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# GENETIC RELATIONSHIPS OF SEVERAL AMBLEMINI SPECIES (BIVALVIA: UNIONIDAE) IN ARKANSAS

RONALD L. JOHNSON, FANG QING LIANG, AND JERRY L. FARRIS
*Arkansas State University*
*Department of Biology*
*State University, Arkansas 72467*

*ABSTRACT* Allozyme analysis of 16 loci was utilized to determine the genetic relationships for four species of mussels in the Tribe Amblemini (*Amblema plicata plicata* (Say), *Plectomerus dombeyanus* (Valenciennes), *Quadrula pustulosa pustulosa* (I. Lea), and *Q. quadrula* (Rafinesque)). Conspecific genetic distance was quite low between populations for each species, with ranges of 0.012–0.113. The congeneric distance for *Q. p. pustulosa* and *Q. quadrula* was determined to be 0.333. Distances between genera ranged from 0.396 to 0.787, similar ranges to those of other unionid species studied. Genetic distance of *Amblema* and *Plectomerus* was greater than that expected based on previous studies, with *Plectomerus* more closely related to *Quadrula* than to *Amblema*.

*KEY WORDS:* Unionidae, genetic analysis, allozyme

## INTRODUCTION

The freshwater bivalves of the family Unionidae are a rich taxonomic group with longstanding difficulties in classification. A historical reliance on conchological features, which exhibit a high degree of phenotypic plasticity, has obscured the existence of convergent and parallel evolution (Kat 1983a, Davis 1984). Attempts to clarify higher taxonomic relationships have resulted in the usage of various soft anatomical and reproductive features for taxonomic analysis (Heard and Guckert 1971, Davis and Fuller 1981, Kat 1983b). More recently, immunologic and allozyme analyses have been utilized (Davis and Fuller 1981, Davis et al. 1981, Kat 1983a, Kat 1983b, Kat and Davis 1984, Stivens and Alderman 1992). Davis et al. (1981) found close genetic relatedness by way of allozyme analysis among species possessing radically different shell morphologies and geographic distributions.

The tribe Amblemini, endemic to North America, is characterized by eight genera and 27 species (Davis and Fuller 1981, Davis 1984, Williams et al. 1993). The tribe Amblemini is characterized by relatively old genera having lineages persisting more than 10 million years (Davis et al. 1981). Genetic distances between genera should therefore be intermediate between more recent groups such as the Pleurobemini and ancient groups, such as the Margaritiferinae and Anodontini (Davis 1984). The genera within this tribe originated as early as 10 million years ago (Davis et al. 1981).

This study utilized cellulose acetate electrophoresis and histochemical staining of allozymes to determine the genetic distance for three genera and four species of the tribe Amblemini. *Amblema plicata plicata* (Say), *Plectomerus dombeyanus* (Valenciennes), *Quadrula pustulosa pustulosa* (I. Lea), and *O. quadrula* (Rafinesque) are dominant community members within the Cache and White rivers, which belong to the Mississippi River drainage system (Christian 1995). Each species studied is widely distributed within the Mississippi River drainage (Williams et al. 1993). No Arkansas mussel populations have been previously studied by allozyme analysis.

Davis (1984) identified a high genetic distance between genera of the Amblemini. However, on the basis of immunologic and morphologic features, Davis and Fuller (1981) placed the genera *Amblema, Megalonaias,* and *Plectomerus* as congeneric. Our primary objective in this study was to test the genetic relationships of these species through biochemical allozyme analysis. Since Christian (1995) and Posey (1997) reported that occasional individuals collected in Arkansas streams possess intermediate anatomic characteristics between *Q. p. pustulosa* and *Q. quadrula*, a secondary objective was to determine the genetic distance between these two species which may be hybridizing.

