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ORIGINAL PAPER

Population genetics of the freshwater mussel, *Amblema plicata* (Say 1817) (Bivalvia: Unionidae): Evidence of high dispersal and post-glacial colonization

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Abstract Over 70% of North American freshwater mussel species (families Unionidae and Margaritiferidae) are listed as threatened or endangered. Knowledge of the genetic structure of target species is essential for the development of effective conservation plans. Because *Ambelma plicata* is a common species, its population genetic structure is likely to be relatively intact, making it a logical model species for investigations of freshwater mussel population genetics. Using mtDNA and allozymes, we determined the genotypes of 170+ individuals in each of three distinct drainages: Lake Erie, Ohio River, and the Lower Mississippi River. Overall, within-population variation increased significantly from north to south, with unique haplotypes and allele frequencies in the Kiamichi River (Lower Mississippi River drainage). Genetic diversity was relatively low in the Strawberry River (Lower Mississippi River drainage), and in the Lake Erie drainage. We calculated significant among-population structure using both molecular markers (A.p. $\Phi_{st} = 0.15$, $\theta_{st} = 0.12$). Using a hierarchical approach, we found low genetic structure among rivers and drainages separated by large geographic distances, indicating high effective population size and/or highly vagile fish hosts for this species. Genetic structure in the Lake Erie drainage was similar to that in the Ohio River, and indicates that northern populations were founded from at least two glacial refugia following the Pleistocene. Conservation of genetic diversity in *Amblema plicata* and other mussel species with similar genetic structure should focus on protection of a number of individual populations, especially those in southern rivers.

Keywords Population genetics · Hierarchical analysis · Post-glacial dispersal · Allozymes · mtDNA · COI

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Introduction

Preservation of biodiversity requires not only the protection of individual taxa, but also the preservation of genetic diversity within each of these taxa. Such diversity is partitioned between variation among individuals within populations and differentiation among populations and groups of populations. The relative proportion of neutral genetic variation within each of

these two components is determined by the opposing forces of genetic drift and gene flow (Hutchison and Templeton 1999). Genetic drift results in loss of within-population genetic diversity over time, while also promoting differentiation of populations (Hartl and Clark 1997). Conversely, gene flow among populations tends to maximize variation within populations, while minimizing differences among these populations. In aquatic invertebrates, dispersal is a major component of gene flow among populations (Bilton et al. 2001). The effects of genetic drift occur more rapidly in small populations, and isolation promotes population differentiation (Newman and Pilson 1997). Decreases in within-population variation due to genetic drift increase the probability of extinction (Frankham 2003; Reed and Frankham 2003; Saccheri et al. 1998). In addition, genetic changes due to isolation will increase among-population genetic structure. Thus, preservation of genetic diversity within a conservation framework requires understanding both within-population genetic variation and patterns of variation among populations across the landscape.

Freshwater mussels of the families Unionidae and Margaratiferidae are a diverse component of North American freshwater ecosystems. In these families, over 70% of the 297 North American taxa are of conservation concern at state, provincial, or federal levels (Williams et al. 1993). As is the case for many aquatic species, threats from habitat loss and destruction (Neves 1999), commercial harvest and pollution (Bogan 1993; Neves 1999), and invasive species such as the zebra mussel (Haag et al. 1993) have jeopardized the existence of many mussel species. Surveys over the past ten years have shown a steep decline in population sizes across most of these mussel taxa (Neves 1999). Because of the high levels of endemism in this group, preservation of North America's freshwater mussel fauna has become a priority for many government agencies and conservation organizations (e.g., USGS, USFWS, Environment Agency (UK), Environment Canada, The Nature Conservancy, Nature Serve, Conserve Online, Outdoor Alabama).

Much of the recent work using genetic markers in freshwater mussels has focused on resolving phylogenetic and phylogeographic relationships (Berg and Berg 2000; King et al. 1999; Mulvey et al. 1997; Roe et al. 2001; Roe and Lydeard 1998; Serb et al. 2003; Grobler et al. 2006) or describing genetic structure across broad geographic ranges (Berg et al. 1998; Machordom et al. 2003). Because riverine systems are organized hierarchically (first order streams giving rise to second order streams, etc.), populations of organisms within these systems are likely to have genetic structure that reflects this type of organization (Meffe and Vrijenhoek 1998). Thus, hierarchical analysis of genetic variation is a particularly useful approach for understanding genetic diversity in freshwater organisms (Bunn and Hughes 1997; Schultheis et al. 2002). However, hierarchical analysis has only been investigated in freshwater mussels at limited spatial scales (Turner et al. 2000).

Our study focuses on *Amblema plicata* (Say 1817), a species of freshwater mussel that is widespread in medium and large rivers of the Mississippi and Great Lakes drainages (Cummings and Mayer 1992; Hoggarth 1995–1996; Metcalfe-Smith et al. 1998; Vaughn and Spooner 2004). Because this species is relatively common, its population genetic structure is likely to be more intact than that of species that have become threatened or endangered. Therefore it is a logical model species for investigations of freshwater mussel population genetics. We quantify within-population genetic variation in a large number of populations across a broad geographic range. We then use a hierarchical approach to examine the distribution of among-population genetic variation at multiple spatial scales (within rivers, among rivers within drainages, and across drainages). We conclude by considering the implications of our findings for understanding population genetic structure of freshwater organisms and for development of conservation strategies.

Materials and methods

Collection

Amblema plicata (the threeridge) were collected from rivers in three distinct drainages: Lake Erie, Ohio River, and Lower Mississippi River drainages (Fig. 1). Collections in the Lake Erie drainage consisted of two collection sites each in the Sydenham and Ausable Rivers of Ontario, Canada and two collection sites on the St. Joseph River (Ohio, USA). The Ohio River drainage samples consisted of a single site of collection in the Ohio, Tennessee, Muskingum, and Licking rivers (Ohio and Kentucky, USA). Additional Ohio River drainage samples were taken from two other tributaries: two collection sites each from the East Fork of the Little Miami River (Ohio, USA) and the Walhonding River (Ohio, USA). The Lower Mississippi River drainage collections were taken from two tributaries in the Red River drainage: four collection sites on the Kiamichi River (Oklahoma, USA), and four sites on the Ouachita River (Arkansas, USA). Additional collections in the Lower Mississippi were taken from a single tributary of the White River drainage: three sties

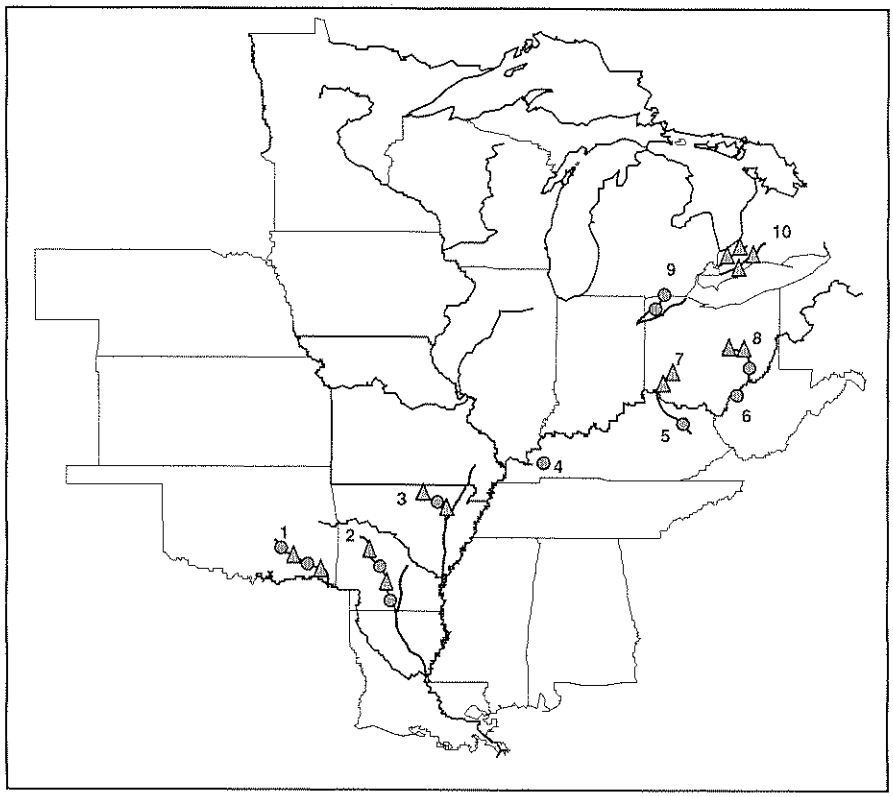

Fig. 1 Map of the central United States and Canada with populations of *Amblema plicata* sampled for this study. Population abbreviations follow those in the text and Table 3. Circles represent sites where only allozyme data were collected, triangles represent sites where both allozyme and mtDNA data were collected. 1 = Kiamichi River (sites KR 1–4); 2 = Ouachita River (OR 1–4); 3 = Strawberry River (STR 1–3); 4 = Tennessee River (TR); 5 = Licking River (LR); 6 = Ohio River (OH); 7 = Little Miami River (LM 1–2); 8 = Walhonding (WR 1–2) and Muskingum rivers (MR); 9 = St. Joseph River (SJ 1–2); 10 = Ausable (AR 1–2) and Sydenham rivers (SR 1–2)

