

CORRELATION BETWEEN MATING SYSTEM AND DISTRIBUTION OF GENETIC VARIATION IN *UTTERBACKIA* (BIVALVIA: UNIONIDAE)WALTER R. HOEH,^{1,5,*} KENNETH S. FRAZER,^{1,**}
EDNA NARANJO-GARCIA,^{2,***} MICHAEL B. BLACK,³
DAVID J. BERG,⁴ AND SHELDON I. GUTTMAN⁵¹Mollusk Division
Museum of Zoology
University of Michigan
Ann Arbor, MI 48109²Estacion de Biologia Chamela
Apartado Postal 51
San Patricio
Jalisco 48980 Mexico³Maurice Lamontagne Institute
Department of Fisheries & Oceans
Quebec G5H 3Z4 Canada⁴Department of Zoology
Miami University
Hamilton, OH 45011⁵Department of Zoology
Miami University
Oxford, OH 45056

ABSTRACT Variation in mating systems (e.g., dioecy versus hermaphroditism, self-fertilization versus cross-fertilization) has been shown to affect the distribution of genetic variation in plants. However, the paucity of this type of variation in closely related taxa has hampered similar evaluations in animals. The freshwater bivalve genus *Utterbackia* (Unionidae: Anodontinae), currently comprising three nominal and one undescribed species, contains gonochoric (dioecious) as well as hermaphroditic species and thus is a model animal system for examining mating system evolution and its effects on the distribution of genetic variation. Comparisons of the level of within- and among-population allozymic variation (at 9 putative genetic loci) in the simultaneous hermaphrodites *Utterbackia imbecillis* (23 populations, 331 individuals) and *U. "imbecillis"* (4 populations, 51 individuals), with those of the gonochoric *U. peggyae* (7 populations, 99 individuals) and *U. peninsularis* (6 populations, 77 individuals) allowed inferences to be made regarding (1) the mating system of *U. imbecillis* and *U. "imbecillis"* and (2) the population genetic structure of these four species. The low levels of within-population variation and marked heterozygote deficiency observed in *U. imbecillis* and *U. "imbecillis"* relative to that in *U. peggyae* and *U. peninsularis*, suggest that there is a high degree of self-fertilization in both hermaphroditic species. However, the among-population variation in the level of heterozygote deficiency (Selander D range: -0.181 to -1.000) is consistent with the hypothesis that the relative amounts of cross-fertilization and self-fertilization vary among populations of *U. imbecillis* and *U. "imbecillis"*. The hypothesis of high levels of self-fertilization in *U. imbecillis* and *U. "imbecillis"* is consonant with the presumed high colonization potential of the former species. The estimates of F_{ST} obtained for the four species of *Utterbackia* suggest a very high level of among-population genetic differentiation (mean F_{ST} range: 0.218-0.818). This observation is quite unexpected for an able colonizer such as *U. imbecillis* (mean F_{ST} = 0.818) unless detailed knowledge of this species' mating system is considered. The combined impact of self-fertilization and founder events in the hermaphroditic *Utterbackia* species likely potentiates among-population genetic differentiation which increases F_{ST} values. These results suggest that the mating system and distribution of genetic variation in unionid populations should be carefully evaluated prior to the enactment of conservation initiatives.

KEY WORDS: Simultaneous hermaphroditism, gonochorism, self-fertilization, cross-fertilization, heterozygote deficiencies, allozymes, genetic variation

INTRODUCTION

Taxa that display variation in mating systems are currently under intense scrutiny because of their utility in addressing ques-

tions of geographic variation and adaptation (Charnov 1982, Rick 1988, Wyatt 1988, Waller 1993, Waser 1993, Barrett and Harder 1996, Reynolds 1996). Mating system variation at relatively low taxonomic levels (among both congeneric species and conspecific populations) is especially useful since the factors responsible for the mating system transitions may still be operative. Furthermore, explicit hypotheses of phylogenetic relationships may permit an assessment of the directionality of character state change in mating systems. Therefore, the presence of gonochorism (dioecy) and simultaneous hermaphroditism within the freshwater bivalve genus *Utterbackia* (Baker 1927) (Bivalvia: Unionidae: Anodontinae),

*Current addresses: Department of Biological Sciences, Kent State University, Kent, OH 44242

**Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME 04609

***Departamento de Zoología, Instituto de Biología, U. A. N. M., Apartado Postal 70-153, Mexico, D. F. 04510 Mexico

combined with an explicit hypothesis of phylogenetic relationships available for the group (Hoeh et al. 1995), renders this taxon a model system for evaluating the forces responsible for mediating mating system evolution. However, the current lack of understanding regarding the details of the fertilization system (e.g., cross-fertilizing versus self-fertilizing) within the hermaphroditic species of *Utterbackia* impedes our ability to evaluate the relative importance of potential mediating forces.

Currently, *Utterbackia* consists of three nominal species—*U. imbecillis* (Say 1829), *U. peggyae* (Johnson 1965), and *U. peninsularis* (Bogan and Hoeh 1995) and one undescribed species (*U. "imbecillis"*) (see Hoeh et al. 1995). The geographic distribution of *U. imbecillis* includes much of the eastern half of the USA, and portions of southern Canada and northern Mexico, and *U. peggyae*, *U. peninsularis*, and *U. "imbecillis"* are restricted to the drainages flowing through the panhandle of Florida, peninsular Florida, and southern Atlantic Slope regions, respectively, of the southeastern USA (Hoeh et al. 1995). The four species within *Utterbackia* are ecologically similar, inhabiting lentic and lotic habitats with relatively low current velocities (Baker 1928, Johnson 1965, Cummings and Mayer 1992). As for most unionids, *Utterbackia* larvae are likely short-duration, obligate parasites of freshwater fish (Fuller 1974). However, host fish data are currently available for only *U. imbecillis* (Hoggarth 1992). A recent cladistic analysis of the genus (Hoeh et al. 1995) resulted in the following phylogenetic hypothesis: [(*U. imbecillis sensu lato*, *U. peninsularis*, *U. peggyae*)]. Together with a paleogeographic hypothesis of Late Cenozoic sea level fluctuations in the southeastern USA, this analysis allowed the inference that the *U. imbecillis s.l.* initially diverged from the *U. peninsularis* lineage approximately 5 million years ago in freshwater drainages of the southeastern USA (Hoeh et al. 1995).

