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GENETIC STRUCTURE OF *QUADRULA QUADRULA* (BIVALVIA: UNIONIDAE): LITTLE VARIATION ACROSS LARGE DISTANCES

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ABSTRACT North American freshwater bivalves of the families Unionidae and Margaritiferidae represent one of the endangered faunas of the world. Effective management of threatened and endangered species requires knowledge not only of abundances of these species but also the degree of variation within species and the geographic distribution of this intraspecific variation. We used allozyme electrophoresis to examine the genetic structure of seven *Quadrula quadrula* populations from the Ohio, Tennessee, and Tensas Rivers. We then considered the implications of our results for the development of effective bivalve conservation strategies. Descriptive measures of genetic variation within populations are quite high (2.1 ± 0.1(se) alleles per locus; 61.4 ± 2.6% polymorphic loci; 0.24 ± 0.01 heterozygosity) relative to other unionids. Genotype frequencies met Hardy-Weinberg expectations at all polymorphic loci. Among-population variation was low and mostly confined to differences between the Tensas River population (lower Mississippi River basin) and the Ohio River basin populations. Significant differences in allele frequencies among populations were only detected at 3 of 10 loci; no differences in allele frequencies were found among Ohio River basin populations. Genetic distances, though all small, were significantly correlated with geographic distance. Estimated gene flow was high among populations, but variation among populations did tend to follow the predictions of an isolation-by-distance model of dispersal. The low levels of among-population genetic variation are remarkable given that these populations are separated by distances as great as 2,500+ river kilometers. High levels of gene flow may ensure that within-population variation remains high and that populations do not become differentiated due to genetic drift. An optimum conservation strategy for this species in the mainstem of the Ohio River would center on the protection of a number of large populations and maintenance of corridors for dispersal of host fishes. Successful protection of threatened and endangered species requires conservation of both abundance and genetic diversity of unionids. Further work is needed to characterize general patterns of genetic structure within freshwater bivalve species.

KEY WORDS: *Quadrula,* Unionidae, genetic structure, geographic patterns, within-population variation, among-population variation, conservation strategies

INTRODUCTION

Freshwater unionoid bivalves (Unionidae and Margaritiferidae) are a principal constituent of many Nearctic riverine and lake communities. Approximately 300 taxa have been described from North America, with a large number of them endemic to the central and southeastern United States (Williams et al. 1993). As the center of diversity of this fauna, the southeastern U.S. may be considered the unionoid "equivalent" of the Amazonian rain forests, with their immense diversity of insect species. Just as the rain forests have seen an enormous loss of biodiversity, North American freshwater ecosystems have suffered precipitous declines in species richness and abundance of bivalve communities, with between 55% and 72% of North American unionoid taxa listed as extinct, threatened, or of special concern (Master 1990, Williams et al. 1993). State and federal agencies have made conservation of the North American unionoid bivalve fauna a priority, and private environmental organizations have begun to publicize the plight of these organisms (Stolzenburg 1992).

The primary threats to the survival of this immensely rich fauna are human-induced sources of stress such as habitat modification (impoundment and channelization of rivers), commercial harvest, and pollution (Bogan 1993, Neves 1993). Recently introduced exotic species, primarily the zebra mussel (*Dreissena polymorpha*), present an additional challenge to the health of native freshwater bivalves (Haag et al. 1993). Unionid populations have declined precipitously in ecosystems such as Lakes Erie and St. Clair, where zebra mussel populations have exploded (Nalepa et al. 1996).

It is now generally accepted that preservation of biodiversity requires maintenance of genetic diversity within threatened species (Schonewald-Cox et al. 1983, Soulé 1987). By definition, populations of threatened and endangered species are often small. They exhibit genetic behavior characteristic of small populations with the "loss of genetic variation resulting in the erosion of evolutionary flexibility" (Meffe 1986). These populations are susceptible to the effects of genetic drift, with its continuing erosion of genetic variation, and to extirpation (Franklin 1980). Maintenance of genetic variation within declining populations is more difficult as these populations become fragmented and gene flow becomes restricted.