on the Strawberry River (Arkansas, USA). Up to 53 mussels were collected from each site. Collections of widespread species such as *A. plicata* can correspond to only part of a population, or in rare cases parts of more than one population. We attempted to minimize re-sampling the same population by sampling locations that were separated by several miles among the river channel. Therefore, in our analysis we will use collection site as a surrogate for a population of *A. plicata*. Individuals were collected by hand with the aid of snorkel or SCUBA, by sight or by feel. At most sites, mussels were carefully opened and the mantle was nondestructively sampled using a mantle biopsy (Berg et al. 1995). Some individuals from large populations were destructively sampled. Tissue was flash-frozen in liquid nitrogen or placed on dry ice, and live individuals were returned to the locations from which they were collected. Tissues were shipped to Oxford, Ohio and stored at − 80°C until processed.

Mitochondrial DNA

Seven to sixteen individuals were used from each of 14 populations for the DNA analysis. We used a subset of the total number of individuals collected from each site for the mitochondrial DNA analysis (a larger sample size is preferable for allozyme analysis). We set up the study in a hierarchy of two populations per river and two rivers in each of three drainages, plus two additional populations from the Strawberry River. A 682-bp fragment of the cytochrome oxidase subunit I (*COI*) gene of the mtDNA was amplified using PCR. The primers used were modified from Folmer et al. (1994), *COI*-22me 5′-GGTCAACAAATCATAAA-GATATTGG-3′ and *COI*-700dy 5′-TCAGGGTGA-CCAAAAAATCA-3′.

Amplification consisted of 0.5 μg of genomic DNA, 25 μl Qiagen PCR master mix (Qiagen), and 0.5 μM primer, in a total volume of 50 μl. Amplifications were carried out on a MJ Research thermocycler using the following conditions: an initial denaturing of 94°C for 2 min, 35 cycles of 94°C denaturing for 30s, 42°C annealing for 30s, 72°C extension for 90s, and a final extension at 72°C for 3 min. The purified *COI* gene was sequenced directly using the same primers.

Sequence reactions were performed using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Inc.). We used standard protocols for sequencing; however, we reduced the total amount of sequencing reagent by 2 μl and substituted 2.5 × buffer in each reaction. Cycle sequencing reactions were purified by standard ethanol, sodium acetate precipitation. Each sample was re-suspended in 20 μl of MegaBACE buffer prior to loading into an ABI

automated 3100 sequencer with DNA sequencing analysis software (ABI). An assumption of the analysis of mtDNA is strict maternal inheritance; however, double uniparental inheritance (DUI) has been found in freshwater mussels (Hoeh et al. 2002). We reduced the chance of DUI occurring in our samples by only using tissue from the mantle, as paternal mtDNA has been found primarily in gonad tissue in mytilids and several unionid species (Hoeh et al. 1996).

Allozymes

One to 53 (N_{mean} = 33.3 across all populations and loci) individuals from 25 populations in three drainages were analyzed at nine allozyme loci. For most populations, all of the individuals sampled were used to calculate population estimates. However, there were times when we were limited by small tissue samples, and therefore a few loci have only a few individuals sampled. All individuals were thawed, ground for a few moments in 2-phenoxyethanol and a few grains of sterile sand to release intracellular fluid, and then centrifuged at speed 5 for 12 min (Marathon Micro A centrifuge, Fisher Scientific).

Allozyme electrophoresis using starch gels was performed using standard recipes and buffer systems (Hebert and Beaton 1993; Richardson et al. 1986). A total of nine loci were resolved from these systems for *A. plicata*: aspartate aminotransferase (*Aat*) [EC 2.6.1.1], isocitrate dehydrogenase (*Idh*) [EC 1.1.1.42], leucine aminopeptidease (*Lap*) [EC 3.4.11.1], malate dehydrogenase (*Mdh-1* and *Mdh-2*) [EC 1.1.1.37], phosphoglucose isomerase (*Pgi*) [EC 5.3.1.9], phosphoglucomutase (*Pgm-1* and *Pgm-2*) [EC 5.4.2.2], and superoxide dismutase (*Sod*) [EC 1.15.1.1]. Loci were considered "polymorphic" when the most common allele was present at a frequency ≤0.95.

Data analysis

The mtDNA sequences were aligned by using Chromas 2.23 (Technelysium Pty., Ltd.) software and MS Wordpad. These alignments were then confirmed using MacClade 4.03 software (Maddison and Maddison 2001). There were no deletions or repeats within the data.

We used Arlequin 2.0 (Schneider et al. 2000) for analysis of molecular variation. This software takes into account both haplotype frequency and molecular sequence divergence. Genetic differentiation was analyzed within-drainage for the Lake Erie, Ohio River, and Lower Mississippi River drainages. We calculated genetic variation partitioned into three hierarchical levels using analysis of molecular variance (AMOVA) (Excoffier et al. 1992): within populations (Φ_{ST}), among populations within rivers (Φ_{SR}), and among rivers (Φ_{RT}). We then calculated among-drainage variation using three hierarchical levels: within populations (Φ_{ST}), among populations within drainages (Φ_{SD}), and among drainages (Φ_{DT}). Significance of the Φ statistics was tested using 1023 nonparametric permutations (Weir and Cockerham 1984). We calculated the pairwise F_{st} values for each combination of populations. The significance of this estimate was calculated using 3023 permutations of the data and was considered significant at $P = 0.05$. We compared the pairwise estimates to a matrix of geographic distance using a Mantel test. Among individual haplotypes, we calculated the mean number of pairwise differences within each population and, after pooling all individuals, we also calculated the total number of pairwise differences of all individual sequences.

In addition, we estimated the phylogenetic relationship among *A. plicata* haplotypes using Paup 8.0b (Swofford 2000) with *Elliptio dilatata* as an outgroup. We employed a Neighbor Joining algorithm using Tamura-Nei (Tamura and Nei 1993) calculation of genetic distance to form a matrix among haplotypes. The robustness of the relationship among haplotypes was tested using bootstrap analysis (1000 replicates), and branch support was considered significant if a node was present in over 50% of the replicate trees. We calculated a parsimony network of all individual gene sequences using the program TCS version 1.13 (Clement et al. 2000), and nesting categories were assigned following Templeton (1998), Templeton and Sing (1993), and Crandall (1996). With this analysis, we could determine the relationship among haplotypes and the frequencies of these haplotypes in our sampling. Ambiguity in the network (loops or circular pathways) was resolved using a coalescence model as outlined in Fetzner and Crandall (2003), where mutations are more probable if they arose from a more frequent haplotype. For haplotypes with more than one pathway, the shortest path was chosen from the most frequent haplotype. We calculated the frequency of closely related haplotypes within each population and mapped those frequency distributions according to where mussels were sampled.

We used Global Positioning System (GPS) latitude and longitude coordinates to mark the geographic position of each sampling location. We then entered these into ArcView digital mapping system (Environmental Systems Research Institute) using geographic maps of the United States and Canada that included rivers of the Mississippi and Great Lakes drainages. Along-river

distances were calculated by tracing the river courses connecting sample sites. Most populations in this study were connected through the main channel of the Mississippi River. A theoretical connection was established to the Canadian populations from the Wabash River, overland into the St. Joseph River (in the vicinity of Fort Wayne, Indiana), the Maumee River, and into Lake Erie. This route is based on historic information outlined in Graf (2002), where geologic evidence suggests that the Wabash River and Lake Erie were briefly connected after the Pleistocene glaciers receded.

Allozyme data analysis

Analysis of the allozyme electrophoretic data was conducted using Genetic Data Analysis (GDA; Lewis and Zaykin 1999), Tools for Population Genetic Analysis (TFPGA; Miller 1997), and BIOSYS-1 (Swofford and Selander 1981). We calculated the following descriptive measures: (1) average number of alleles for each locus, (2) allele frequency, (3) number of private alleles (present in only one population; Slatkin 1981), (5) genotype frequency, (6) percentage of polymorphic loci (loci with the most common allele frequency ≤0.95), and (7) mean direct-count heterozygosity per locus (H). We tested for differences in mean heterozygosity using a one-way analysis of variance (ANOVA) among drainages followed by a Bonferroni multiple comparison test. Agreement of genotype frequencies with Hardy–Weinberg expectations was tested with exact tests of goodness-of-fit (TFPGA). A sequential-comparison Bonferroni technique was used to minimize type I error (Lessios 1992).