## MATERIALS AND METHODS

Genetic distance was determined within populations for the lower regions of the Cache and White rivers in Arkansas. Three sequential downstream mussel beds were chosen for each river: miles 37, 36, and 35 (sites A, B, and C, respectively) for the Cache River, and miles 63.5, 57.2, and 48.5 for the White River (sites D, E, and F, respectively). Four species of bivalves were studied, with 12–36 individuals of two species collected from each site. Hookah rig diving was used to obtain the mussels in the summer of 1994. Mussels were brought back to the laboratory on ice and processed immediately or frozen at −70°C. Voucher specimens have been deposited in the Unionacea collection of the Arkansas State University Museum of Zoology (ASUMZ).

Adductor muscles were homogenized in equal volumes (w/v) of Tris-HCl buffer (pH 7.0). Electrophoresis of homogenate was performed on cellulose acetate plates at 200 volts for 15 min in TG buffer (0.025 M Tris; 0.192 M Glycine) at room temperature (2 mA/plate). Nine enzyme systems representing 16 loci were selected for analysis based upon their expression in adductor muscle. The enzymatic loci were as follows: fumarase (FUM-1, FUM-2; E.C. No. 4.2.1.2); glutamate-oxaloacetate transferase (GOT-1, GOT-2; E.C. No. 2.6.1.1); isocitrate dehydrogenase (IDH-1, IDH-2; E.C. No. 1.1.1.42); lactate dehydrogenase (LDH-1, LDH-2; E.C. No. 1.1.1.27); malate dehydrogenase (MDH-1, MDH-2; E.C. No. 1.1.1.37); malic enzyme (ME-1, ME-2; E.C. No. 1.1.1.40); mannose phosphate isomerase (MPI-1, MPI-2; E.C. No. 5.3.1.8); phosphoglucose isomerase (PGI-1; E.C. No. 5.3.1.9); and phosphoglucomutase (PGM-1, PGM-2; E.C. No. 2.7.5.1) (IUBNC 1984). The distance of migration for each specific enzyme was visualized by histochemical staining (Hebert and Beaton 1989).

Individual genotypes were used as original data with allele frequencies, locus heterozygosity, genetic identity, and Nei's unbiased genetic distance determined using the program BIOSYS-1 (Swofford and Selander 1989).

TABLE 1.

Allele frequencies, mean direct count heterozygosities (H), and polymorphism (P) of each of the four species at each site[a]