A significant difference in sexuality exists between *Utterbackia imbecillis s.l.* and both *U. peggyae* and *U. peninsularis*. Like the majority of unionid bivalves, *U. peggyae* and *U. peninsularis* are gonochoric whereas *U. imbecillis* and *U. "imbecillis"* are simultaneous hermaphrodites (i.e., produce mature oocytes and spermatozoa concurrently; Sterki 1998a, Sterki 1998b, Ortmann 1910, Ortmann 1911, Allen 1924, Baker 1927, Baker 1928, van der Schalie 1970, Heard 1975, Kat 1983). Based on a parsimony criterion, it was hypothesized that a single transition, from gonochorism to simultaneous hermaphroditism, occurred in the ancestor of the *U. imbecillis s.l.* lineage subsequent to its divergence from the *U. peninsularis* lineage (Hoeh et al. 1995). This hypothesis suggests that simultaneous hermaphroditism is a relatively recently evolved form of sexuality derived from a gonochoric ancestral taxon.

Variability in the mating systems of simultaneous hermaphroditic taxa (i.e., from obligate cross-fertilizing to obligate self-fertilizing) is well known in plants (Allard 1975, Stebbins 1970, Stebbins 1974, Jain 1976, Schoen 1982, Schemske and Lande 1985, Holtsford and Ellstrand 1990, Waller 1993, Barrett and Harder 1996) and has been documented in mollusks (Tompa et al. 1984, McCracken and Selander 1980, Foltz et al. 1984, O Foighil and Eernisse 1987, O Foighil and Eernisse 1988, Eernisse 1988). The following data suggest that this type of mating system variability exists within *U. imbecillis*.

Extrapolating from relative cross-sectional areas of stained paraffin sections from a standard position in the visceral mass, Kat (1983) reported a bimodal distribution of testicular to ovarian tissue volume ratios among 15 populations of *Anodonta imbecillis*

(*sic*) (*Utterbackia imbecillis*; it is possible that several of these populations actually represented *U. "imbecillis"*). Among Kat's "central geographic range" populations, relatively low density populations from running water had significantly higher average ratios of male to female gonadal tissue than did high density populations from standing water. Given the current understanding of the distributions of *U. imbecillis* and *U. "imbecillis"*, it seems unlikely that this particular result can be attributed to species-specific gonadal allocations between *U. imbecillis* and *U. "imbecillis"*.

Kat's (1983) preferred explanation for the bimodality phenomenon was that selection adjusted sperm production to facilitate efficient cross-fertilization in local populations. Under this cross-fertilization efficiency hypothesis, relatively high density, low current velocity populations would require smaller amounts of spermatozoa to effect efficient cross-fertilization than would low density, high current velocity populations. Kat also suggested that sperm limitation could reduce fecundity in the latter situation. However, unless it is assumed that *U. imbecillis* only exists in extremes of both population density and current velocity, this hypothesis does not explain the bimodal nature of the data. Alternatively, sex allocation theory predicts a strong ovarian bias in the gonad of selfing hermaphrodites and an approximately equal amount of testicular and ovarian tissue in cross-fertilizing hermaphrodites (Charnov 1982). Thus, Kat's (1983) populations with relatively high ratios of male to female gonadal tissue may be predominantly cross-fertilizing whereas those with relatively low ratios may be predominantly self-fertilizing. This hypothesis was noted by Kat (1983) but rejected because of lack of substantiating data.

If self-fertilization has played a role in the genesis of the variation in gonadal allocation observed in *Utterbackia imbecillis*, one could predict that this species' population genetic structure would be very different from that of the gonochoric (i.e., obligately cross-fertilizing) *U. peggyae* and *U. peninsularis*. Prolonged selfing should result in a decay of heterozygosity (Crow and Kimura 1970). Even though total allozymic monomorphism is not unknown in gonochoric species, it is the expected consequence of a prolonged selfing regime combined with genetic drift (Wright 1969). Thus, the occurrence of considerable within-population genetic diversity is contrary to the expectations of prolonged selfing in combination with drift (Hamrick et al. 1979, Schoen 1982, Loveless and Hamrick 1984, Hamrick and Godt 1990), whereas selfing is but one of several mechanisms that might produce highly homozygous strains.

Given the above, with respect to within-population genetic structure, a predominantly self-fertilizing *U. imbecillis* would be expected to have a much lower level of variation than the obligately cross-fertilizing *U. peggyae* and *U. peninsularis*. Furthermore, the observed frequency of heterozygotes in a predominantly self-fertilizing *U. imbecillis* population should be much lower than the frequencies predicted by Hardy-Weinberg equilibria (Crow and Kimura 1970). Additionally, predominantly self-fertilizing species would likely have higher among-population levels of genetic differentiation than would cross-fertilizing species (Levin 1978, Loveless and Hamrick 1984, Holtsford and Ellstrand 1989, Hamrick and Godt 1990). Founder effects, due to a small number (often one?) of homozygous colonizing individuals, are likely responsible for the generality of this phenomenon among self-fertilizing lineages.

In order to evaluate Kat's (1983) cross-fertilizing/self-fertilizing hypothesis as a possible explanation for the observed

gonadal allocation in *U. imbecillis*, the present study analyzed a total of 40 populations of *Utterbackia* for allozyme variation at nine presumptive loci. The patterns of within-population genetic variation obtained from the allozyme analyses were used to categorize the mating system of 23 populations of *U. imbecillis* and four populations of *U. "imbecillis."* Genetic evidence consistent with the hypothesis of high levels of self-fertilization for Kat's (1983) low testicular: ovarian tissue ratio *U. imbecillis* populations would provide sufficient justification to reject Kat's cross-fertilization efficiency hypothesis for a more parsimonious alternative. Among-population patterns of genetic variation were also examined to allow for a more complete evaluation of potential correlates between population genetic structure and mating system in *Utterbackia*. Furthermore, the observed population genetic structures within *Utterbackia* were evaluated with regard to potential conservation initiatives.