We calculated unbiased genetic distances (Nei 1978) among populations. We also performed a cluster analysis of all samples based on the unweighted pair group method with arithmetic averages (UPGMA) (Sokal and Sneath 1963). Geographic distances were calculated using digital mapping software as described above. We performed a Mantel test to evaluate the correlation between matrices of genetic distance and geographic distances among populations using Arlequin 2.0. This analysis was used as a test for isolation-by-distance.

We calculated among-population variation using exact tests and Weir and Cockerham's (1984) method of calculating Wright's (1965) F-statistics (θ). The exact tests were used to determine whether significant differences in allele frequencies existed among populations (Rousset and Raymond 1995) and were followed by a sequential-comparison Bonferroni technique to minimize type I error (Lessios 1992). For among-population estimates of θ, we calculated mean θ for each locus (± 1 SD) using the jackknife method at individual loci, and a 95% CI for θ across loci using the bootstrap method (1,000 repetitions). We also calculated population structure at increasing geographic levels similar to the hierarchical analysis of population structure using mtDNA. However, for these analyses, populations in the Muskingum, Ohio, Tennessee, and Licking rivers were not used, as they do not fit into our hierarchical analysis. Using TFPGA, we calculated the genetic structure among populations (θ_{ST}), among rivers within drainages (θ_{RD}), and among drainages (θ_{DT}). We also calculated pairwise F-statistics of population structure and conduced a third test of isolation-by-distance using a Mantel test.

MtDNA Results

We sequenced a 631 bp section of the *COI* gene for 170 individuals. From those, we found 36 unique haplotypes. Sequences were deposited in, and are available from, Genbank (National Sequence Database, http://www.ncbi.nlm.nih.gov/Genbank/).

Within-population structure

Haplotype richness was significantly negatively correlated with latitude($R = -0.60$; $P = 0.02$). Populations within the Sydenham and Ausable Rivers (Ontario) averaged 3 and 4 haplotypes; similarly the Walhonding and Little Miami samples (Ohio) averaged 4.5 and 5 haplotypes. Populaitons within the Strawberry River (Arkansas) averaged 4 haplotypes. Populations within the Ouachita and Kiamichi rivers (southern Arkansas and Oklahoma, respectively) averaged 5.5 and 6.5 haplotypes per site (Table 1).

The number of haplotypes unique to a particular river was strongly negatively correlated with latitude ($R = -0.88$; $P < 0.001$). The Sydenham and Ausable sites had 0 and 2 unique haplotypes, respectively. The Walhonding and Little Miami sites had 2 and 5 unique haplotypes. In the Lower Mississippi drainage, the Strawberry sites had 6 unique haplotypes, and the Ouachita and Kiamichi sites had 6 and 10 unique haplotypes, respectively (Table 1).

The maximum within-population sequence divergence was significantly negatively correlated with latitude ($R = -0.69$; $P = 0.006$). The Sydenham and Ausable sites had a maximum difference of 3 bp between pairs of individuals within a site. Values for other drainages were 3–5 bp for the Ohio River sites, 2–5 for the Strawberry River sites, and 6–8 for the Ouachita and Kiamichi river sites. Thus, overall *COI* variation increased significantly with decreasing latitude.

Table 1 Relative frequencies of haplotypes at the mtDNA COI gene across all populations sampled. Population labels follow Fig. 1

Haplotype: N=	KR1 13	KR2 14	OR1 10	OR2 17	STR1 7	STR2 8	LM1 12	LM2 12	WR1 12	WR2 12	SR1 14	SR2 14	AR1 15	AR2 10
AP1		0.429	0.700	0.647	0.143	0.500	0.500	0.333	0.417	0.583	0.357	0.143	0.667	0.200
AP2		0.071					0.083		0.167	0.083	0.500	0.500	0.267	0.400
AP3							0.333	0.250	0.083		0.143	0.357		
AP4	0.308													
AP5	0.231													
AP6	0.154													
AP7	0.154													
AP8	0.154													
AP9		0.071		0.059										
AP10		0.071												
AP11		0.143												
AP12		0.071												
AP13		0.071												
AP14		0.071												
AP15			0.100	0.059										
AP16			0.100											
AP17			0.100											
AP18				0.059										0.100
AP19				0.059										
AP20				0.059										
AP21				0.059										
AP22					0.714									
AP23					0.143									
AP24						0.125								
AP25						0.125								
AP26						0.125								
AP27						0.125								
AP28							0.083							
AP29								0.167						
AP30								0.083						
AP31								0.083						
AP32								0.083						
AP33									0.167	0.167				
AP34									0.167	0.167				
AP35													0.067	0.100
AP36														0.200

Among-population structure

Comparisons of population structure within drainages for mtDNA showed that there was significant structure among populations within the Lake Erie and Lower Mississippi drainages. Within the Ohio River drainage, there was no significant genetic structure at any level of the hierarchy. Within Lake Erie and the Lower Mississippi River, our results show that the majority of the population structure occurred among populations within river (Lake Erie $\Phi_{SR} = 0.15$, $P = 0.001$; Lower Mississippi $\Phi_{SR} = 0.16$, $P = 0.001$). Genetic structure among rivers within a drainage was not significant within any of the drainages.

In the overall analysis, populations of *A. plicata* contain 82% of the total genetic variation. Correspondingly, there was significant mtDNA structure among all *A. plicata* populations ($\Phi_{ST} = 0.18$, $P < 0.001$). Of that genetic structure, more variation was among populations within a drainage ($\Phi_{SD} = 0.13$, $P < 0.001$) than among drainages ($\Phi_{DT} = 0.06$, $P = 0.022$). Pairwise F_{st} estimates did not increase with geographic distance between sampling sites, and non-adjacent populations did not show higher genetic structure in this analysis (Fig. 2A). The highest pairwise F_{st} estimates were consistently from the following sites: Sydenham River, Ausable River, Strawberry River, and Kiamichi River (Table 2). Mismatch analysis of total mtDNA haplotypes showed an average of 1.9 bp difference between sequences (0.3% sequence divergence), with a range of 0–8 bp difference. These haplotype differences are mirrored in the parsimony network, where we found two common groups of haplotypes which were, on average, 2 bp different (Fig. 3). Both these groups were widely distributed