| Locus | Population | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AP-A | AP-B | PD-A | PD-B | PD-C | QP-D | QP-E | QP-F | QQ-D | QQ-E | QQ-F | QQ-C |
| FUM-1 | | | | | | | | | | | | |
| (N) | 35 | 23 | 31 | 12 | 36 | 12 | 20 | 15 | 32 | 24 | 36 | 36 |
| A | 0.500 | 0.826 | 0.048 | 0.000 | 0.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| B | 0.500 | 0.174 | 0.935 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| C | 0.000 | 0.000 | 0.016 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| FUM-2 | | | | | | | | | | | | |
| (N) | 35 | 1 | 31 | 18 | 36 | 12 | 20 | 15 | 32 | 24 | 36 | 36 |
| A | 0.771 | 1.000 | 0.968 | 1.000 | 1.000 | 0.500 | 0.975 | 0.867 | 0.047 | 0.000 | 0.000 | 0.000 |
| B | 0.143 | 0.000 | 0.032 | 0.000 | 0.000 | 0.500 | 0.025 | 0.133 | 0.953 | 1.000 | 1.000 | 1.000 |
| C | 0.086 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| GOT-1 | | | | | | | | | | | | |
| (N) | 35 | 24 | 31 | 18 | 36 | 12 | 20 | 15 | 32 | 24 | 36 | 36 |
| A | 0.357 | 0.000 | 0.113 | 0.167 | 0.000 | 0.000 | 0.050 | 0.000 | 0.063 | 0.104 | 0.000 | 0.000 |
| B | 0.614 | 1.000 | 0.790 | 0.694 | 1.000 | 1.000 | 0.950 | 0.933 | 0.938 | 0.458 | 1.000 | 1.000 |
| C | 0.029 | 0.000 | 0.097 | 0.139 | 0.000 | 0.000 | 0.000 | 0.067 | 0.000 | 0.438 | 0.000 | 0.000 |
| GOT-2 | | | | | | | | | | | | |
| (N) | 35 | 24 | 31 | 18 | 36 | 12 | 20 | 15 | 32 | 24 | 36 | 36 |
| A | 0.029 | 0.000 | 0.839 | 0.972 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| B | 0.100 | 0.000 | 0.161 | 0.028 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| C | 0.057 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| D | 0.800 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| E | 0.014 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| IDH-1 | | | | | | | | | | | | |
| (N) | 35 | 23 | 31 | 18 | 36 | 12 | 20 | 14 | 32 | 24 | 36 | 36 |
| A | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.667 | 0.975 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| B | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.333 | 0.025 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| IDH-2 | | | | | | | | | | | | |
| (N) | 35 | 23 | 31 | 18 | 36 | 12 | 16 | 15 | 32 | 24 | 36 | 36 |
| A | 0.943 | 1.000 | 0.968 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 | 0.021 | 0.000 | 0.000 |
| B | 0.057 | 0.000 | 0.032 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.979 | 1.000 | 1.000 |
| LDH-1 | | | | | | | | | | | | |
| (N) | 0 | 0 | 0 | 0 | 0 | 11 | 20 | 15 | 32 | 24 | 36 | 36 |
| A | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.636 | 0.525 | 0.600 | 0.906 | 0.875 | 0.764 | 0.792 |
| B | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.364 | 0.475 | 0.400 | 0.031 | 0.042 | 0.167 | 0.208 |
| C | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.063 | 0.083 | 0.069 | 0.000 |
| D | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| LDH-2 | | | | | | | | | | | | |
| (N) | 0 | 0 | 1 | 1 | 1 | 10 | 17 | 13 | 32 | 24 | 36 | 36 |
| A | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| B | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| C | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| D | 0.000 | 0.000 | 1.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| MDH-1 | | | | | | | | | | | | |
| (N) | 35 | 24 | 30 | 18 | 36 | 12 | 20 | 15 | 32 | 24 | 36 | 36 |
| A | 0.129 | 0.167 | 0.267 | 0.361 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| B | 0.529 | 0.500 | 0.467 | 0.444 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| C | 0.329 | 0.292 | 0.267 | 0.194 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| D | 0.014 | 0.042 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| MDH-2 | | | | | | | | | | | | |
| (N) | 35 | 24 | 31 | 18 | 36 | 12 | 20 | 15 | 32 | 24 | 36 | 36 |
| A | 0.000 | 0.042 | 0.806 | 0.500 | 1.000 | 1.000 | 0.950 | 0.933 | 1.000 | 1.000 | 1.000 | 1.000 |
| B | 0.914 | 0.958 | 0.194 | 0.500 | 0.000 | 0.000 | 0.050 | 0.067 | 0.000 | 0.000 | 0.000 | 0.000 |
| C | 0.086 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| ME-1 | | | | | | | | | | | | |
| (N) | 34 | 23 | 27 | 18 | 36 | 12 | 20 | 15 | 32 | 24 | 36 | 35 |
| A | 0.206 | 0.130 | 0.222 | 0.167 | 0.000 | 0.000 | 0.050 | 0.033 | 0.344 | 0.104 | 0.139 | 0.000 |
| B | 0.206 | 0.217 | 0.704 | 0.722 | 1.000 | 1.000 | 0.925 | 0.967 | 0.656 | 0.625 | 0.861 | 0.986 |
| C | 0.485 | 0.522 | 0.074 | 0.111 | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | 0.271 | 0.000 | 0.014 |
| D | 0.103 | 0.130 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

continued on next page

TABLE 1.