MATERIALS AND METHODS

In an attempt to secure adequate sampling of the genetic variability within *Utterbackia*, 23 populations of *U. imbecillis* (331 individuals), four populations of *U. "imbecillis"* (51 individuals), seven populations of *U. peggyae* (99 individuals), and six populations of *U. peninsularis* (77 individuals) were sampled. Sample size and locality information are presented in Appendix I. A diligent effort was made to collect all specimens at a site. Voucher specimens, representing *U. peggyae*, *U. peninsularis*, *U. imbecillis*, and *U. "imbecillis,"* have been deposited in the Museum of Zoology, University of Michigan (Mollusk Division #253578, 253579, 253580, and 253581, respectively). Soon after specimen collection, non-gravid gill tissues were excised and cleaned of macroscopic parasites and debris, frozen in liquid nitrogen, and stored at -70°C . Tissues were homogenized with a glass pestle in 1.5 ml microcentrifuge tubes. The gill tissues contained sufficient water to eliminate the need for homogenization buffer. The resultant homogenates were centrifuged at $13,605 \times g$ for 10 min 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 nine polymorphic, putative genetic loci using four buffer systems (Appendix II). Stain recipes followed Shaw and Prasad (1970), Siciliano and Shaw (1976), and Murphy et al. (1990).

Data analyses were carried out using the BIOSYS-1 (Swofford and Selander 1981) computer program. In order to evaluate the patterns of within-population genetic variation, the mean number of alleles per locus, number of polymorphic loci, expected and observed heterozygosities, departure of variable loci from H-W expectations (significance test using exact probabilities; significance criterion, $p \leq .05$), and heterozygote deviation at variable loci relative to H-W expectations (Selander's D, 1970; [Ho-He]/He) were calculated for each population. Selander's D can range from -1 (no heterozygous individuals) to $+1$ (all individuals heterozygous) with zero being the expected value if the population is in H-W equilibrium. Mann-Whitney U tests were employed to evaluate the significance of differences between the gonochoric and hermaphroditic species for the above within-population descriptive statistics. Among-population patterns of genetic variation were evaluated using Wright's (1978) F_{ST} .

RESULTS

A summary of the within-population genetic variation for the 40 populations of *Utterbackia* examined is presented in Table 1.

The multilocus genotypes generated for the 558 individuals are available upon request from the senior author. Of the 23 *U. imbecillis* populations, 13 were monomorphic at all nine loci. For the remaining 10 populations, the number of polymorphic loci ranged from one to three. Two of the four *U. "imbecillis"* populations were monomorphic at all assayed loci, and the remaining two populations were each polymorphic at a single locus. The number of polymorphic loci per population in *U. peggyae* and *U. peninsularis* ranged from 1 to 3 and 1 to 4, respectively. The mean number of polymorphic loci per population for *U. imbecillis*, *U. "imbecillis,"* *U. peggyae*, and *U. peninsularis* was 0.74, 0.50, 2.57, and 2.00, respectively. The mean number of alleles per locus per population was 1.08 for *U. imbecillis*, 1.06 for *U. "imbecillis,"* 1.38 for *U. peggyae*, and 1.26 for *U. peninsularis*. Mann-Whitney U tests detected no significant differences in either number of polymorphic loci per population or mean number of alleles per locus between *U. imbecillis* and *U. "imbecillis"* and between *U. peggyae* and *U. peninsularis*. However, there was a significant difference in both number of polymorphic loci per population ($U = 44.5$, $p = .0002$) and mean number of alleles per locus ($U = 35.5$, $p < .0001$) between the combined gonochoric and combined hermaphroditic species groupings.

Eleven of 17 (65%) and 1 of 2 (50%) of the population by polymorphic locus pairs were not within H-W expectations for *U. imbecillis* and *U. "imbecillis,"* respectively. In contrast, only 1 of 18 (6%) and 1 of 12 (8%) were not for *U. peggyae* and *U. peninsularis*, respectively. The range of heterozygote deviation scores (Selander's D, 1970) for the genetically variable populations of *U. imbecillis*, *U. "imbecillis,"* *U. peggyae*, and *U. peninsularis* was -1.000 to -0.181 , -1.00 to -0.632 , -0.177 to 0.197 , and -0.120 to 0.364 , respectively. The mean heterozygote deviation score for *U. imbecillis* was -0.661 , for *U. "imbecillis"* -0.816 , for *U. peggyae* 0.031 , and for *U. peninsularis* 0.032 . Mann-Whitney U tests detected no significant differences in either number of loci with significant departures from H-W expectations or heterozygote deviation scores between *U. imbecillis* and *U. "imbecillis"* and between *U. peggyae* and *U. peninsularis*. However, there was a significant difference in both number of loci with significant departures from H-W expectations ($U = 35.0$, $p = .0193$) and heterozygote deviation scores ($U = 0$, $p < .0001$) between the combined gonochoric and combined hermaphroditic species groupings.

Among-population genetic differentiation was relatively high in the four species of *Utterbackia*. Mean F_{ST} for *U. imbecillis*, *U. "imbecillis,"* *U. peggyae*, and *U. peninsularis* was 0.818 (based on 5 loci), 0.218 (1 locus), 0.348 (6 loci), and 0.600 (5 loci), respectively. No clear pattern relating mating system and levels of among-population genetic differentiation emerged from this analysis. For example, the two hermaphroditic species, *U. imbecillis* and *U. "imbecillis,"* had both the highest and lowest mean F_{ST} values, respectively, for the genus. However, the relatively low mean F_{ST} value for *U. "imbecillis"* (0.218) may be due to both the limited number of populations (four) and the few variable loci (one). Overall, the relatively high F_{ST} values observed in *Utterbackia* may be due, in part, to the sampling design of the present study. Most of the drainages represented contained a single sampled population of each species. Expanding the within-drainage sampling for each species would likely reduce their global F_{ST} values.