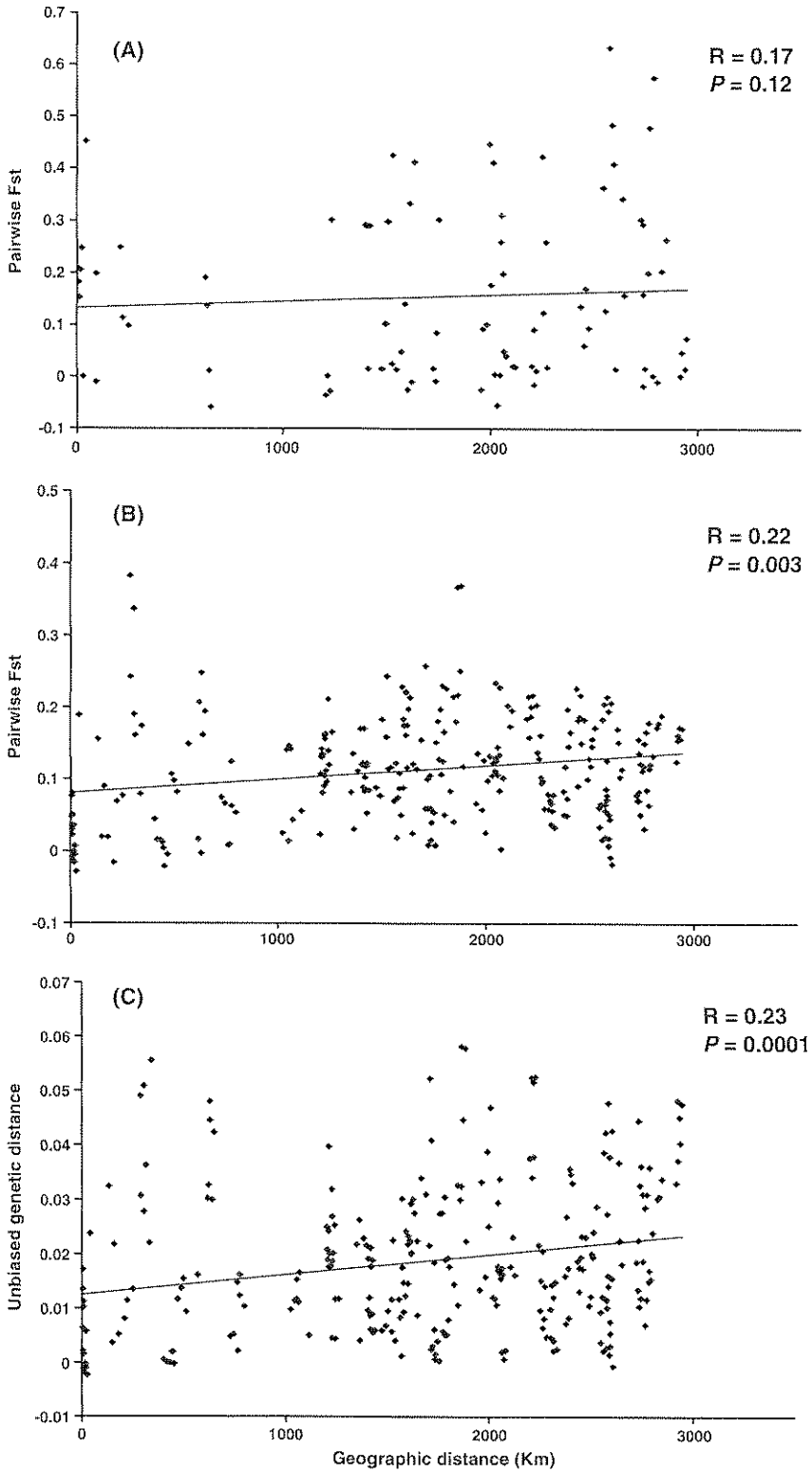

Fig. 2 Results of Mantel tests between pairwise geographic distance among sampling sites (km), and (**A**) pairwise F_{st} estimates using mtDNA haplotypes (**B**) pairwise estimates of F_{st} using allozymes and (**C**) pairwise estimates of Nei's unbiased genetic distance using allozymes. Correlation coefficients and probabilities are given for each analysis

geographically (Fig. 4). A third group was located almost exclusively in a Kiamichi River population (Fig. 4).

We compared a pairwise matrix of genetic distances among *COI* sequences using the Tamura-Nei model for all haplotypes that occurred at least once in the

Table 2 Pairwise population estimates of Fst using the COI gene, for 14 populations of Amblema plicata

Population	SR1	SR2	AR1	AR2	WR1	WR2	LM1	LM2	STR1	STR2	OR1	OR2	KR1	KR2
SR1	0.000													
SR2	0.113	0.000												
AR1	0.093	0.446*	0.000											
AR2	–0.025	0.101	0.176*	0.000										
WR1	0.015	0.298*	0.025	0.048	0.000									
WR2	0.102	0.425*	0.014	0.140*	–0.059	0.000								
LM1	–0.055	0.198	0.040	0.019	0.011	0.091	0.000							
LM2	0.004	0.259*	0.049	0.020	–0.015	0.021	0.016	0.000						
STR1	0.364*	0.633*	0.408*	0.342*	0.293*	0.303*	0.422*	0.259*	0.000					
STR2	0.127	0.484*	0.015	0.157*	0.016	–0.018	0.123	0.018	0.290*	0.000				
OR1	0.200*	0.575*	–0.009	0.264*	0.074	0.016	0.170	0.094	0.412*	–0.010	0.000			
OR2	0.159*	0.478*	0.002	0.203*	0.047	0.001	0.136*	0.060	0.332*	–0.025	–0.036	0.000		
KR1	0.249*	0.451*	0.310*	0.208*	0.190*	0.205*	0.302*	0.153*	0.291*	0.181*	0.301*	0.247*	0.000	
KR2	0.098	0.411*	0.004	0.136*	0.011	–0.009	0.085	0.016	0.289*	–0.028	0.001	–0.010	0.198*	0.000

Estimates were tested for significance at $P = 0.05$ using 3023 nonparametric permutations of the data, a (*) symbol indicates significance at $P = 0.05$. Population labels follow Fig. 1

study (data not shown). For the most part, genetic distances were not significant among haplotypes and the resulting tree showed little resolution of relationships. However, there were two short branches (two haplotypes each, 76 and 73%, respectively) that had significant bootstrap support. These four haplotypes composed 13% of all individuals found in the combined Kiamichi and Ouachita River samples. Further, these haplotypes did not occur outside of these two rivers.

Allozyme results

The allozyme analysis mean sample size per locus was 33.3 individuals from each population. Sample sizes

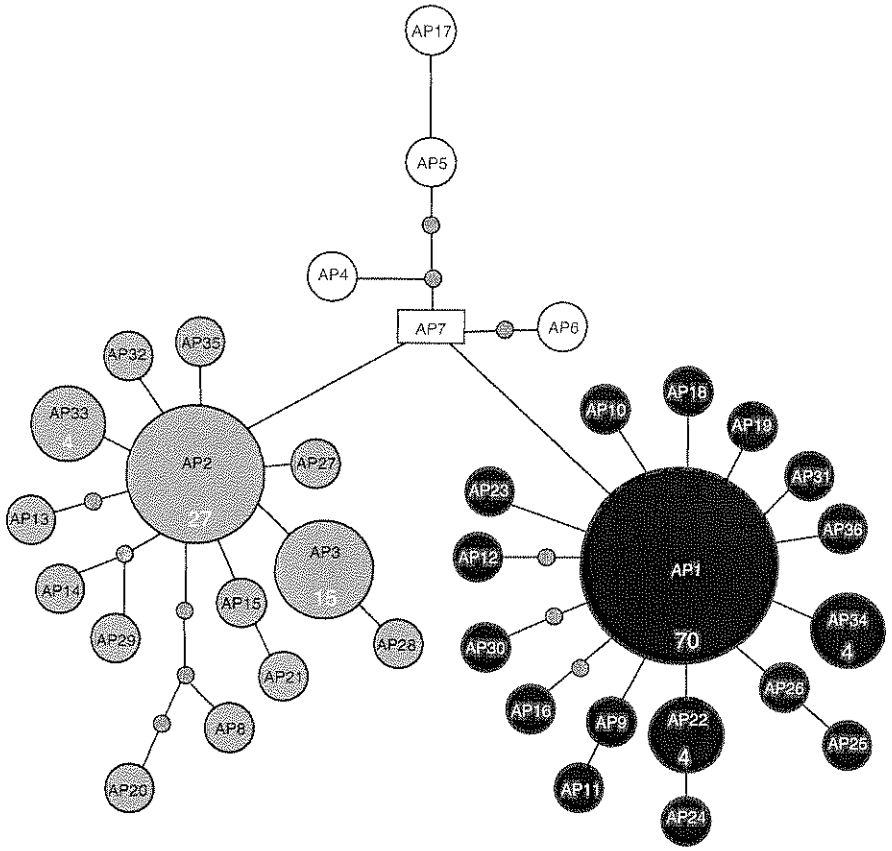

Fig. 3 Parsimony network of mtDNA haplotypes at the COI gene in *Amblema plicata*, where a circle represents a unique haplotype encountered in our analysis and a line connecting two circles represents a single base pair difference between two haplotypes. Rectangular individual is based on 95% root probability; small hatched circles represent intermediate haplotypes that were not encountered in our sampling. Size of the circle represents the frequency of the haplotype in this analysis (numbers are given for $N > 3$ haplotypes). Shading of the haplotype groups are coordinated with the map in Fig. 4. Haplotype labels follow Table 1

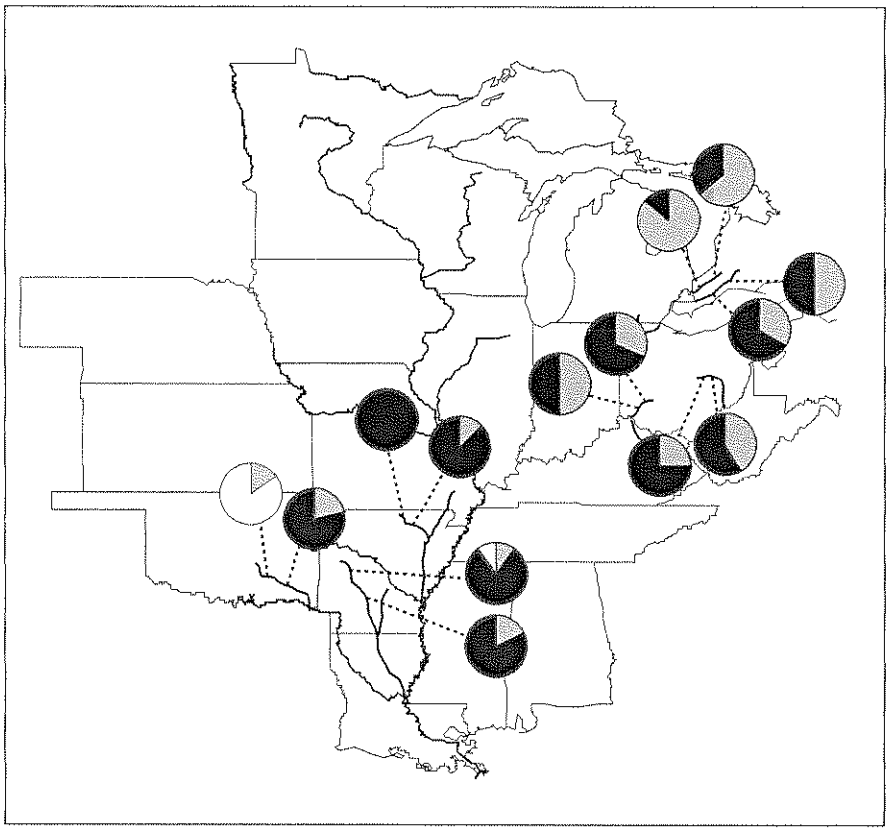

Fig. 4 A map of the central United States and Canada with populations of *Amblema plicata* sampled for mtDNA analysis. Pie charts represent haplotype group frequencies within each sampling location; group color is consistent with Fig. 3

varied for each locus-by-population combination (Table 3).