continued

| Locus | Population | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AP-A | AP-B | PD-A | PD-B | PD-C | QP-D | QP-E | QP-F | QQ-D | QQ-E | QQ-F | QQ-C |
| ME-2 | | | | | | | | | | | | |
| (N) | 35 | 24 | 31 | 18 | 36 | 12 | 20 | 15 | 32 | 24 | 36 | 36 |
| A | 0.886 | 0.875 | 0.032 | 0.000 | 0.000 | 0.500 | 0.100 | 0.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| B | 0.114 | 0.125 | 0.903 | 0.833 | 1.000 | 0.500 | 0.875 | 0.933 | 0.000 | 0.000 | 0.000 | 0.000 |
| C | 0.000 | 0.000 | 0.065 | 0.167 | 0.000 | 0.000 | 0.025 | 0.067 | 0.000 | 0.000 | 0.000 | 0.000 |
| MPI-1 | | | | | | | | | | | | |
| (N) | 35 | 24 | 31 | 18 | 36 | 12 | 20 | 15 | 32 | 24 | 36 | 36 |
| A | 0.329 | 0.000 | 0.113 | 0.667 | 1.000 | 1.000 | 1.000 | 0.967 | 0.000 | 0.646 | 0.028 | 0.000 |
| B | 0.671 | 1.000 | 0.758 | 0.333 | 0.000 | 0.000 | 0.000 | 0.033 | 1.000 | 0.354 | 0.917 | 1.000 |
| C | 0.000 | 0.000 | 0.129 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.056 | 0.000 |
| PGI-1 | | | | | | | | | | | | |
| (N) | 35 | 24 | 31 | 18 | 36 | 12 | 20 | 15 | 32 | 24 | 36 | 36 |
| A | 1.000 | 1.000 | 0.935 | 1.000 | 1.000 | 0.167 | 0.425 | 0.000 | 0.750 | 0.792 | 0.806 | 0.375 |
| B | 0.000 | 0.000 | 0.065 | 0.000 | 0.000 | 0.833 | 0.575 | 1.000 | 0.250 | 0.208 | 0.194 | 0.028 |
| C | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.597 |
| PGM-1 | | | | | | | | | | | | |
| (N) | 35 | 22 | 31 | 18 | 36 | 12 | 20 | 15 | 32 | 24 | 36 | 36 |
| A | 0.843 | 0.977 | 0.403 | 0.278 | 0.431 | 0.000 | 0.125 | 0.167 | 0.000 | 0.167 | 0.000 | 0.000 |
| B | 0.157 | 0.023 | 0.468 | 0.722 | 0.569 | 1.000 | 0.875 | 0.833 | 0.328 | 0.417 | 0.194 | 0.542 |
| C | 0.000 | 0.000 | 0.129 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.609 | 0.333 | 0.792 | 0.458 |
| D | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.063 | 0.083 | 0.014 | 0.000 |
| PGM-2 | | | | | | | | | | | | |
| (N) | 35 | 19 | 1 | 1 | 1 | 11 | 18 | 15 | 29 | 21 | 34 | 20 |
| A | 0.629 | 1.000 | 0.000 | 0.000 | 0.000 | 0.500 | 0.444 | 0.300 | 0.293 | 0.571 | 0.574 | 0.575 |
| B | 0.257 | 0.000 | 0.000 | 0.000 | 0.000 | 0.500 | 0.528 | 0.067 | 0.483 | 0.214 | 0.132 | 0.425 |
| C | 0.114 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.028 | 0.433 | 0.224 | 0.214 | 0.250 | 0.000 |
| D | 0.000 | 0.000 | 1.000 | 1.000 | 1.000 | 0.000 | 0.000 | 0.167 | 0.000 | 0.000 | 0.044 | 0.000 |
| E | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.033 | 0.000 | 0.000 | 0.000 | 0.000 |
| H (dc) | 0.167 | 0.064 | 0.042 | 0.056 | 0.049 | 0.188 | 0.128 | 0.117 | 0.062 | 0.069 | 0.060 | 0.041 |
| (SD) | 0.081 | 0.044 | 0.024 | 0.023 | 0.049 | 0.092 | 0.057 | 0.057 | 0.033 | 0.028 | 0.031 | 0.027 |
| P | 0.857 | 0.286 | 0.769 | 0.538 | 0.077 | 0.375 | 0.500 | 0.438 | 0.375 | 0.438 | 0.375 | 0.250 |

[a] *A. p. plicata* (AP), *P. dombeyanus* (PD), *Q. p. pustulosa* (QP), and *Q. quadrula* (QQ) in three collecting sites in the Cache (A, B, C) and White rivers (D, E, F), Arkansas.