Overall, both individuals and populations of the simultaneously hermaphroditic *U. imbecillis* and *U. "imbecillis"* had less genetic variation than the gonochoric *U. peggyae* and *U. peninsularis*.

APPENDIX I.

Locality information and sample sizes for the 40 populations of *Utterbackia* examined in this study.
Utterbackia imbecillis (n = 331)

- 1) ACi-Apalachicola River, below Lake Seminole, Chattahoochee, Gadsden Co., FL (n = 33)
- 2) BHCi-Ocmulgee River, at the Ben Hill/Coffee Co. line boat ramp, GA (n = 11)
- 3) CACi-C100A canal, Miami, Dade Co., FL (n = 4)
- 4) CCLi-Lake Corpus Christi, at Lake Corpus Christi State Park, San Patricio Co., TX (n = 7)
- 5) CDi-Cedar River, below Wiggins Lake, Gladwin Co., MI (n = 32)
- 6) DCLi-Deep Creek, at US Route 258, Edgecombe Co., NC (n = 5)
- 7) DRLi-mouth of Dead River Lake, off of the Pascagoula River, Jackson Co., MS (n = 7)
- 8) GALi-Pond at Suntree Country Club, Brevard Co., FL (n = 33)
- 9) GLCi-Gantt Lake, off of US Route 29, Covington Co., AL (n = 14)
- 10) KLTi-Kentucky Lake, at Paris Landing State Park, Henry Co., TN (n = 18)
- 11) KRK8i-Kokosing River, below Knox Lake, Knox Co., OH (n = 7)
- 12) KRK9i-Kankakee River, at US Route 45, Kankakee, Kankakee Co., IL (n = 6)
- 13) KRMi-Kankakee River, Momence, Kankakee Co., IL (n = 4)
- 14) LCHi-Lake Cass, Hillsborough Co., FL (n = 16)
- 15) LRGi-Little River, at FL Route 12, Gadsden Co., FL (n = 5)
- 16) LTCi-Ochlockonee River, Lake Talquin, Coe's Landing, Leon Co., FL (n = 12)
- 17) MCI-Mill Creek, below Starve Hollow Lake, Jackson Co., IN (n = 12)
- 18) MCWi-Mantua Creek, at Lambs Road bridge, Pitman, Gloucester Co., NJ (n = 7)
- 19) OSFi-Oklawaha River, at FL Route 314, Marion Co., FL (n = 24)
- 20) PCi-Pickering Creek, at PA Route 23, Chester Co., PA (n = 12)
- 21) PRWi-Pearl River, at Walkiah Bluff, Pearl River Co., MS (n = 4)
- 22) SRI-Canal off of the Suwannee River, at Dilger's Campground, Dixie Co., FL (n = 34)
- 23) UPPi-Pond off of Poinciana Blvd., Polk Co., FL (n = 24)

U. imbecillis (n = 51)

- 1) CRHi-Combahee River, at US Route 17A, Hampton Co., SC (n = 11)
- 2) FPCi-Fisher's Pond, Mt. Pleasant, Cabarrus Co., NC (n = 20)
- 3) ORTi-Ohoopsee River, at GA Route 147, Tattnall Co., Ga (n = 7)
- 4) SRGi-Saluda River, at Saluda Dam Road, Pickens Co., SC (n = 13)

U. peggyae (n = 99)

- 1) ACHp-Attapulugus Creek, at FL Route 159, Gadsden Co., FL (n = 16)
 - 2) CSLp-Chumuckla Springs Lake, Santa Rosa Co., FL (n = 15)
 - 3) HCCp-Holmes Creek, at US Route 90, Holmes Co., FL (n = 8)
 - 4) LRGp-Little River, at FL Route 12, Gadsden Co., FL (n = 16)
 - 5) LTCp-Ochlockonee River, Lake Talquin, Coe's Landing, Leon Co., FL (n = 15)
 - 6) SHFp-sink hole, at Florida Caverns State Park, Jackson Co., FL (n = 19)
 - 7) YRCp-Yellow River, at US Route 90, Okaloosa Co., FL (n = 10)
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continued in next column

APPENDIX I.

continued

U. peninsularis (n = 77)

- 1) GCPs-Gator Creek, at FL Route 471, Polk Co., FL (n = 14)
 - 2) HRHs-Hillsborough River, at FL Route 579, Hillsborough Co., FL (n = 13)
 - 3) HRPs-Hillsborough River, at FL Route 39, Pasco Co., FL (n = 5)
 - 4) NRBs-New River, at County Route 231, Bradford Co., FL (n = 5)
 - 5) RCAs-Rocky Creek, at FL Route 235, Alachua Co., FL (n = 20)
 - 6) SRs-canal off of the Suwannee River, at Dilger's Campground, Dixie Co., FL (n = 20)
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Extreme heterozygote deficits were commonly observed in both *U. imbecillis* and *U. "imbecillis."* Neither *U. peggyae* nor *U. peninsularis* exhibited significant wide-scale heterozygote deficiencies similar to those observed in many gonochoric marine bivalve species (Singh and Green 1984, Zouros and Foltz 1984, Gaffney et al. 1990).

DISCUSSION

Mating Systems in Utterbackia imbecillis s.l.