Genetic differentiation of populations within a species is a function of gene flow between the populations, with greater gene flow leading to less interpopulation variation (Slatkin 1987). The minimum spatial scale at which differentiation of populations can be detected can vary from several meters (Guttman et al. 1989) to hundreds of kilometers (Berg and Garton 1994), depending on

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gene flow and dispersal ability among the populations studied. Reports of genetic variation in freshwater bivalves of the family Unionidae have concentrated on relationships among species or genera (Davis and Fuller 1981, Kat 1983a, Hoeh 1990, Stiven and Alderman 1992, Hoeh et al. 1995) or have examined patterns within species over broad geographic areas (Kat and Davis 1984, Hoeh et al. 1995, Mulvey et al. 1997). These studies have not examined the partitioning of genetic variation within and among populations. However, if government agencies and conservation organizations are to properly manage and protect threatened and endangered unionids, "normal" patterns of intrapopulation variation and gene flow between populations must be determined. These patterns can then be used to develop conservation strategies designed to preserve genetic structure of endangered species.

In this paper, we characterize the population genetic structure of *Quadrula quadrula,* a common freshwater bivalve typical of medium to large river systems of the southeastern and midwestern U.S. Specifically, we describe allozyme variation within and among seven populations from the Ohio, Tennessee, and Tensas Rivers. The observed levels of variation are used to estimate the degree of gene flow and differentiation among populations from 3 to 2,600 river kilometers apart. We place these results in a conservation biology framework, using *Q. quadrula* as a model for endangered and threatened unionids.

MATERIALS AND METHODS

Sample Collection and Electrophoresis

Bivalves were collected from beds in the Ohio, Tennessee, and Tensas Rivers in 1993 (Fig. 1). Samples were taken from four consecutive beds in a 31 km stretch of the Ohio River near Middleport, OH (River Mile (RM) 238.6, RM 252.6, RM 255.3, and RM 257.7) and from a site 68 km downstream near Huntington, WV (RM 300) (Fig. 1). The Tennessee River was sampled in Kentucky Lake, located 72 river km from its confluence with the Ohio River and approximately 1,100 river km from the upstream Ohio River sites. The Tensas River was sampled at Tyndel, LA, about 340 river km upstream of its confluence with the Mississippi River, more than 1,500 river km away from Kentucky Lake, and more than 2,500 river km from the Ohio River sites. Thus, within-population variation was estimated for populations across a broad geographic range within the lower Mississippi River basin, whereas among-population variation could be measured over very short distances (Ohio River) and over successively greater distances.

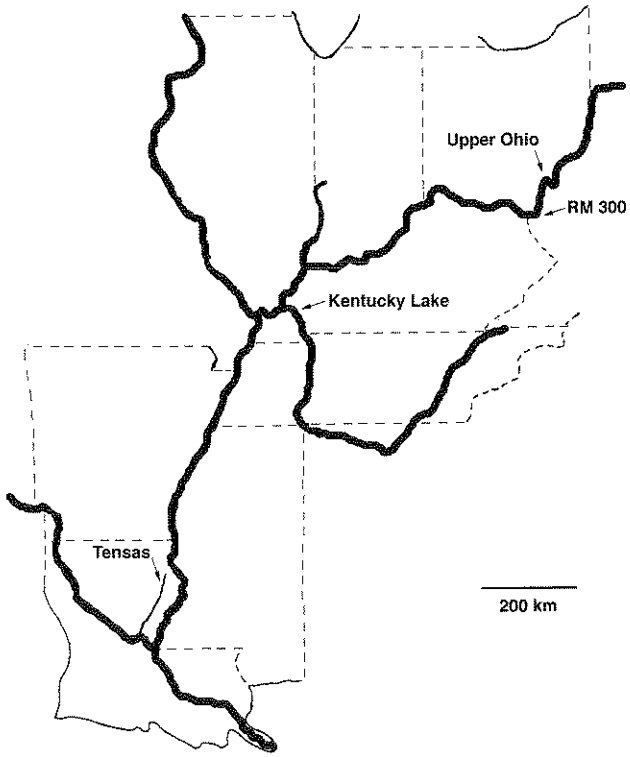

Figure 1. Populations of *Quadrula quadrula* sampled in this study. Population abbreviations follow those of the text. "Upper Ohio" sites are sites RM 238.6, RM 252.6, RM 255.3, and RM 257.7 on the Ohio River.

Individuals were collected by SCUBA, with the exception of the Tensas River, where it was shallow enough to collect while wading. At each site, 27–92 individuals were collected. For the three downstream sites, all bivalves were shucked and the mantle and adductor muscles were flash-frozen in liquid nitrogen and stored at −70°C until analyzed. For the other Ohio River populations, 20 individuals were destructively sampled, and the remainder were nondestructively sampled using a mantle biopsy (Berg et al. 1995). These tissue samples were stored as above.