## RESULTS

### *Allele Frequencies*

Allele frequencies were determined for each of the four species at each site. Twelve polymorphic loci were identified for *A. p. plicata* out of the 14 loci studied from sites A and B in the Cache River (Table 1). Ten polymorphic loci were identified for *P. dombeyanus* out of 13 loci in sites A, B, and C of the Cache River. *Plectomerus* individuals from site C exhibited a very high degree of monomorphism, with only PGM-1 polymorphic. Six of the 16 loci studied for *Q. p. pustulosa* were monomorphic for each of the three sites (D, E, and F) in the White River. *Quadrula quadrula* had fewer polymorphic loci (7) than did *Q. p. pustulosa* (10). Three populations (sites D, E, and F) of *Q. quadrula* were studied within the White River and a single population within the Cache River (site C). The Cache River population of *Q. quadrula* possessed 11 monomorphic loci, greater than all of the White River populations combined (10).

### *Genetic Distance and Identity*

Genetic distance (Nei's unbiased) and identity were determined between populations for the four species studied. A genetic distance of 0.040 (I = 0.961) was determined for the two populations of *A. p. plicata* (Fig. 1). Genetic distance for *P. dombeyanus* ranged from 0.040 to 0.113 (I = 0.893–0.961). Genetic distance for each of the three populations of *Q. p. pustulosa* ranged from 0.027 to 0.050 (I = 0.951–0.973). Populations of *Q. quadrula* ranged from 0.012 to 0.084 (I = 0.919–0.988).

There was a mean genetic distance of 0.333 ± 0.066 (I = 0.717) between the two species of *Quadrula* (Table 2). *Amblema p. plicata* and *Q. p. pustulosa* had the greatest mean genetic distance of 0.814 ± 0.058, and *P. dombeyanus* and *Q. p. pustulosa* were the most closely related genera with a genetic distance of 0.396 ± 0.081. Figure 1 demonstrates the intraspecific and interspecific genetic distances of these species. The cophenetic correlation for the UPGMA phenogram was 0.916, indicating a good fit of the phenogram to the original data matrix.

## DISCUSSION

### *Conspecific Genetic Distance*

On the basis of analyzing electrophoretic data from a diversity of taxa, Nei (1978) identified several benchmark criteria for establishing levels of taxonomic differentiation. Populations of vari-