Heterozygote deficiencies in marine bivalves have been attributed to (1) inbreeding, (2) inadvertently sampling multiple differentiated subpopulations at a single locality (Wahlund effect; Wahlund 1928), (3) selection against heterozygotes, (4) null alleles, (5) aneuploidy, and (6) molecular imprinting (Gaffney et al. 1990). The relatively large number of populations with substantial heterozygote deficiencies noted in the present study makes it unlikely that sampling differentiated subpopulations is the major explanation for the observed heterozygote deficiencies in *U. imbecillis* and *U. "imbecillis."* Furthermore, the absence of substantial heterozygote deficiencies in the congeneric and ecologically similar, but gonochoric, *U. peggyae* and *U. peninsularis* does not support this explanation. Since particular populations of *U. imbecillis* had multiple polymorphic loci displaying significant heterozygote deficiencies (populations 1, 10, 22) (Table 1), invoking selection against heterozygotes at multiple, presumably independent loci to explain the overall heterozygote deficiencies observed in *U. imbecillis* seems unwarranted. Certainly, this type of selection is not generally operative in *U. peggyae* or *U. peninsularis* (see Table 1). The syntopic occurrences of *U. imbecillis* with *U. peggyae* (at LRG) and *U. peninsularis* (at SR; see Appendix I), though the disparity of the respective heterozygote deficits was maintained, do not support a hypothesis of differential underdominant selection based on habitat. Likewise, there is no reason to expect that null alleles, aneuploidy, or molecular imprinting would be more common in *U. imbecillis* and *U. "imbecillis"* than in *U. peggyae* or *U. peninsularis*.

Given the above discussion, an absolute linkage between large heterozygote deficiencies and simultaneous hermaphroditism among these species is suggestive. The most likely explanation for the relatively large heterozygote deficiencies observed in certain populations of *U. imbecillis* and *U. "imbecillis"* is a high level of inbreeding resulting from substantial self-fertilization in those particular populations. Indeed, Johnston et al. (1998) detected significant non-zero selfing rates in two of seven *U. imbecillis* populations examined individually and in the mean selfing rate for the

APPENDIX II.

Presumptive loci scored and buffer systems used in this study.

Enzyme	No. Loci Scored	Abbreviation	E.C. No.	Buffer System
Aspartate aminotransferase	1	AAT	2.6.1.1	TMME 7.4
Cytosol aminopeptidase	1	CAP	3.4.11.1	TC 8.0
Dihydroliipoamide dehydrogenase	1	DDH	1.8.1.4	MC 5.5
Esterase (alpha naphthyl acetate)	1	EST	3.1.1.-	MC 6.0
Fumarate hydratase	1	FUMH	4.2.1.2	TMME 7.4
Glucose-6-phosphate isomerase	1	GPI	5.3.1.9	MC 5.5
Glycerol-3-phosphate dehydrogenase	1	G3PDH	1.1.1.8	TC 8.0
Isocitrate dehydrogenase	1	IDH	1.1.1.42	MC 6.0 NADP
Phosphoglucomutase	1	PGM	5.4.2.2	MC 6.0
Total = 9				

References for the electrophoretic buffer systems are as follows: tris-maleic acid-magnesium chloride-EDTA pH 7.4 (TMME 7.4, Spencer *et al.*, 1964), tris-citrate pH 8.0 (TC 8.0, Selander *et al.* 1971), 3-amino propyl morpholine-citrate pH 6.0 (MC 6.0, Clayton and Tretiak 1972). The MC5.5 system was identical in composition to the MC 6.0 system except that the gel buffer was pH 5.5. When NADP was added to a particular electrophoretic system, 20 mg was added to the gel (425 ml) and 10 mg was added to the cathodal (-) electrode tray.

species; significant non-zero mean selfing rates were not detected in *U. peggyae* or *U. peninsularis*, as expected for gonochoric species. The self-fertilization hypothesis could be further tested by comparing nuclear genotypes of brooded offspring with those of the brooding parent.

Since the ratio of the volume of testicular to ovarian tissue in the gonad of a simultaneous hermaphrodite is expected to vary with mating system (Charnov 1982, Eernisse 1988), Kat's (1983) gonadal allocation data can be used to test the 'variable levels of self-fertilization' hypothesis. A positive correlation between levels of within-individual genetic variability and testicular: ovarian allocation ratios for particular populations of *Utterbackia imbecillis* would corroborate the 'variable levels of self-fertilization' hypothesis. Four populations of *U. imbecillis* were both included in this study and that of Kat (1983): BHCi, LTCi, PCi, and SRi. BHCi and PCi are from lotic habitats and LTCi and SRi are from standing water habitats (Appendix I). LTCi is allozymically monomorphic whereas SRi has a heterozygote deviation score of -1.000. BHCi and PCi have heterozygote deviation scores of -0.753 and -0.758, respectively. In Kat's (1983) study, individuals from LTCi and SRi had relatively low testicular to ovarian tissue volume ratio estimates (0.09 and 0.18, respectively); individuals from BHCi and PCi had relatively high testicular to ovarian tissue ratios (0.47 and 0.36, respectively). The evident positive correlation between the levels of within-individual genetic variability and the testicular to ovarian tissue ratio estimates (Kat 1983) for these four populations is in strong accord with predictions from sex allocation theory, i.e., self-fertilizing hermaphroditic individuals are expected to produce less spermatozoa than cross-fertilizing hermaphroditic individuals (Charnov 1982). Additional estimates of *U. imbecillis* gonadal tissue volumes and their correlation with rates of self-fertilization were presented in Johnston *et al.* (1998). Therein, individuals from high selfing rate populations had a significantly lower proportion of testicular tissue, in support of sex allocation theory.

In the absence of additional data, the stark contrast between the heterozygote deviation scores of *Utterbackia imbecillis*/*U. "imbecillis"* and *U. peggyae*/*U. peninsularis* are most readily explained by recourse to the high levels of inbreeding produced by self-fertilization. This hypothesis is supported by the (1) overall levels of within-population variation; (2) number of loci with significant departures from H-W equilibrium; (3) levels of heterozygote defi-

cit observed in the simultaneously hermaphroditic species compared with those observed in the obligately cross-fertilizing *U. peggyae* and *U. peninsularis* (Table 1); (4) the positive correlation, observed in *U. imbecillis*, between the levels of within-individual genetic variability determined herein and the testicular to ovarian tissue volume ratio estimates of Kat (1983); and (5) the positive correlation, observed in *U. imbecillis*, between the rates of cross fertilization and the proportion of male gonadal tissue (Johnston *et al.* 1998). In addition, the wide range of among-population variation in heterozygote deviation scores (see Table 1) suggests that the relative frequency of self-fertilization likely varies among populations of *U. imbecillis* and *U. "imbecillis."*