Allozyme electrophoresis using starch and cellulose acetate gels was performed for eight enzyme systems, using standard recipes (Harris and Hopkinson 1976, Hebert and Beaton 1989) and buffer systems (Selander et al. 1971, Clayton and Tretiak 1972, Hebert and Beaton 1989). A total of 10 loci were resolved from these systems (Table 1). The number of loci analyzed was limited by the small amounts of tissue available for many individuals. At least 20 individuals were analyzed for all loci for each population; additional individuals were analyzed for each variable locus. Loci were considered "polymorphic" when the most common allele was present at a frequency of 0.95. Because we were often limited in the amount of tissue available from each individual and occasionally gels did not produce results, sample size varied from 3 to 92 for each locus-by-population combination (Table 2).

Statistical Analyses

Electrophoretic results were analyzed using standard population genetic techniques contained in BIOSYS-1 (Swofford and Selander 1981). Descriptive statistics calculated for each population included percent polymorphic loci, mean number of alleles per locus, and average direct-count heterozygosity. Among-population variation in mean number of alleles per locus and average direct-count heterozygosity was analyzed using one way analysis of variance. Comparison of measured genotype frequencies with Hardy-Weinberg expectations were evaluated using the "Exact Probabilities" procedure of BIOSYS-1 with the "sharper" sequential-comparison Bonferroni (s-cB) technique to adjust significance levels described by Lessios (1992). For polymorphic loci, allele frequencies among populations were compared using contingency χ^2 analysis with s-cB. Where necessary, rare alleles were pooled to meet the assumptions of this procedure.

Allele frequency differences were integrated across loci by calculating genetic distances for all pairs of populations (Nei 1978, Wright 1978). Genetic similarity of populations was determined by construction of dendrograms using modified Rogers' genetic dis-

TABLE 1.

Enzyme systems, gels, and buffers used with *Quadrula quadrula*.

Enzyme System	Number of Loci	E. C. Number	Gel Type	Buffer System	
Esterase	2	3.1.1.1	Starch	TC 6.7	1 visible locus[a] 1 UV locus[b]
Glucosephosphate isomerase	1	5.3.1.9	Starch	CT	
Malate dehydrogenase	2	1.1.1.37	Starch	CT	
Mannose phosphate isomerase	1	5.3.1.8	Starch	LiOH	
Peptidase	1	3.4.11	Cellulose	TG	leucyl-tyrosine used as dipeptide
Phosphoglucomutase	2	2.7.5.1	Starch	TC 6.7	
Superoxide dismutase	1	1.15.1.1	Starch	LiOH	

Number of loci refers to the number of scoreable allozyme loci for a system.

[a] α-Naphthyl acetate used as substrate.

[b] 4-Methylumbelliferyl acetate used as substrate.

CT from Clayton and Tretiak (1972); TC 6.7 and LiOH from Selander et al. (1971); TG from Hebert and Beaton (1989).

tance and the unweighted-pair-group method (UPGMA) to cluster populations (Sokal and Sneath 1963). The dendrogram used a population of *Quadrula apiculata* (Cantonwine and Berg unpubl) as an outgroup and bootstrap values for the nodes of the dendrogram were obtained from 1,000 replicates using the software package Tools for Population Genetic Analysis (TFPGA) (Miller 1998). Correlation of genetic and geographic distance was determined using a Mantel test in TFPGA, with geographic distance measured as river distances (rather than straight-line distances) between sites. Among-population genetic variation was further analyzed by calculating values of F_{ST} (θ of Weir and Cockerham 1984) for polymorphic loci. Values were bootstrapped (1,000 replicates) to obtain 95% confidence intervals. The number of migrants per generation (Nm) among populations was estimated from F_{ST}, assuming a stepping-stone model of dispersal (Slatkin and Barton 1989).

RESULTS

Within-population Variation

Seven of the 10 loci sampled were polymorphic in at least one population (Table 2). One other locus (EST-UV) was variable but not polymorphic according to the 95% criterion (see above), whereas 2 loci (MDH-1 and SOD) were fixed for a single allele in all populations (minimum of 27 individuals sampled per population). Within-population genetic variation was similar for all populations, with the percentage of polymorphic loci varying from 50% to 70% (Table 2). Mean number of alleles per locus was not significantly different among populations ($n = 10$, $F_{6,63} = 1.43$, $p = .22$), nor was average direct-count heterozygosity ($n = 10$, $F_{6,63} = 0.13$, $p = .99$). All 45 polymorphic locus-by-population combinations had genotype frequencies that were not significantly different from Hardy-Weinberg expectation at an experiment-wise error rate of $\alpha = 0.05$.