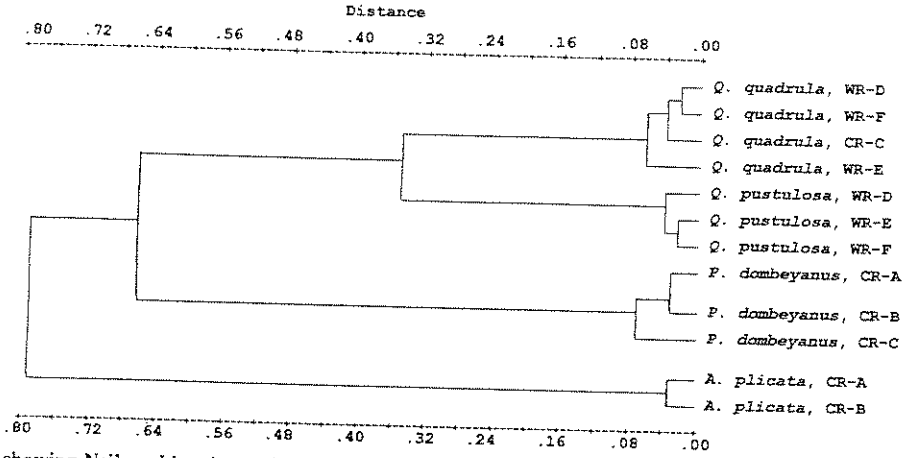


**Figure 1. Phenogram showing Nei's unbiased genetic distance (1978) between populations species and genera of *A. plicata*, *P. dombeyanus*, *Q. pustulosa*, and *Q. quadrula* in the White (WR) and Cache River (CR), Arkansas. Cophenetic correlation = 0.916.**

ous taxa sharing a genetic identity of 0.90 were typical of conspecifics, somewhat greater than that determined by Davis (1984) for freshwater mussel populations (I = 0.87). Conspecific genetic distances of the present study fell within those as determined by Davis (1984) and Nei (1978), with the least genetic similarity for a single population of *P. dombeyanus* (Site C), which exhibited high monomorphism (I = 0.893 to Site A). Intraspecific genetic identities for other Unionid mussel species studied are generally high, although values as low as 0.822 (D = 0.196) were reported for *Elliptio complanata* (Lightfoot) conspecifics (Kat and Davis 1984) (Table 3). Berg et al. (1998) identified low genetic divergence between populations of *Q. quadrula* ($\bar{x}$ = 0.009; range 0.0005–0.037), even populations separated by 1,550 river miles. These authors concluded that these seven populations studied could be reduced to two interactive metapopulations, and attributed the low distances to high gene flow. Our *Q. quadrula* mussel populations were much closer in geographic distance yet possessed greater genetic distances and significant differences between loci (Johnson et al. 1998).

Gene flow between populations serves to minimize genetic distances between populations (Slatkin 1987), particularly populations of close proximity such as in the present study. Dispersal can occur at the gamete, juvenile, and adult stage of the life cycle to facilitate gene flow. Mussels are sessile animals for which glochidia and the downstream flow of gametes are the possible stages whereby significant migration can occur.

Fish hosts play an important role in the genetic structure of mussel populations by facilitating gene flow. Generalism for host selection may improve opportunities for transformed juvenile to migrate from one site to another, whereas the tendency for migration of the individual host species during the glochidial attachment phase will alter gene flow. Kat (1983b) found that *Lampsilis* species utilizing anadromous hosts shared greater genetic identities than did species using territorial or strictly freshwater hosts. The flathead catfish (*Pylodictis olivaris* (Rafinesque)) is the sole host identified for the glochidia of *Q. quadrula*, whereas *A. p. plicata* and *Q. p. pustulosa* use a wide range of fish hosts, including the flathead catfish (Howard and Anson 1923, Wilson 1916, Howard 1914, Coker et al. 1921, Stein 1968). No host has been identified for glochidia of *P. dombeyanus* (Oesch 1984). Host diversity may provide the glochidia of *A. p. plicata* and *Q. p. pustulosa* a greater capacity for migrating from one site to another when compared with glochidia of *Q. quadrula* and possibly *P. dombeyanus*. These latter two species had the greatest genetic distances between populations. The flathead catfish has large diurnal migrations (Coon and Dames 1989), which would serve to enhance gene flow for *A. p. plicata*, *Q. p. pustulosa*, and *Q. quadrula*.

Several researchers have correlated migration distance and the capacity for gene flow (Murray and Clarke 1984, Slatkin 1987, Johnson et al. 1988). It was expected for the present study that conspecific populations more distantly separated would exhibit greater genetic distance. This was indeed the case for *P. dombeyanus* and *Q. p. pustulosa*, as upstream and downstream sites exhibited a greater genetic distance than did central sites. However, for *Q. quadrula*, site C of the Cache River shared a greater genetic identity with sites D and F of the White River than did site E, positioned between sites D and F (Fig. 1). Site C of the Cache River is located 71.5 river miles from the nearest White River site. There is no obvious explanation for this anomaly, although similar phenomena were observed by Hornbach et al. (1980) for *Sphaerium*.