In order to categorize populations of *U. imbecillis s.l.* by mating system type (i.e., predominantly self-fertilizing, mixed selfing and cross-fertilizing, or predominantly cross-fertilizing), criteria were used that are similar to those used by McCracken and Selander (1980) to determine the mating system assignments for terrestrial slugs. From these somewhat arbitrary criteria, 14 of the 23 (60.9%) assayed populations of *U. imbecillis* are categorized as predominantly self-fertilizing (i.e., populations having no polymorphic loci or having a heterozygote deviation score of -1.00; populations 3-6, 9, 11-14, 16, 18, 21-23; Table 1), seven (30.4%) as having a mixed mating system involving some level of cross-fertilization (i.e., populations having a heterozygote deviation score greater than -1.00 but markedly less than the lowest value seen in the obligately cross-fertilizing *U. peggyae* and *U. peninsularis* (-0.177); populations 1, 2, 8, 10, 15, 17, 20; Table 1), and two (8.7%) as having a predominantly cross-fertilizing mating system (i.e., populations having a heterozygote deviation score similar to the lowest value seen in *U. peggyae* or *U. peninsularis*; populations 7 and 19; Table 1). Similarly, three *U. "imbecillis"* populations (75%) are categorized as predominantly self-fertilizing (populations 2-4; Table 1) and one (25%) population (population 1; Table 1) is categorized as mixed mating. Natural populations of pulmonate gastropods as well as most other hermaphroditic molluscan taxa are thought to be predominantly cross-fertilizing (Foltz *et al.* 1984, Tompa *et al.* 1984, O Foighil and Eernisse 1987, Jarne *et al.* 1993, Jarne and Charlesworth 1993). Therefore, the inference that most of the *U. imbecillis s.l.* populations examined herein are undergoing some degree of self-fertilization runs contrary to expectations. No strong correlations are evident between these mat-

TABLE 1.
Within-population variation at 9 loci in *Utterbackia*.

	N	Mean # Alleles per locus & S.E.	# Polymorphic Loci (%)	# Loci with a Significant Departure from H-W Exp.	Heterozygote Deviation (D)	Mating System
Hermaphroditic Species						
<i>U. imbecillis</i> (n = 331)						
1) ACi	33	1.33 ± 0.17	3 (33)	3	-0.968	M
2) BHCi	11	1.11 ± 0.11	1 (11)	1	-0.753	M
3) CACi	4	1.00 ± 0.00	0 (0)	—	—	S
4) CCLi	7	1.00 ± 0.00	0 (0)	—	—	S
5) CDi	32	1.00 ± 0.00	0 (0)	—	—	S
6) DCLi	5	1.00 ± 0.00	0 (0)	—	—	S
7) DRLi	7	1.11 ± 0.11	1 (11)	0	-0.204	C
8) GALi	33	1.11 ± 0.11	1 (11)	1	-0.745	M
9) GLCi	14	1.00 ± 0.00	0 (0)	—	—	S
10) KLTi	18	1.33 ± 0.17	3 (33)	2	-0.825	M
11) KRK8i	7	1.00 ± 0.00	0 (0)	—	—	S
12) KRK9i	6	1.00 ± 0.00	0 (0)	—	—	S
13) KRMi	4	1.00 ± 0.00	0 (0)	—	—	S
14) LCHi	16	1.00 ± 0.00	0 (0)	—	—	S
15) LRGi	5	1.11 ± 0.11	1 (11)	0	-0.571	M
16) LTCi	12	1.00 ± 0.00	0 (0)	—	—	S
17) MCI	12	1.22 ± 0.15	2 (22)	1	-0.606	M
18) MCWi	7	1.00 ± 0.00	0 (0)	—	—	S
19) OSFi	24	1.22 ± 0.15	2 (22)	0	-0.181	C
20) PCi	12	1.11 ± 0.11	1 (11)	1	-0.758	M
21) PRWi	4	1.00 ± 0.00	0 (0)	—	—	S
22) SRi	34	1.22 ± 0.15	2 (22)	2	-1.000	S
23) UPPi	24	1.00 ± 0.00	0 (0)	—	—	S
<i>U. "imbecillis"</i> (n = 51)						
1) CRHi	11	1.11 ± 0.11	1 (11)	0	-0.632	M
2) FPCi	20	1.00 ± 0.00	0 (0)	—	—	S
3) ORTi	7	1.00 ± 0.00	0 (0)	—	—	S
4) SRGi	13	1.11 ± 0.11	1 (11)	1	-1.000	S
Mean	14.1	1.08	0.70 (7.7)	1.00	-0.687	
Gonochoric Species						
<i>U. peggyae</i> (n = 99)						
1) ACHp	16	1.56 ± 0.34	3 (33)	0	0.152	C
2) CSLp	15	1.33 ± 0.17	3 (33)	0	0.029	C
3) HCCp	8	1.44 ± 0.24	3 (33)	0	0.197	C
4) LRGP	16	1.44 ± 0.24	3 (33)	1	-0.158	C
5) LTCp	15	1.56 ± 0.34	3 (33)	0	-0.177	C
6) SHFp	19	1.22 ± 0.15	2 (22)	0	0.057	C
7) YRCp	10	1.11 ± 0.11	1 (11)	0	0.118	C
<i>U. peninsularis</i> (n = 77)						
1) GCPs	14	1.22 ± 0.15	2 (22)	0	-0.120	C
2) HRHs	13	1.22 ± 0.22	1 (11)	0	0.056	C
3) HRPp	5	1.22 ± 0.15	2 (22)	0	0.364	C
4) NRBs	5	1.11 ± 0.11	1 (11)	0	0.000	C
5) RCAs	20	1.22 ± 0.15	2 (22)	0	-0.055	C
6) SRs	20	1.56 ± 0.24	4 (44)	1	-0.056	C
Mean	13.5	1.32	2.31 (25.4)	0.15	0.031	

Mating system designations are the following: C = predominantly cross-fertilizing; M = mixed (cross- and self-fertilizing); S = predominantly self-fertilizing.

ing system assignments and (1) type of aquatic habitat (lentic versus lotic systems) or (2) geographic location (cf. Table 1 and Appendix I). However, the two populations of *U. imbecillis* that have relatively small heterozygote deficiencies (DRLi and OSFi) are located at relatively low latitudes.