Among-population Variation

Allele frequencies did not vary significantly among all populations for four polymorphic loci (EST, GPI, MDH-2, PEP; $\chi^2 = 25.2$, $n = 12$, experimentwise error rate of $\alpha = 0.05$; Table 2). Allele frequencies varied significantly among populations for the other three polymorphic loci (MPI, PGM-1, PGM-2; $\chi^2 = 49.2$, $n = 12$, experimentwise $\alpha = 0.05$). However, when the Tensas River population was omitted from the analysis, there were no significant differences in allele frequencies for these three loci among the remaining populations ($\chi^2 = 29.6$, $n = 10$, experimentwise $\alpha = 0.05$). For PGM-1, this was primarily due to the relatively high frequency of allele 1 in the Tensas River population and the presence of allele 2 in only the Kentucky Lake and Tensas River populations (Table 2). Allele 4 was present at relatively high frequency in the RM 300 population and at low frequency in the Tensas River population. For PGM-2, allele 2 was at least twice as common as allele 1 in all populations except the Tensas (Table 2). In the latter population, alleles 1 and 2 were present in similar frequencies. For MPI, the differences are apparently the result of the cumulative effect of differences in frequencies for each of the four alleles and the presence of allele 4 only in the two most downstream populations (Table 2). Thus, populations from the Ohio River basin do not have significant differences in allele frequencies at any polymorphic loci. For three of seven polymorphic loci, the Tensas River population has significantly different allele frequencies than the Ohio River basin populations.

Genetic distances were lowest among populations from the Ohio River basin, whereas distances between each of these populations and the Tensas River population were higher (Table 3). Cluster analysis shows that populations from the upper Ohio River are most similar; of these, the three populations that were closest geographically (RM 252.6, 255.3, and 257.7—a total of 8.3 km apart) had the lowest average genetic distances. The RM 238.6 population clustered with downstream populations (RM 300 and Kentucky Lake). These populations are more similar to other Ohio River populations than to the Tensas River population (Fig. 2). The relatively high (55.5%) bootstrap value for the Ohio Basin node reinforces the conclusion that the Tensas River population is different from these populations, whereas the generally low bootstrap values at the other nodes within the Ohio Basin are consistent with a lack of differentiation among these populations. The tight positive correlation of genetic distance with geographic distance ($r = 0.83$, $n = 21$, $p = .003$; Fig. 3) matches the predictions of an isolation-by-distance model of dispersal. Values of F_{ST} were low but significantly greater than zero over all populations; exclusion of the Tensas River population resulted in even lower, but still significant, values (Table 4). The mean value of Nm was 2.4 times larger when the Tensas River population was excluded from the analysis.

TABLE 2.

Allele frequencies at variable loci and descriptive measures of within-population genetic variation for *Quadrula quadrula*.

Locus	Allele	Population						
		RM 238.6	RM 252.2	RM 255.3	RM 257.7	RM 300	Kentucky Lake	Tensas River
EST[a]	1	—	—	—	—	—	0.01	—
	2	1.00	1.00	1.00	1.00	0.98	0.93	0.94
	3	—	—	—	—	0.03	0.06	0.06
	(n)	27	48	46	47	40	75	92
EST-UV	1	—	—	—	—	—	0.01	—
	2	0.98	1.00	1.00	1.00	1.00	0.97	0.98
	3	0.02	—	—	—	—	0.01	0.02
	4	—	—	—	—	—	0.01	—
	(n)	27	43	47	47	40	74	88
GPI[a]	1	—	—	—	—	—	—	0.02
	2	0.37	0.26	0.32	0.31	0.30	0.20	0.21
	3	0.63	0.74	0.68	0.69	0.71	0.80	0.77
	(n)	27	46	47	50	39	74	90
MDH-2[a]	1	0.24	0.27	0.36	0.26	0.26	0.36	0.26
	2	0.76	0.73	0.64	0.75	0.74	0.64	0.74
	(n)	27	45	47	49	36	73	88
MPI[a,b]	1	0.16	0.34	0.33	0.34	0.15	0.31	0.12
	2	0.54	0.31	0.29	0.39	0.51	0.47	0.51
	3	0.30	0.35	0.38	0.27	0.34	0.21	0.36
	4	—	—	—	—	—	0.01	0.01
	(n)	25	40	41	41	40	75	73
PEP[a]	1	—	—	—	—	—	—	0.01
	2	0.50	0.36	0.41	0.50	0.59	0.60	0.64
	3	—	—	—	—	0.03	—	0.01
	4	0.50	0.64	0.59	0.50	0.39	0.40	0.33
	(n)	3	14	16	13	40	57	39
PGM-1[a,b]	1	0.03	0.03	0.03	0.03	0.08	0.07	0.29
	2	—	—	—	—	—	0.01	0.02
	3	0.91	0.91	0.94	0.97	0.75	0.87	0.68
	4	0.06	0.05	0.03	—	0.17	0.05	0.02
	(n)	16	29	36	35	36	59	64
PGM-2[a,b]	1	0.22	0.23	0.22	0.26	0.29	0.33	0.52
	2	0.78	0.77	0.78	0.73	0.71	0.67	0.47
	3	—	—	—	0.01	—	—	0.01
	(n)	27	46	46	48	40	75	92
Mean sample size per locus		23.3 (2.5)	39.7 (3.3)	40.8 (3.1)	41.3 (3.6)	66.5 (4.8)	37.2 (1.9)	74.4 (7.7)
Mean number of alleles per locus		1.9 (0.2)	1.8 (0.2)	1.8 (0.2)	1.8 (0.2)	2.5 (0.4)	2.0 (0.3)	2.6 (0.4)
Polymorphic loci (%)		60	60	60	50	70	60	70
Mean heterozygosity		0.26 (0.09)	0.22 (0.07)	0.24 (0.08)	0.20 (0.07)	0.27 (0.07)	0.23 (0.07)	0.27 (0.07)