Within-population structure

Comparisons of observed allozyme genotype frequencies with Hardy-Weinberg expectations showed that 10 of 70 population-by-locus comparisons at polymorphic loci had genotype frequencies significantly different from H-W expectation. In total, we found seven populations out of H-W at the *MDH-2* locus (KR3, OR1, OR4*, STR3*, WR2, SJ1*, and SR2) and four loci out of H-W within the Walhonding 2 (WR2) population (*IDH-1**, *PGM-1*, *GPI-1**). However, after Bonferroni corrections 5 of 70 comparisons had genotype frequencies significantly different from H-W expectation (indicated with a asterisk above). All deviations from H-W were due to lower than expected heterozygosity in these populations.

There was no significant correlation with latitude for allozyme heterozygosity. Mean heterozygosity (H) was 0.11 over all locations (Table 2), but was varied among populations (range 0.03–0.23). We calculated the lowest mean H in Strawberry River populations (n = 3, H_{mean} = 0.04, range = 0.03–0.04) which were much lower than other Lower Mississippi drainage populations (therefore, Strawberry River populations are treated as a separate group in the following analyses of heterozygosity). Calculations of mean H within drainage was, on average, low in the Lake Erie drainage (n = 6, H_{mean} = 0.09, range 0.05–0.12). Mean H was similar between the Ohio River and Lower Mississippi drainages (Ohio River drainage n = 8, H_{mean} = 0.12, range 0.04–0.23; Ouachita and Kiamichi Rivers n = 7, H_{mean} = 0.12, range 0.10–0.16). Results showed a statistically significant difference in heterozygosity among drainages (df = 3, F = 4.15, P = 0.02) with the Strawberry River populations significantly lower than all other areas (Lake Erie, Ohio River, and Lower Mississippi drainages). Percentage of polymorphic loci (% P) was variable among populations (14.8–61.1%), but we found no geographic pattern. Similarly, mean number of alleles per locus was variable (1.2–2.1), with no geographic pattern (Table 3).

Among-population structure

Allele frequencies at allozyme loci varied among populations, and there were significant allele frequency differences among populations at 5 of the 8 polymorphic loci across all populations. Of those, three alleles were positively correlated with latitude: *Est-3* allele 2

Table 3 Allozyme allele frequencies and descriptive statistics for *Amblema plicata* populations from three drainages

Locus		Lower Mississippi River Drainage											Ohio River Drainage								Lake Erie Drainage					
		KR1	KR2	KR3	KR4	OR1	OR2	OR3	OR4	STR1	STR2	STR3	MA	LR	KL	OH	LM1	LM2	WR1	WR2	SJ1	SJ2	AR1	AR2	SR1	SR2
AAT-1		30	25	17	16	30	32	20	46	19	20	23	42	33	52	36	30	30	43	36	8	29	1	6	27	6
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
EST-3		35	35	26	18	20	27	10	31	19	20	23	42	33	52	36	42	30	43	33	24	25	2	26	27	26
	1	–	–	–	–	–	–	–	0.048	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	0.729	0.743	0.750	0.833	1.000	1.000	1.000	0.839	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.967	0.698	0.924	1.000	1.000	1.000	1.000	1.000	1.000
	3	0.271	0.257	0.250	0.167	0.000	0.000	0.000	0.097	–	–	–	–	–	–	–	–	0.033	0.302	0.076	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	0.016	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IDH-1		40	43	42	27	30	32	20	43	19	20	23	42	33	52	36	42	30	42	40	8	29	1	26	27	26
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.950	1.000	1.000	1.000	1.000	1.000	1.000
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.050	–	–	–	–	–	–
PGM-1		43	44	47	28	29	32	20	41	19	20	23	42	33	51	36	41	28	43	39	23	27	6	26	27	26
	1	–	–	–	–	0.086	0.063	0.100	0.110	0.105	0.150	0.196	0.190	0.152	0.118	0.139	0.037	0.089	0.209	0.192	0.087	0.019	0.083	–	0.019	0.019
	2	1.000	1.000	1.000	1.000	0.914	0.828	0.850	0.854	0.895	0.850	0.804	0.798	0.848	0.882	0.833	0.963	0.911	0.779	0.769	0.913	0.926	0.750	1.000	0.981	0.981
	3	–	–	–	–	–	0.109	0.050	0.037	–	–	–	0.012	–	–	0.028	–	–	0.012	0.038	–	0.056	0.167	–	–	–
PGM-2		40	43	43	27	28	32	20	45	19	20	23	42	33	52	36	42	30	43	33	22	29	1	26	27	26
	1	0.325	0.209	0.279	0.278	–	–	–	–	–	–	–	0.012	–	–	–	–	–	0.023	0.030	–	–	–	–	–	–
	2	–	–	–	–	0.036	0.047	0.025	0.311	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	0.675	0.791	0.721	0.722	0.893	0.875	0.875	0.600	1.000	1.000	1.000	0.750	0.742	0.673	0.597	0.881	0.900	0.616	0.606	0.909	0.707	1.000	0.692	0.796	0.750
	4	–	–	–	–	0.071	0.078	0.100	0.089	–	–	–	0.226	0.242	0.317	0.361	0.119	0.100	0.360	0.364	0.091	0.293	–	0.308	0.204	0.250
	5	–	–	–	–	–	–	–	–	–	–	–	0.012	0.015	–	0.042	–	–	–	–	–	–	–	–	–	–
GPI-1		36	39	34	25	30	32	20	46	19	20	23	41	33	51	35	42	30	21	28	18	6	1	26	27	6
	1	–	–	–	–	0.017	–	0.025	0.011	–	–	–	–	–	–	–	–	–	0.071	0.071	0.083	0.083	–	–	0.037	0.083
	2	1.000	1.000	1.000	0.980	0.983	1.000	0.975	0.989	1.000	1.000	1.000	0.939	0.848	0.980	0.943	1.000	0.983	0.643	0.839	0.917	0.750	1.000	0.788	0.944	0.917
	3	–	–	–	0.020	–	–	–	–	–	–	–	0.049	0.015	0.010	0.057	–	–	–	–	–	0.167	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–	0.012	0.136	0.010	–	–	0.017	0.286	0.089	–	–	–	0.212	0.019	–
MDH-1		37	42	36	28	30	32	20	46	19	20	23	42	33	52	36	42	30	43	40	26	29	1	26	27	26
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.988	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.012	–	–	–	–	–	–	–	–	–
MDH-2		27	31	24	12	27	31	19	44	19	20	23	42	33	1	5	42	30	43	40	26	6	1	6	27	6
	1	–	–	–	–	0.296	0.161	0.237	0.091	0.026	0.025	–	0.345	0.485	–	–	–	0.033	0.384	0.375	0.038	0.250	0.083	–	0.111	–
	2	0.741	0.726	0.833	0.667	0.667	0.452	0.711	0.739	0.974	0.975	0.826	0.655	0.515	1.000	0.300	0.976	0.967	0.605	0.512	0.750	0.667	0.750	1.000	0.704	0.667
	3	0.259	0.274	0.167	0.333	0.037	0.129	0.053	0.080	–	–	0.022	–	–	–	0.500	0.024	0.000	0.012	0.112	0.212	0.083	0.167	–	0.185	0.333
	4	–	–	–	–	–	0.226	–	0.091	–	–	0.152	–	–	–	0.200	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	0.032	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SOD-1		41	38	44	28	30	32	20	46	19	20	23	42	33	52	36	20	30	43	22	26	29	20	1	1	1
	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.977	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.023	–	–	–	–	–	–	–
Mean Sample size per locus		36.6 (1.8)	37.8 (2.1)	34.8 (3.5)	23.2 (2.1)	28.2 (1.1)	31.3 (0.6)	18.8 (1.1)	43.1 (1.6)	19 (0.0)	20 (0.0)	23 (0.0)	41.9 (0.1)	33 (0.0)	46.1 (5.6)	32.4 (3.4)	38.1 (2.6)	29.8 (0.2)	40.4 (2.4)	34.6 (2.1)	20.1 (2.4)	23.2 (3.3)	6.3 (2.7)	18.8 (3.6)	24.1 (2.9)	16.6 (3.8)