It should be emphasized that these mating system categories,

based on the distribution of within-population genetic variation, are rough approximations. Equating complete genetic monomorphism with a predominantly self-fertilizing mating system is especially tenuous because such homogeneity has been detected in obligately cross-fertilizing species (Bonnell and Selander 1974, Schnell and Selander 1981). However, the lack of completely

monomorphic populations in the obligately cross-fertilizing (gonochoric) species of this study is consistent with the rationale for these particular mating system assignments. The lower within-population levels of genetic variation observed in the hermaphroditic *Utterbackia imbecillis* and *U. imbecillis*, relative to congeneric gonochores, and the extremely high mean F_{ST} value for the well-sampled *U. imbecillis* suggest that mating system differences have had an impact on the intraspecific distribution of genetic variation within *Utterbackia*. Significant levels of selfing, in combination with genetic drift, have likely reduced the within-population component of genetic variation in the hermaphroditic species of *Utterbackia* while augmenting genetic differentiation among populations of *U. imbecillis* through founder effects.

Inferences Regarding the Dispersal Capability of Utterbackia imbecillis

Species in the predominantly gonochoric Unionidae typically exhibit a relatively high degree of drainage-basin philopatry (LaRocque 1967, Burch 1975a, McMahon 1991). For example, no unionid species is native to both sides of the North American continental divide. Additionally, very few species have ranges that include portions of both the Mississippi River and Atlantic Slope drainages. In these instances, it is apparent that mountain ranges are acting as formidable barriers to dispersal for unionid bivalves. Even in cases where there are no obvious barriers to dispersal (e.g., the low coastal plain habitat that is currently separating *U. peggyae* and *U. peninsularis*) or obvious habitat differences, unionid species' distributional patterns suggest that inter-drainage colonization is a relatively rare event.

In contrast, the distributional ranges exhibited by the simultaneous hermaphroditic, freshwater bivalves of the family Sphaeriidae are relatively cosmopolitan in nature (LaRocque 1967, Burch 1975b). Many native North American sphaeriids have transcontinental distributions (McMahon 1991, Burch 1975b). Self-fertilization is apparently common in sphaeriid bivalves (Thomas 1959, Mackie 1984) and the ability of single, self-fertilizing sphaeriid individuals to colonize new habitats is postulated as one of the major factors responsible for the relatively cosmopolitan species distributions in this taxon (McMahon 1991). Therefore, the self-fertilization potential of *Utterbackia imbecillis* may largely account for its atypically (i.e., for a gonochoric unionid bivalve) widespread geographic distribution in eastern North America. Additional evidence consistent with relatively high dispersal capability for *U. imbecillis* includes the (1) postulated southeastern USA origin for this now-widespread species (Hoeh et al. 1995), (2) lack of among-population resolution for *U. imbecillis* in a phylogenetic analysis of *Utterbackia* (Hoeh et al. 1995) and (3) reports of recent range expansion into areas where it has been historically absent (Starrett 1971, Fuller and Hartenstine 1980, Kat 1983, Hoeh et al. 1995).

Further investigation is necessary to understand a somewhat paradoxical finding of this study with regard to *U. "imbecillis."* An initial hypothesis postulated that this geographically restricted, simultaneous hermaphrodite was predominantly cross-fertilizing (Hoeh et al. 1995). However, analyses of within-population genetic variability (Table 1) suggest that *U. "imbecillis"* populations, like many of those of *U. imbecillis*, are not predominantly cross-fertilizing. If self-fertilization typically facilitates dispersal in freshwater bivalves, what factor(s) is limiting the geographic range of *U. "imbecillis?"*

The range of *U. "imbecillis"* is in an area of the southern Atlantic Slope that has a relatively large number of ichthyofaunal endemics (Lee et al. 1980, Shute et al. 1981, Wiley and Mayden 1985, Hocutt et al. 1986, Swift et al. 1986, Wood and Mayden 1992). If *U. "imbecillis"* has an obligate host-fish association with one or more of the endemic fish species of the region, this would preclude its movement into other drainages lacking the required fish hosts. A determination of the host-fish requirements of *U. "imbecillis"* is necessary to test this hypothesis. However, the dependence of *U. "imbecillis"* on other unique attributes of the drainages of the southern Atlantic Slope cannot be ruled out at the present time. A relatively recent transition to predominantly self-fertilization is another possibility that would explain the relatively small geographic range of *U. "imbecillis."*

Evolution of Mating Systems in Utterbackia imbecillis

Of broad interest (Ghiselin 1969, 1974, Maynard Smith 1978, Charnov 1982, Lloyd 1988, Jarne and Charlesworth 1993) is the elucidation of the processes involved in the maintenance of the wide range of mating systems (predominantly cross-fertilizing through predominantly self-fertilizing) inferred for *Utterbackia imbecillis* s.l. in this report. This range of mating systems may represent multiple optimal end points generated by local selective regimes or, in the case of the mixed-mating system populations, merely transient states on the way toward either predominantly cross- or self-fertilization. However, the lack of (1) long-term studies of the interpopulational variation in mating systems, (2) knowledge of ecological requirements, and (3) knowledge of fertilization biology in *U. imbecillis* currently precludes a deeper understanding of the evolutionary forces shaping the mating system in this species.