Two additional loci (MDH-1 and SOD) were fixed for the same allele in all populations. Population abbreviations follow those of the text. Numbers in parentheses are standard errors. (n) = sample size.

[a] Polymorphic locus (95% criterion).

[b] Significant variation in allele frequencies (contingency chi-square, $p < .05$) when Tensas River population is included.

DISCUSSION

Within-population Variation

Our study shows that populations of *Quadrula quadrula* may contain significant amounts of within-population genetic variation. A study of *Q. quadrula* in Arkansas rivers found considerably lower levels of within-population variation and higher levels of differentiation among populations (Johnson et al. 1998). The grand mean of direct-count heterozygosity for *Q. quadrula* from our study was 0.24 (range 0.20–0.27), whereas Arkansas populations showed an average heterozygosity of 0.06 (range 0.04–0.07). In fact, we report some of the highest levels of genetic variation found in unionid populations when compared with other published studies (Badino 1982, Davis 1984, Davis and Mulvey 1993, Kat 1983b, Stiven and Alderman 1992, van der Bank 1995, Nagel et al. 1996, Hoeh et al. 1998, Johnson et al. 1998). The grand mean of average heterozygosity from these other studies of unionids is only 0.09 (range 0.00–0.31 for 44 studies). Almost all (91%) previously reported values of heterozygosity were lower than the minimum value for the *Q. quadrula* populations we sampled. A similar situation occurs when comparing average number of alleles per locus and proportion of polymorphic loci among populations. Based on these comparisons, the populations of *Q. quadrula* that we sampled contain high levels of genetic variation compared with other unionid populations. Our values are similar to those reported for the giant marine clams *Tridacna gigas* and *T. maxima* (Benzie and Williams 1992a, Benzie and Williams 1992b). Reports across large numbers of taxa show that *Q. quadrula* contains above-

TABLE 3.

Matrix of genetic distances among pairs of populations of *Quadrula quadrula*.

Population	Ohio RM 238.6	Ohio RM 252.2	Ohio RM 255.3	Ohio RM 257.7	Ohio RM 300	Kentucky Lake	Tensas River
Ohio RM 238.6	—	0.000	0.000	0.000	0.001	0.000	0.017
Ohio RM 252.2	0.087	—	0.000	0.000	0.010	0.011	0.035
Ohio RM 255.3	0.087	0.040	—	0.000	0.010	0.012	0.037
Ohio RM 257.7	0.062	0.058	0.059	—	0.003	0.005	0.025
Ohio RM 300	0.065	0.111	0.112	0.089	—	0.004	0.015
Kentucky Lake	0.094	0.104	0.101	0.073	0.077	—	0.010
Tensas River	0.143	0.170	0.173	0.147	0.112	0.101	—

Nei's (1978) unbiased genetic distances are above the diagonal and modified Rogers' distances (Wright 1978) are below the diagonal. Distances calculated using 10 allozyme loci. Population abbreviations follow those of the text.

average levels of variation within populations (Nevo 1978). The high degree of within-population genetic variation implies that these populations of *Q. quadrula* have large effective population sizes and that there have not been significant genetic bottlenecks in the recent past.