TABLE 2.

**Values of Nei's (1978) genetic identity (above the diagonal) and genetic distance (below the diagonal) for Amblemini in the Cache and White rivers, Arkansas.**

| Population | *A. p.* | *P. d.* | *Q. p.* | *Q. q.* |
|---|---|---|---|---|
| *A. p. plicata* | *** | 0.507 | 0.443 | 0.455 |
| *P. dombeyanus* | 0.680 | *** | 0.673 | 0.469 |
| *Q. p. pustulosa* | 0.814 | 0.396 | *** | 0.717 |
| *Q. quadrula* | 0.787 | 0.758 | 0.333 | *** |

*Genetic Distance between Species*

A congeneric genetic distance for *Quadrula* was determined to be 0.333. This distance is greater than that expected considering that possible hybrids may have been observed for these species (Christian 1995, Posey 1997). It is possible that a third species exists intermediate to both *Quadrula* species studied; this has yet to be investigated. This is a greater genetic distance than congenerics of various taxa considered by Nei (1978) (mean D =

TABLE 3.

Mean values of D and standard deviations for pairwise comparisons of conspecific populations within the family Unionidae.

| Conspecific Population Comparisons | No. Populations | $\bar{x}$ ± S.D. | Range | Source |
|---|---|---|---|---|
| Tribe Anodontini | | | | |
| *Anodonta anatina* (Linnaeus) | 18 | 0.064 ± N/A | 0.000–0.252 | Nagel et al. 1996 |
| *A. cataracta* (Say) | 5 | 0.034 ± 0.037 | 0.001–0.081 | Davis et al. 1981 |
| *A. cygnea* (Linnaeus) | 3 | 0.008 ± N/A | 0.000–0.012 | Nagel et al. 1996 |
| *Pseudanodonta complanata* (Rossmassler) | 2 | 0.000 ± N/A | N/A | Nagel et al. 1996 |
| Tribe Lampsilini | | | | |
| *Lampsilis cariosa* (Say) | 3 | 0.071 ± 0.021 | 0.041–0.091 | Stiven and Alderman (1992) |
| *L. radiata* (Gmelin) | 5 | 0.018 ± 0.010 | 0.001–0.033 | Kat and Davis (1984) |
| *L. radiata* (Gmelin) | 3 | 0.015 ± 0.005 | 0.009–0.021 | Kat (1983b) |
| *Leptodea ochracea* (Say) | 2 | 0.018 | N/A | Stiven and Alderman (1992) |
| Tribe Pleurobemini | | | | |
| *Elliptio complanata* (Lightfoot) | 15 | 0.043 ± 0.025 | 0.014–0.196 | Davis et al. (1981); Kat and Davis (1984) |
| *E. crassidens* (Lamarck) | 3 | 0.010 ± 0.006 | 0.005–0.018 | Davis (1984) |
| *E. icterina* (Conrad) | 9 | 0.097 ± 0.050 | 0.025–0.184 | Davis et al. (1981) |
| *E. mcmichaeli* (Clench & Turner) | 2 | 0.014 | N/A | Davis (1984) |
| Tribe Amblemini | | | | |
| *A. p. plicata* (Say) | 2 | 0.035 | N/A | Present study |
| *P. dombeyanus* (Valenciennes) | 2 | 0.074 ± 0.030 | 0.040–0.113 | Present study |
| *Q. p. pustulosa* (I. Lea) | 3 | 0.034 ± 0.013 | 0.027–0.050 | Present study |
| *Q. quadrula* (Rafinesque) | 7 | 0.009 ± 0.011 | 0.0005–0.037 | Berg et al. (1997) |
| *Q. quadrula* (Rafinesque) | 4 | 0.051 ± 0.030 | 0.012–0.084 | Present study |

0.222}, whereas congeneric genetic distances for Unionids range from 0.010 to 1.323 for differing clades (Table 4). Genetic distances should be correlative with time of divergence for genera, as is demonstrated by greater genetic distances for older lineages such as Anodonini and Lampsilini, as compared with lower genetic distances for the more recent Pleurobemini (Davis 1984). These intermediate values obtained for Amblemini are consistent with the geological record.