The fertilization biology of the gonochoric, let alone the simultaneously hermaphroditic, unionid bivalves is, at best, poorly understood. Fertilization in unionids is thought to take place within the female suprabranchial chambers or in the marsupia (gills modified for larval brooding) in gonochoric species, but the details of the process are unknown (Coker et al. 1921, Matteson 1948). However, it appears that in multiple species of gonochoric unionids, representing different subfamilies, spherical spermatozoa aggregates are released by males prior to fertilization (Utterback 1931, Edgar 1965, Lynn 1994). That these aggregates are released from male unionids from diverse taxa suggests that this conserved feature may be essential for cross-fertilization. Data from *Pyganodon grandis* (Unionidae: Anodontinae) suggest that females collect these spermatozoa aggregates with their gills and store the spermatozoa in their nephridia (W. R. Hoeh and R. J. Trdan unpubl. data). Subsequently, the spermatozoa are likely released from the nephridial pore as oocytes are released from the adjacent genital opening, thus effecting fertilization.

In simultaneously hermaphroditic unionids, a common gonoduct carries both oocytes and autospermatozoa to the suprabranchial chambers (Mackie 1984). This suggests that the oocytes of a simultaneous hermaphrodite may encounter autospermatozoa in the common gonoduct before encountering allospermatozoa (which may be retained in the suprabranchial chambers or brood pouches?). The use of a common gonoduct may favor self-fertilization unless the release of male and female gametes can be displaced in time. Observations made during the course of a preliminary examination of the reproductive biology of *Utterbackia imbecillis* (Hoeh et al. 1986) noted a concurrent release of oocytes

and, presumably, autospermatozoa in the gonoducts of individuals from four of the populations examined in the present study (ACi, BHCi, CDi, MCi). Furthermore, the spermatozoa within the gonoducts were not contained in spherical aggregates as mentioned above for male unionids. The individuals examined were from populations that were likely undergoing significant amounts of self-fertilization (see discussion above and Table 1).

These limited observations on the fertilization biology of *U. imbecillis* suggest that (1) the use of a common gonoduct in simultaneous hermaphrodites may predispose the mating system towards self-fertilization and (2) all spermatozoa may not be capable of effecting cross-fertilization. More information regarding the details of fertilization biology for both gonochoric and hermaphroditic unionids is required to address these possibilities. Specifically, comparative information on the fertilization biology of individuals of *U. imbecillis* from populations that were categorized, herein, as self-fertilizing, mixed mating, and cross-fertilizing would be most illuminating. Questions still to be addressed include (1) Does the selfing rate vary among individuals within *U. imbecillis s.l.* populations? (2) Is the selfing rate within a *U. imbecillis s.l.* population relatively fixed or plastic over time? (3) Is the selfing rate within populations of *U. imbecillis s.l.* correlated with particular environmental variables? The answers to these questions will be of broad interest and utility to evolutionary, conservation, and mollusk biologists.

Conservation Implications

Bivalves in the family Unionidae are one of the most endangered groups of North American aquatic macroinvertebrates (Bogan 1993, Williams et al. 1993, Lydeard and Mayden 1995). However, unionid conservation and management plans have been hampered by a relative lack of knowledge concerning (1) unionid ecological requirements (e.g., fish host and dietary), (2) the actual extent of unionid biodiversity, (3) the distribution of genetic variation within species, and (4) mating systems. Studies such as this one are useful for assessing the distribution of genetic variation within species and to evaluate the effects of mating system variation on the distribution of genetic variation (Hamrick and Godt 1990). Knowledge of a species' genetic structure can provide important insights into population-level processes such as colonization, extinction, and gene flow as well as for conservation/management initiatives (Slatkin 1977, 1985, 1993, Falk and Holsinger 1991, McCauley 1991, 1992).

The superior colonization potential of self-fertilizing species, such as *Utterbackia imbecillis*, may be partially offset, in an evolutionary sense, by the reduction of within-population genetic variation due to the combined effects of selfing and founder events. For instance, the relatively large geographic range of *U. imbecillis* will likely act to buffer this species from complete extinction. However, the relatively low levels of within-population genetic variation observed in *U. imbecillis s.l.*, with respect to *U. peggyae* and *U. peninsularis*, may predispose individual populations of these hermaphroditic species to increased probability of extinction due to reduced ability to cope with both natural (Thorpe et al. 1981, Allendorf and Leary 1986, Lively et al. 1990) and anthropogenic (Guttman 1994) environmental change. Therefore, extinction and colonization are potentially important population-level processes within *Utterbackia*, and metapopulation-dynamic models may be especially applicable to the hermaphroditic *Utterbackia* species (Hastings and Harrison 1994).

The relatively high F_{ST} values reported herein for four species of *Utterbackia* indicate that much of the genetic variation within each species is distributed among populations, suggesting relatively low levels of historical gene flow. An especially high F_{ST} value (0.818) was obtained for *U. imbecillis*. This latter result, which is counter intuitive for an able colonizer such as *U. imbecillis*, is likely an outcome of significant levels of self-fertilization, which leads to a within-individual reduction of genetic variability, combined with frequent population extinctions and single-individual founder events facilitating the fixation of distinct alleles in different populations (Wade and McCauley 1988, McCauley 1991, McCauley 1992). Therefore, the normally homogenizing effects of dispersal are likely potentiating among-population differentiation within *U. imbecillis*.

Overall, the relatively high F_{ST} values observed herein within four species of *Utterbackia* have significant implications for potential conservation plans for these species. Since the among-population component of genetic variation is relatively high, any conservation initiative to preserve a significant amount of the genetic diversity within species of *Utterbackia* would necessitate the protection of multiple populations of each species. One potential strategy, consistent with the findings of this report, would be to protect one or more populations within each distinct drainage basin for each *Utterbackia* species. Additionally, transplantation initiatives, to aid population recovery efforts, should take into consideration the genetic structure of the target species. Transplantations of individuals between genetically distinct populations could lead to (1) the detriment of the recipient population due to outbreeding depression if cross-fertilization occurs (Templeton 1986), (2) the introduction of genotypes that are poorly adapted to the new ecological conditions (Huenneke 1991), and (3) loss of the original genotypes due to the potential superior competitive ability of the transplanted individuals' genotypes. Clearly, the preservation of a species' overall genetic diversity is desirable for its long-term survival and is dependent upon an understanding of the genetic structure of the constituent populations (Hamrick et al. 1991, Saccheri et al. 1998).

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