Table 3 Continued

Locus	Lower Mississippi River Drainage							Ohio River Drainage							Lake Erie Drainage					
	KR1	KR2	KR3	KR4	OR1	OR2	OR3	OR4	STR1	STR2	STR3	MA	LR	KL	OH	LM1	LM2	WR1	WR2	SJ1	SJ2	AR1	AR2	SR1	SR2
Mean number of allele per locus	1.3 (0.2)	1.3 (0.2)	1.3 (0.2)	1.4 (0.2)	1.7 (0.3)	1.9 (0.5)	1.8 (0.3)	2.2 (0.4)	1.2 (0.1)	1.2 (0.1)	1.3 (0.2)	1.9 (0.4)	1.7 (0.3)	1.6 (0.3)	1.8 (0.3)	1.4 (0.2)	1.6 (0.2)	2.1 (0.3)	2.1 (0.3)	1.6 (0.2)	1.8 (0.3)	1.4 (0.3)	1.2 (0.1)	1.7 (0.3)	1.4 (0.2)
Polymorphic loci (%)	33.3	33.3	33.3	33.3	33.3	33.3	33.3	44.4	11.1	11.1	22.2	44.4	44.4	22.2	44.4	11.1	22.2	55.6	66.7	44.4	44.4	22.2	22.2	33.3	33.3
Mean Heterozygosity	0.16 (0.08)	0.13 (0.06)	0.12 (0.06)	0.11 (0.05)	0.11 (0.06)	0.11 (0.06)	0.11 (0.05)	0.10 (0.04)	0.03 (0.02)	0.04 (0.03)	0.04 (0.03)	0.13 (0.06)	0.17 (0.08)	0.07 (0.05)	0.14 (0.08)	0.04 (0.02)	0.06 (0.03)	0.23 (0.07)	0.15 (0.30)	0.08 (0.03)	0.21 (0.10)	0.09 (0.06)	0.10 (0.06)	0.09 (0.05)	0.05 (0.03)

For mean population estimates, standard deviations are in parentheses. Population labels follow Fig. 1

($R = 0.53$, $P = 0.007$), *Pgm-2* allele 4 ($R = 0.58$, $P = 0.002$), and *Gpi-1* allele 2($R = 0.41$, $P = 0.040$); and two alleles were negatively correlated: *Est-3* allele 3 ($R = -0.50$, $P = 0.010$), and *Pgm-2* allele 1 ($R = -0.52$, $P = 0.007$). In past studies allele frequencies were significantly correlated with temperature clines including populations of other freshwater mussel taxa, *Dreissena polymorpha* (Elderkin et al. 2001). Allele frequency shifts have been caused by natural selection on physiological adaptations in *Colias* butterflies (Watt 1977) acorn barnacles, *Semibalanus balanoides* (Schmit and Rand 1999) and blue mussels, *Mytilus edulis* (Hilbish and Koehn 1985). Therefore in populations of *A. plicata* allele frequency changes correlated with latitude could be due to natural selection on one or more of these alleles. There were no private alleles according to our criterion.

Overall among-population structure using allozymes was significant ($\theta_{ST} = 0.112$; 95% CI = 0.145–0.081). When the genetic structure was divided into a hierarchy, structure was low both within rivers ($\theta_{SR} = 0.017$) and among drainages ($\theta_{DT} = 0.019$). However, genetic structure was relatively high among rivers ($\theta_{RD} = 0.102$).

Unbiased genetic distance estimates calculated among populations had a range of 0.001–0.058 (Table 4). The Kiamichi River sites were more similar to each other than to any other population, and therefore grouped together on a solitary branch in the analysis (Fig. 5). The remainder of the tree showed no geographic relationship among populations. The Mantel test between Nei's unbiased genetic distance (allozyme loci) and geographic distance showed a positive correlation (Fig. 2C), as did the analysis of pairwise Fst estimates and geographic distance (Fig. 2B).

Discussion

Within-population structure

For the freshwater mussel *Amblema plicata*, most of the genetic variation is found within populations. Average haplotype diversity within populations at the *COI* locus in *A. plicata* is higher than values reported for other freshwater mussels. . While we found an average of 4.8 haplotypes per population and a total of 39 haplotypes, a study of *COI* variation found only 3 haplotypes throughout the range of *Lasmigona subviridis* (King et al. 1999). Also, in populations of *Margaritifera margaritifera* from several drainages in Spain, only two variable sites were found in a 657 bp segment of the COI gene (Machordom et al. 2003).

Table 4 Pairwise estimates of population differentiation (F_{st}) above the diagonal and Nei's (1978) unbiased genetic distance below the diagonal using nine allozyme markers for 25 populations of *Amblema plicata*. Population labels follow Fig. 1

Population	MR	LR	TR	OH	KR1	KR2	KR3	KR4	OR1	OR2	OR3	OR4
MR		0.012	0.008	0.155	0.179	0.166	0.169	0.150	0.020	0.065	0.008	0.077
LR	0.002		0.017	0.161	0.215	0.205	0.216	0.183	0.051	0.083	0.050	0.117
TR	0.015	0.030		–0.003	0.197	0.181	0.184	0.151	0.074	0.074	0.050	0.087
OH	0.032	0.036	0.045		0.207	0.195	0.252	0.126	0.198	0.073	0.165	0.146
KR1	0.036	0.048	0.030	0.043		–0.001	–0.004	–0.005	0.163	0.160	0.139	0.096
KR2	0.031	0.042	0.028	0.038	0.000		0.000	–0.009	0.134	0.134	0.112	0.091
KR3	0.031	0.044	0.022	0.049	0.000	0.000		0.005	0.147	0.163	0.120	0.085
KR4	0.029	0.039	0.027	0.027	0.000	0.000	0.001		0.130	0.107	0.105	0.081
OR1	0.003	0.007	0.018	0.036	0.027	0.020	0.022	0.019		0.048	–0.013	0.076
OR2	0.012	0.016	0.030	0.018	0.032	0.024	0.030	0.021	0.007		0.035	0.080
OR3	0.001	0.008	0.012	0.033	0.025	0.019	0.020	0.018	0.000	0.006		0.050
OR4	0.016	0.027	0.015	0.035	0.020	0.018	0.017	0.018	0.014	0.017	0.011	
SJ1	0.012	0.022	0.012	0.026	0.020	0.012	0.016	0.011	0.005	0.010	0.002	0.014
SJ2	0.001	0.004	0.010	0.023	0.029	0.024	0.025	0.020	0.004	0.012	0.015	
STR1	0.018	0.033	0.011	0.058	0.028	0.020	0.019	0.023	0.009	0.021	0.006	0.019
STR2	0.017	0.033	0.011	0.058	0.029	0.022	0.020	0.024	0.010	0.022	0.006	0.019
STR3	0.016	0.030	0.015	0.045	0.030	0.022	0.023	0.023	0.009	0.012	0.006	0.018
AR1	0.011	0.022	0.017	0.034	0.024	0.015	0.019	0.015	0.003	0.005	0.000	0.016
AR2	0.022	0.031	0.005	0.052	0.034	0.031	0.026	0.030	0.023	0.037	0.018	0.023
SR1	0.008	0.015	0.010	0.020	0.019	0.013	0.016	0.010	0.004	0.009	0.002	0.012
SR2	0.014	0.023	0.012	0.009	0.017	0.012	0.016	0.007	0.010	0.012	0.008	0.014
LM1	0.016	0.031	0.005	0.049	0.023	0.018	0.015	0.019	0.010	0.022	0.006	0.015
LM2	0.014	0.028	0.005	0.051	0.023	0.017	0.015	0.020	0.008	0.021	0.005	0.014
WR1	0.022	0.016	0.040	0.056	0.048	0.045	0.045	0.048	0.032	0.044	0.031	0.036
WR2	0.004	0.002	0.025	0.022	0.040	0.037	0.039	0.033	0.013	0.019	0.012	0.023