Genotype frequencies of *Quadrula quadrula* populations were not significantly different from Hardy-Weinberg expectation (HWE). We have found similar results for *Elliptio dilatata* from streams in Ohio (Berg and Guttman, unpubl). Other studies of unionids have shown considerable variation in the proportion of loci that show significant differences from HWE. Among three species of unionids in North Carolina, the proportion of loci deviating from HWE varied between 5% and 75%, and all of these deviations were heterozygote deficiencies (Stiven and Alderman 1992). Genotype frequencies of Arkansas unionids, including *Q. quadrula* and *Q. pustulosa,* were different from HWE in 67% of polymorphic locus-by-population combinations (Johnson et al. 1998), with most of these deviations being heterozygote deficits. A similar pattern was seen in European anodontines, where 64% of polymorphic locus-by-population combinations showed heterozygote deficits (Nagel et al. 1996). In the genus *Utterbackia,* the gonochoric species *U. peggyae* and *U. peninsularis* exhibited deviation from HWE at only 6% and 8% of all locus-by-population combinations, respectively (Hoeh et al. 1998). In contrast, two simultaneously hermaphroditic species, *U. imbecillis* and an undescribed *U. "imbecillis,"* showed heterozygote deficits at 65% and 50% of all locus-by-population combinations. Hoeh et al. (1998) concluded that deviations from HWE, in particular heterozygote deficiencies, may be explained by the mating systems employed by unionid species (e.g., self-fertilization versus cross-fertilization). Thus, our results are completely consistent with those expected of a gonochoric species with high levels of gene flow among populations.

Among-population Variation

Variation among populations was quite low; the vast majority of this among-population variation occurs between the Tensas River site and the sites in the Ohio River basin. Even over river distances of >1000 km, no differences in allele frequencies were found among populations in the Ohio River basin, whereas the Tensas River site differed from the Ohio River populations at only three of seven polymorphic loci. Gene diversity analyses show that little of the total heterozygosity within *Quadrula quadrula* is accounted for by among-population variation. When compared with other studies of unionids, the differences between the Tensas River population and the Ohio basin populations of *Q. quadrula* were low. Genetic distances among populations in this study averaged 0.009 (range <0.0005–0.037) for all populations; exclusion of the Tensas River site lowered average distance to 0.004 (<0.0005–

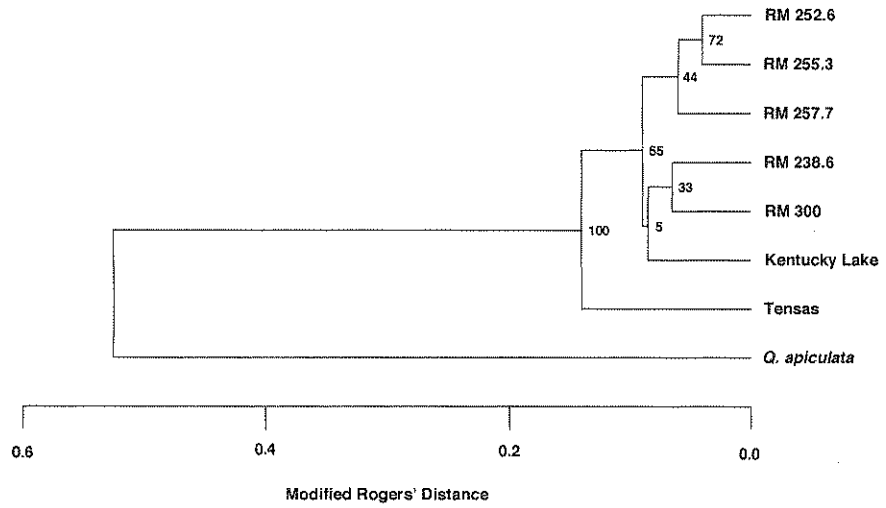

Figure 2. Modified Rogers' genetic distances (Wright 1978) for populations of *Quadrula quadrula.* Distances calculated using 10 allozyme loci. Values at the nodes represent the percentages of 1,000 bootstrapped trees that produced these nodes.

