACi	A	A	A	A	B	A	B	A	A	B	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A
SRi	A	A	A	A	C	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
KLTi	A	A	A	A	C	A	B	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
MCi	A	A	A	A	C	A	C	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
BHCi	A	A	A	A	B	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
PCi	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
PRWi	A	A	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
OSFi	A	A	A	A	C	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
UPPi	A	A	A	A	B	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
GALi	A	A	A	A	C	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
GLCi	A	A	A	A	B	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
DCLi	A	A	A	A	A	A	A	A	A	C	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
ORGi	A	A	A	A	B	A	C	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
CCLi	A	A	A	A	B	A	C	A	A	C	A	A	A	A	A	A	D	A	A	A	A	A	A	A	A
PBJi	A	A	A	A	B	A	C	A	A	A	A	A	A	A	A	A	C	A	A	A	A	A	B	A	A
PGRi	A	A	A	A	B	A	C	A	A	A	A	A	A	A	A	A	C	A	A	A	A	B	B	A	A
DRLi	A	A	A	A	B	A	C	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	B	A	A
ORTi	A	A	A	A	B	A	C	B	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	C
CRHi	A	A	A	A	B	A	C	B	A	D	A	B	A	A	A	A	A	A	A	A	A	A	A	A	C
FPCi	A	A	A	A	B	A	C	B	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	B
CSLp	A	A	A	A	A	A	D	A	A	B	A	A	B	A	A	B	E	A	A	A	B	A	C	A	D
ERFp	A	A	A	A	A	A	D	A	A	B	A	A	B	A	A	B	F	A	A	A	B	A	C	A	A
YRCp	A	A	A	A	A	A	D	A	A	A	A	C	B	A	A	B	F	A	A	A	B	A	D	A	D
HCCp	A	A	A	A	A	A	D	A	A	E	A	A	C	C	A	C	F	A	A	A	B	A	C	A	A
SHFp	A	A	A	A	A	A	D	A	A	A	A	A	D	D	A	A	F	A	A	A	B	A	E	A	E
LTCp	A	A	A	A	A	A	D	A	A	A	B	A	D	A	A	A	F	A	A	A	C	C	F	A	A
ACHp	A	A	A	A	A	A	D	A	A	A	B	A	D	A	A	A	F	A	A	A	B	C	F	A	A
ORTp	A	A	A	A	A	A	D	A	A	A	A	A	D	A	A	A	F	A	A	A	B	B	G	A	A
LRGp	A	A	A	A	A	A	D	A	A	F	A	A	D	A	A	A	F	A	A	A	B	D	F	A	A
SRs	A	A	A	A	B	B	C	A	A	A	C	A	G	A	A	D	H	A	A	A	C	G	H	A	F
RCAs	A	A	A	A	B	B	C	A	A	A	A	A	H	A	B	D	G	A	A	A	C	A	H	A	F
NRBs	A	A	A	A	B	B	C	A	A	A	A	A	E	A	A	D	G	A	A	A	B	A	H	A	F
GCPs	A	A	A	A	B	B	C	A	B	A	B	A	F	A	A	D	G	A	A	A	B	E	G	A	G
HRPs	A	A	A	A	B	B	C	A	B	A	B	A	F	A	A	D	G	A	A	A	B	E	I	A	G
HRHs	A	A	A	A	B	A	C	A	C	A	A	A	F	A	A	D	G	A	A	A	B	F	J	A	G
PCc	A	B	A	A	A	C	A	B	D	G	A	A	I	A	A	E	D	A	A	A	D	H	K	B	A
LSGf	A	C	A	A	A	C	A	B	D	G	A	A	A	A	A	A	D	A	A	A	D	I	K	C	A
HLMl	A	D	A	A	A	C	C	A	D	A	A	A	J	A	C	A	I	A	A	A	D	J	K	D	A
MCg	A	B	A	A	A	C	E	A	E	H	D	A	K	E	D	A	D	A	A	A	E	I	H	E	A