Population	SJ1	SJ2	STR1	STR2	STR3	AR1	AR2	SR1	SR2	LM1	LM2	WR1	WR2
MR	0.070	0.019	0.134	0.129	0.106	0.041	0.118	0.050	0.083	0.149	0.107	0.090	0.019
LR	0.081	0.030	0.217	0.215	0.180	0.121	0.155	0.088	0.115	0.243	0.190	0.063	0.009
TR	0.124	0.053	0.142	0.141	0.147	0.043	0.056	0.025	0.014	0.075	0.066	0.142	0.023
OH	0.135	0.111	0.369	0.367	0.250	0.114	0.258	0.116	0.025	0.383	0.337	0.174	0.079
KR1	0.146	0.172	0.214	0.221	0.198	0.133	0.189	0.118	0.115	0.217	0.183	0.171	0.156
KR2	0.129	0.156	0.176	0.184	0.161	0.121	0.178	0.091	0.085	0.182	0.150	0.172	0.155
KR3	0.139	0.172	0.175	0.184	0.170	0.138	0.167	0.106	0.122	0.171	0.139	0.172	0.162
KR4	0.100	0.118	0.222	0.229	0.175	0.064	0.172	0.071	0.031	0.227	0.187	0.162	0.125
OR1	0.058	0.056	0.118	0.120	0.088	0.045	0.151	0.034	0.087	0.138	0.095	0.136	0.071
OR2	0.079	0.066	0.170	0.170	0.088	0.018	0.170	0.061	0.056	0.203	0.161	0.155	0.078
OR3	0.037	0.033	0.084	0.083	0.053	–0.018	0.114	0.016	0.051	0.103	0.059	0.112	0.051
OR4	0.071	0.078	0.122	0.122	0.102	–0.008	0.103	0.066	0.072	0.129	0.098	0.122	0.083
SJ1		0.007	0.125	0.128	0.107	–0.021	0.098	0.044	0.015	0.156	0.120	0.100	0.059
SJ2	0.006		0.227	0.230	0.166	–0.005	0.082	0.016	0.004	0.212	0.165	0.059	0.009
STR1	0.005	0.018		–0.016	0.035	0.102	0.196	0.123	0.228	0.040	0.008	0.217	0.187
STR2	0.006	0.019	0.000		0.022	0.084	0.203	0.132	0.234	0.059	0.015	0.215	0.185
STR3	0.005	0.019	0.002	0.002		0.003	0.173	0.105	0.145	0.105	0.054	0.201	0.158
AR1	0.000	0.012	0.002	0.002	0.001		0.189	0.019	–0.016	0.244	0.117	0.098	0.026
AR2	0.015	0.009	0.016	0.018	0.023	0.024		0.069	0.077	0.122	0.108	0.126	0.112
SR1	0.001	0.000	0.011	0.012	0.012	0.005	0.012		–0.029	0.088	0.077	0.136	0.066
SR2	0.000	0.002	0.016	0.018	0.016	0.008	0.014	0.000		0.183	0.159	0.126	0.057
LM1	0.005	0.012	0.002	0.003	0.006	0.006	0.008	0.006	0.010		–0.001	0.248	0.207
LM2	0.004	0.012	0.000	0.001	0.004	0.004	0.009	0.007	0.012	0.000		0.194	0.161
WR1	0.041	0.018	0.053	0.052	0.052	0.047	0.034	0.033	0.039	0.048	0.042		0.029
WR2	0.022	0.003	0.038	0.038	0.034	0.025	0.029	0.014	0.016	0.033	0.030	0.010	

However, these latter studies, while examining multiple drainage basins, reported much smaller total sample sizes. A second explanation is that these two taxa may have lower effective population sizes and *A. plicata* maintains high within population diversity due to relatively high effective population size. Deviations from Hardy-Weinberg equilibrium are common in bivalve mollusks, especially for heterozygote deficiencies (Reviewed by Zouros and Foltz 1984; Gaffney et al. 1990; Bierne et al. 1998). Possible explanations for this range from electrophoretic effects of null alleles (Foltz 1986), to non-random mating, selection, and Wahlund

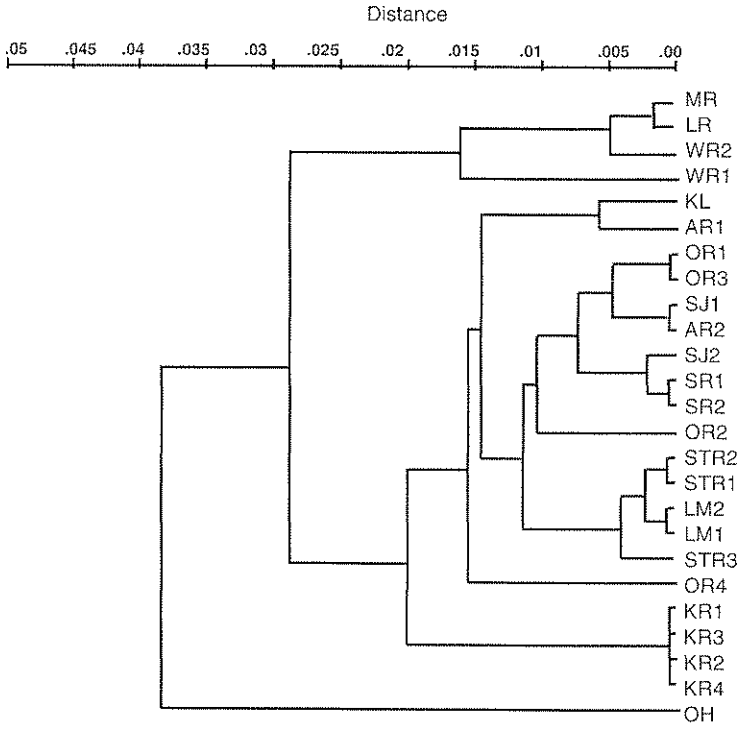

Fig. 5 UPGMA phenogram based on unbiased genetic distance (Nei 1978) for populations of *Amblema plicata*. Distances calculated using nine allozyme loci. Population labels follow Fig. 1.

effect (Gaffney et al. 1990; Bierne et al. 1998). Our estimates of direct count heterozygosity are similar to those reported previously for *A. plicata* ($H = 0.09$; range 0.05–0.14) (Johnson et al. 1998; Mulvey et al. 1997), but lower than those reported for *Q. quadrula* (mean = 0.24 range = 0.20–0.27)(Berg et al. 1998). Also, *A. plicata* has higher average H than the average of 58 unionid species—$H = 0.05$; range 0.01–0.29 (Table 3, Curole et al. 2004). The same is true for other allozyme measures of within-population variation (% P and average number of alleles per locus, Curole et al. 2004).

Population genetic variation can be affected by the geologic history and/or the dispersal abilities of the species under investigation (Wares and Turner 2003). In North America, the Pleistocene glaciation had a profound effect on the genetic structure of terrestrial invertebrates (Hewitt 1996) and many freshwater fishes (Bernatchez and Wilson 1998; Hewitt 1996). During this time, many species retreated into (multiple) glacial refugia, thus isolating populations from one another and allowing them to lose within-population diversity due to genetic drift. With the receding of the ice sheet, freshwater species dispersed from these refugia to re-colonize suitable habitat (Mandrak and Crossman 1992; Mayden 1988). Newly-founded populations tend to have reduced genetic diversity as they result from chance founding (Hewitt 1996). Consistent with this scenario, *A. plicata* populations exhibited lower diversity within populations in glaciated regions than in regions unaffected by the Pleistocene glaciers. Thus, we observed a pattern of fewer haplotypes per population, fewer unique haplotypes, lower divergence among haplotypes, and lower average heterozygosity within the Lake Erie drainage compared to the Lower Mississippi drainage. Interestingly, genetic diversity was also low within the Strawberry River populations at both mtDNA and allozyme loci. Overall, Strawberry River populations had significantly lower heterozygosity when compared to all other areas (allozymes). This may indicate either a population bottleneck in the recent past, or recent founding by only a few individuals (Hartl and Clark 1997).

Among-population structure

We found significant genetic structure over all populations of *A. plicata*. This finding was primarily among populations within rivers from the Lake Erie and Lower Mississippi drainages. Relatively little structure was found within the Ohio River drainage or at larger spatial scales. Surprisingly, when our results are compared to those of other studies of mussel taxa, estimates of among-population structure in *A. plicata* are relatively low. Although average within-population sequence divergence increased with latitude, total average sequence divergence was relatively low when compared to other mussel taxa. The average sequence

divergence over all individuals was as low as was reported for other mussels such as *Lasmigona subvirdis* (King et al. 1999) and *Margartifera margaritifera* (Machordom et al. 2003), even though we sampled over a greater geographic range. In populations of *Elliptio dilatata* and *Cumberlandia monodonta*, we have found higher average sequence divergence among individuals at similar spatial scales (C. L. Elderkin and D. J. Berg, unpublished data). At the same mtDNA locus, *Lampsilis hydiana* showed greater genetic structure at much smaller geographic scales within Arkansas rivers (Turner et al. 2000). Our estimates of Nei's unbiased genetic distance for allozyme loci (mean = 0.019, range 0.000 to 0.058) were closer to the low values reported for *Quadrula quadrula* (mean = 0.004, range < 0.0005–0.037; Berg et al. 1998) than those reported for six other mussel species (mean = 0.047, range 0.00 to 0.12; Nagel et al. 1996; Stiven and Alderman 1992). Although these estimates were lower than those of other mussels, we found significant isolation-by-distance (allozymes) among populations of *A. plicata*, where non-adjacent populations were more likely to have increased genetic distance and/or population structure (Wright 1942; Kimura and Weiss 1964). These results are similar to those reported for *Quadrula quadrula* where genetic distance was relatively low, but was significantly correlated to geographic distance (Berg et al. 1998).