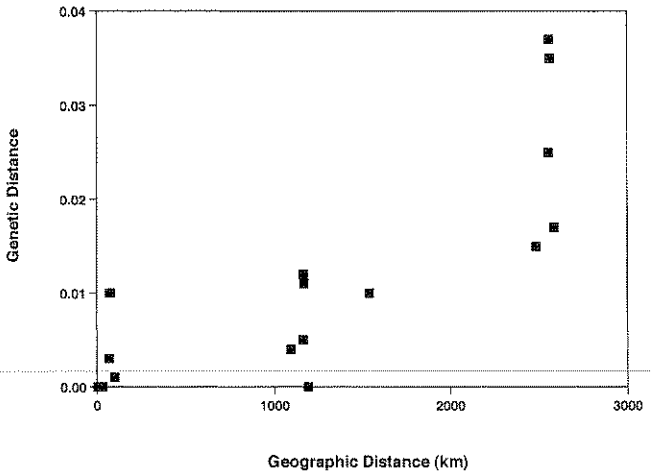

Figure 3. Correlation of unbiased genetic distance (Nei 1978) and geographic distance (river km) between pairs of populations of *Quadrula quadrula*. The correlation is significant (Mantel test; r = 0.83, n = 21, p = .003).

0.012). Published measures of unbiased genetic distance (Nei 1978) among unionid populations averaged 0.047 (0.000–0.252) for six species (Nagel et al. 1996, Stiven and Alderman 1992). Three populations of *Anodonta cygnea* showed little differentiation (mean genetic distance of 0.008; range 0.000–0.012) over large geographic distances (Nagel et al. 1996). However, other species of *Anodonta* reported from the same study showed mean values between 0.063 and 0.108 among populations within the same river basin. Most published studies of unionid genetic structure have calculated standard genetic distance (Nei 1972) rather than unbiased genetic distance. Comparison of our calculations of standard genetic distance with those of such studies (Davis and Mulvey 1993, Davis et al. 1981, Kat 1983a, Badino 1982) show that genetic distances among populations of *Q. quadrula* are very low, even though populations were separated by as much as 2,500+ river kilometers.

The dendrogram created from the cluster analysis and the tight correlation of genetic distance with geographic distance are both consistent with isolation-by-distance (Wright 1943, Slatkin 1993); this process increases genetic differentiation as populations become separated by larger distances. Given the method of dispersal available to unionids (downstream transport of spermatozeugmata, host fish transport of glochidia larvae up and down rivers), this isolation-by-distance likely arises due to a one-dimensional stepping-stone pattern of dispersal (Slatkin 1985). However, the degree of gene flow among populations is apparently sufficient (greater than 1 migrant per generation) to prevent significant differentiation of populations by genetic drift (Slatkin and Barton 1989). Gene flow estimates among populations of *Anodonta anatina* from several European Atlantic coast drainages were sufficient for the authors of the study to classify this species as "panmictic" (Nagel et al. 1996), although the values reported were much lower than we report (*A. anatina* mean Nm = 1.2, *Quadrula quadrula* mean Nm = 7.8).

Genetic Structure of Quadrula quadrula

Our results suggest that populations of *Quadrula quadrula* have high levels of gene flow within the Ohio and lower Mississippi River basins and that this has two consequences for genetic structure of these populations. The first is that this gene flow maintains high levels of within-population genetic variation relative to that seen in other unionids, and genetic drift does not play a significant role in determining the frequencies of alleles within populations. Secondly, the high levels of gene flow inhibit differentiation among populations. What little differentiation that has occurred is a function of isolation-by-distance. Because gene flow among unionid populations over spatial scales greater than a few meters is a function of host fish movements, our results suggest that there is sufficient movement of host fishes to allow glochidia to disperse to at least the next bed in a river. The flathead catfish (*Pylodictis olivaris*) is a known host of *Q. quadrula*, and other large-river species within the genus *Quadrula* utilize various catfishes and large centrarchids (crappie—*Pomoxis* spp.; largemouth bass—*Micropteris salmoides*) (Watters 1994). Because these hosts are all common large-river species, they are capable of movement among beds that are separated by distances greater than a kilometer. Such minimal dispersal would then be best described by a one-dimensional stepping-stone model. It is certainly possible that such host fishes may move sufficient distances to "skip" adjacent beds and thus disperse their glochidia much greater distances. As a result, we can best characterize the genetic structure of *Q. quadrula* in the Ohio and lower Mississippi basins as a single "metapopulation" (Hastings and Harrison 1994) in which gene flow prevents "extinction" of some alleles and fixation of others.

Genetic structure is known to be a function of dispersal ability in other molluscs. Highly vagile species such as the zebra mussel (*Dreissena polymorpha*) show low values of F_{ST} (Marsden et al. 1995). Giant clams (*Tridacna gigas* and *T. maxima*) show high levels of within-population variation, few deviations of allele frequencies from Hardy-Weinberg expectations, and little or no ge-

TABLE 4.

F_{ST} (Θ of Weir and Cockerham 1984) values for polymorphic loci and average migration rates.

Populations	Locus	Fst	Nm
All	EST	0.024	
	GPI	0.010	
	MDH-2	0.003	
	MPI	0.026	
	PEP	0.018	
	PGM-1	0.089	
	PGM-2	0.064	
	Overall	0.031	7.815
		(0.013–0.056)	
Ohio R. Basin	EST	0.034	
	GPI	0.010	
	MDH-2	0.004	
	MPI	0.019	
	PEP	0.032	
	PGM-1	0.042	
	PGM-2	0.009	
	Overall	0.013	18.981
		(0.006–0.020)	

Nm–number of migrants per generation for populations of *Quadrula quadrula*. Comparisons are among all populations and among populations from the Ohio River basin (Tensas River population excluded). Numbers in parentheses are 95% confidence intervals from bootstrapping of 1,000 replicates.

netic differentiation among populations in the south Pacific Ocean (Benzie and Williams 1992a, Benzie and Williams 1992b). Where genetic differentiation occurs, the patterns are consistent with isolation-by-distance. Marine snails of the genus *Littorina* exhibit a strong negative correlation between genetic differentiation among populations and dispersal ability (Janson 1987). The results of our study show that although life histories of marine molluscs and freshwater unionoid bivalves are quite different, *Quadrula quadrula* has genetic structure similar to that of zebra mussels, giant clams, and littorinids with planktonic larvae. In all of these cases, high variation within populations and little differentiation among populations are likely due to high levels of gene flow between populations.

Conservation Implications

As the field of conservation biology has grown, efforts have been made to consider genetic variation when developing strategies for management and conservation of rare species (reviewed in Avise 1994). Recent work has focused on providing a conceptual framework of the organization of genetic variation in species such that management agencies and conservation organizations are able to design strategies that conserve genetic diversity. A key piece of this conceptual framework is the definition of Evolutionarily Significant Units (ESUs) and Management Units (MUs) within ESUs (Moritz 1994). ESUs tend to be concordant with morphological species, and MUs are subdivisions within ESUs. With this approach, MUs are defined ". . . as populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles" (Moritz 1994). The results of our study show that *Quadrula quadrula* in the Ohio and lower Mississippi basins is composed of at least 2 MUs—one in the mainstem of the Ohio and lower Tennessee Rivers and one in the Tensas River. However, it is possible that additional MUs were undetected because of the great distance between Kentucky Lake and the Tensas River, and because we did not sample the mitochondrial genome. Conservation of genetic variation within ESUs implies the protection of each MU within it. Thus, a conservation strategy designed to protect *Q. quadrula* or any other unionid species with similar genetic structure would need to "customize" efforts for each MU.

Understanding the genetic structure of *Quadrula quadrula* in the Ohio and lower Mississippi basins provides insight for the development of effective conservation strategies for this species and those threatened and endangered (T&E) species that have similar genetic structure. Our results suggest that any single population of *Q. quadrula* contains most of the genetic diversity of the populations along the mainstems of the Ohio and lower Tennessee Rivers. As such, only a small number of populations scattered throughout this stretch of rivers would need to be conserved to ensure conservation of most of the total genetic diversity within this region. However, each of these populations must be large enough that they are able to maintain the high levels of within-population variation. Under these circumstances, an optimal conservation strategy would be to protect a number of populations, each of which remains large. Such a strategy would be compatible with the establishment of sanctuaries at multiple locations along a river system and the maintenance of corridors for movement of host fishes among these sanctuaries. The distances between these sanctuaries should then be a function of host fish dispersal distances. This type of strategy would also work for T&E species that are locally abundant but limited to only a small number of populations. Of course, populations of T&E species are often quite small and in these cases, efforts must be made to protect all individuals.

Recent studies have noted declines in many species of unionids, not just those that are listed as threatened or endangered (Neves 1997, Howells et al. 1997, other studies reported in Cummings et al. 1997). If government agencies and other conservation organizations are to be successful in protecting species so that listing is not necessary, steps must be taken to conserve both abundance and genetic diversity of unionids. This effort requires an understanding of the genetic structure of these species and thus, there is a great need for additional studies to determine whether the model of genetic structure we present is generally applicable to unionids or whether other patterns exist. If other patterns are found, the implications for development of conservation strategies for species exhibiting these patterns must be explored. There is no doubt that understanding the genetic structure of unionid species is essential if this unique part of North America's freshwater fauna is to be preserved.

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