In freshwater rivers, the distance between two populations is affected by the network of connectivity among tributaries to large rivers (Bunn and Hughes 1997; Fetzner and Crandall 2003; Wares and Turner 2003). Our analysis integrated these networks into nested hierarchies to determine the level of connectivity within the network (Bunn and Hughes 1997). From our results, we conclude that for *A. plicata*, there is little genetic structure among drainages or among rivers within drainages. Similar patterns of genetic structure have been found in stream insects; this pattern has been attributed to differential recruitment of offspring due to nonrandom mating and/or limited dispersal within streams (Hughes et al. 2000). However, if this were the case, mussels would likely show significant deviations of genotype frequencies from Hardy–Weinberg expectation, as is seen with aquatic insects (Hughes et al. 2000). The fact that we observed H–W deviations in our study implies that this process or processes might be responsible for the observed pattern of genetic structure. Low genetic structure on large geographic scales could be due to several factors including high effective population size (Waples 1998; Whitlock and McCauley 1999) or high gene flow across large geographic distances. Populations of *A. plicata* have been historically widespread and common (Cummings and Mayer 1992) and were even commercially harvested until a few years ago. In the three drainages sampled, *A. plicata* is an abundant species and is commonly found in large numbers (Cummings and Mayer 1992; Hoggarth 1995–1996; Metcalfe-Smith et al. 1998; Vaughn and Spooner 2004). Therefore, high effective population size and low genetic drift is a viable hypothesis for the lack of genetic structure at large geographic scales.

Gene flow among populations of freshwater mussels is most likely affected by migration patterns of fish host species. Freshwater mussels disperse as glochidia, larvae which are obligate parasites on fish hosts (McMahon 1991). *Amblema plicata* populations most likely are obligate to one or more fish hosts that are widespread in the Lower Mississippi, Ohio River, and Lake Erie drainages. Potential hosts for *A. plicata* include species from the perch and sunfish families (Percidae and Centrarchidae, respectively; Division of Molluscs, The Ohio State University Museum of Biological Diversity, Mussel Host Database, http://www.128.146.250.63/bivalves/), both of which are common and widespread throughout eastern North America. In other freshwater invertebrates, dispersal ability was found to be a major component of among-population genetic structure (Bilton et al. 2001), and degree of isolation was negatively correlated with dispersal ability in aquatic insects from small streams (Miller et al. 2002). One explanation for the genetic structure of *A. plicata* is high gene flow at large geographic distances, most likely due to widespread and/or highly vagile host species. Potential hosts such as percids and centrarchids are known to travel large distances during migration; for instance, smallmouth bass (Centrarchidae: *Micropterus dolomei*) have been known to travel up to 75 km (Lyons and Kanehl 2002). Our results include widespread distribution of haplotypes (mtDNA) and alleles (allozymes) and shallow genetic divergences (mtDNA and allozymes), further suggesting high gene flow (Avise 2000; Berendzen et al. 2003). Use of such abundant, widespread fish hosts is at least partially responsible for this mussel being among the most common species in this highly endangered taxonomic group. Although fish migration distance is high, *A. plicata* populations are geographically far apart, making it unlikely that a single dispersal event would occur between the most distant sites. Our data suggest a stepping-stone model where distant populations have a greater genetic distance on average than adjacent populations (Kimura and Weiss 1964). Further, diversity indices and allele frequencies correlated with latitude suggest that *A. plicata* expanded out of southern refugia.

We found significant among-population structure within the Lake Erie and Lower Mississippi River drainages, which most likely is due to their geologic history. The Lake Erie drainage populations shows significant among-population genetic structure, which may be due to multiple migration routes into the drainage following the Pleistocene deglaciation. Most freshwater fishes crossed into Ontario using one of nine dispersal routes following the recession of the glacier (Mandrak and Crossman 1992). Because mussels are obligate to fish hosts for dispersal, it follows that mussels in the Great Lakes drainage were brought there through these routes via their respective hosts. Two of the dispersal routes lead directly into Lake Erie (Fort Wayne route from the west and Mohawk route from the east). From the distribution of haplotypes (mtDNA) and allele frequencies (allozymes), it appears that our results are consistent with the hypothesis that Canadian Great Lakes populations migrated into Lake Erie through the Fort Wayne route, where the glacial Lake Maumee flowed out of the Maumee basin into the Ohio River (Graf 2002). However, the significant genetic structure in the Lake Erie populations (and lack of structure in Ohio River populations) indicates that Canadian Great Lakes populations used at least one other migration route into the drainage (Mandrak and Crossman 1992). These results are consistent with clinal genetic variation found in other species where multiple glacial refugia have been identified (Bernatchez and Wilson 1998). Our understanding of past dispersal by *A. plicata* into the Great Lakes drainage would be enhanced by further sampling of the northwestern area of the species range, including the drainage basins of the upper Great Lakes and the upper Mississippi River, including the Missouri River.

Populations of *A. plicata* within the Lower Mississippi River drainage had the highest among-population structure. During the Pleistocene, southern rivers were most likely refuges for many freshwater species (Mayden 1988; Bernatchez and Wilson 1998; Berendzen et al. 2003). The region drained by the Kiamichi and Ouachita rivers has been identified as having a high degree of endemism in freshwater taxa (Crandall 1998; Mayden 1985; Vaughn et al. 1996) and shows population genetic structure at relatively small geographic scales for several of these endemic taxa (Fetzner and Crandall 2003; Turner et al. 2000). For *A. plicata*, the Kiamichi River is a region containing a unique assemblage of haplotypes (mtDNA) and genetic distance estimates based on allele frequency (allozymes) showed a clustering of Kiamichi populations. Surprisingly, when our results are compared to other invertebrates, *A. plicata* has relatively little genetic structure endemic to the region (Fetzner and Crandall 2003; Turner et al. 2000). *A. plicata* populations are found in the other rivers of the southern Mississippi River drainage, which we would predict from our results contain high variation and possibly other unique populations similar to the Kiamichi populations. We suggest further sampling of *A. plicata* populations in the southern Mississippi River drainage.

Conservation implications

Conservation of freshwater mussels is imperative as population numbers are declining, even for species that as yet have no conservation status (Neves 1999). Quantifying genetic diversity and understanding the partitioning of this diversity is necessary for identifying units for conservation (Moritz 1994a, 1994b). *Amblema plicata* is a relatively common mussel species in the three drainages we sampled (Cummings and Mayer 1992; Hoggarth 1995–1996; Metcalfe-Smith et al. 1998; Vaughn and Spooner 2004). However, there was a possibility that, although this species is widespread, populations could be discrete nonexchangeable units. Further analysis of local adaptation (especially to potential fish hosts), and ecological differences among populations beyond genetic differences is necessary prior to the development of any comprehensive conservation effort (Frazer and Bernatchez 2001; Moritz 2002). An optimal strategy for protection of genetic diversity within this species might be based on protection of several populations in each of the regions we sampled. Particular attention should be paid to southern river systems, such as the Kiamichi, which seem to contain unique haplotypes and an even larger proportion of the total genetic variation. Further sampling in the southern portion of the range is necessary to determine the extent of genetic structure within this drainage.

Summary

Overall, genetic structure of *A. plicata* populations follow a pattern typical of a species that has high effective population sizes, and/or high dispersal among populations even at large geographic scales. Colonization of all extant populations sampled was likely the result of dispersal from closely related populations, possibly from the Kiamichi or Ouachita rivers or other parts of the Lower Mississippi drainage, where we measured the highest genetic diversity. As the Pleistocene ice sheet receded, Lake Erie populations were probably established via dispersal

from the Ohio River drainage into glacial Lake Maumee and from at least one other glacial refugium. *Amblema plicata* populations in post-glacial areas have lower within-population diversity than, and have similar genetic composition to, populations in the central Ohio River drainage. Further sampling of the species' northern and southern range is necessary to determine the total genetic structure of this species, and may identify other glacial refugia. When we partition genetic structure into a hierarchy, *A. plicata* has relatively low structure when compared to other freshwater invertebrates, consistent with a hypothesis of large effective population sizes and/or high dispersal. Across much of its current range, *A. plicata* shows increasing genetic diversity from north to south but has less genetic structure than other mussel species. Mussel species exhibiting this pattern of genetic structure will likely require management at several spatial scales (within river and among regions) if genetic resources are to be effectively conserved.

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