Tribal genetic distances for the present study ranged from 0.396 for *Quadrula* versus *Plectomerus* to 0.787 for *Amblema* versus *Quadrula*, with a mean genetic distance of 0.687 ± 0.152. These intergeneric values are greater than that identified by Mulvey et al. (1997) comparing *Amblema* and *Megalonaias* (0.516). In comparing studies of other tribes these values are similar to those obtained by Davis (1984) ($\bar{x}$ = 0.651; range 0.358–0.935), yet lower than those of Stivens and Alderman (1992) {range 0.825–1.146}.

Of particular interest was the genetic relationship of *P. dombeyanus* to *A. p. plicata*. On the basis of immunologic and

TABLE 4.

Mean values of D and standard deviations for pairwise comparisons of congeneric and tribal species within the family Unionidae.

| Congeneric Species Comparisons | No. Species | $\bar{x}$ ± S.D. | Range | Source |
|---|---|---|---|---|
| Tribe Anodontini | | | | |
| *Anodonta* | 3 | 0.840 ± 0.353 | 0.373–1.323 | Kat (1983a) |
| *Anodonta* | 3 | 0.501 ± 0.094 | 0.417–0.632 | Nagel et al. (1996) |
| Tribe Lampsilini | | | | |
| *Lampsilis* | 3 | 0.342 ± 0.141 | 0.350–0.420 | Stiven and Alderman (1992) |
| *Lampsilis* | 3 | 0.958 ± 0.358 | 0.213–1.224 | Kat (1983b) |
| Tribe Pleurobemini | | | | |
| *Elliptio* | 7 | 0.210 ± 0.117 | 0.010–0.446 | Davis et al. (1981) |
| *Uniomerus* | 3 | 0.308 ± 0.165 | 0.216–0.498 | Davis (1984) |
| Tribe Amblemini | | | | |
| *Amblema* | 3 | 0.237 ± N/A | 0.012–0.276 | Mulvey et al. (1997) |
| *Quadrula* | 2 | 0.360 | N/A | Present study |
| Tribal Comparisons | No. Genera/Species | | | |
| Tribe Anodontini | 2/4 | 0.610 ± 0.052 | 0.565–0.682 | Nagel et al. (1996) |
| Tribe Lampsilini | 2/4 | 0.962 ± 0.148 | 0.726–1.146 | Stiven and Alderman (1992) |
| Tribe Pleurobemini | 3/5 | 0.431 ± 0.161 | 0.208–0.725 | Davis (1984) |
| Tribe Amblemini | 1/2 | 0.321 | N/A | Davis (1984) |
| Tribe Amblemini | 2/5 | 0.516 ± N/A | N/A | Mulvey et al. (1997) |
| Tribe Amblemini | 3/4 | 0.687 ± 0.152 | 0.396–0.814 | Present study |

morphologic data, Davis and Fuller (1981) suggested that the genera *Amblema, Plectomerus,* and *Megalonaias* were congeneric. This close genetic relationship was not identified by Lydeard et al. (1996) nor in the present study; *P. dombeyanus* was most closely related to *Q. p. pustulosa.* Lydeard et al. (1996) studied the phyletic relationships among Unionidae combining sequence data from the 16S rRNA gene and morphologic/reproductive data. They identified a closer relationship of *A. plicata* and *P. dombeyanus* than to *Quadrula,* and questioned the taxonomic utility of the Tribe Amblemini. Other researchers have identified incongruence between anatomical and genetic relationships among freshwater bivalves (e.g., Hornbach et al. 1980, Davis 1981; Hoeh 1990), yet good congruence in immunologic and allozyme data (Davis and Fuller 1981). Further evidence of the uncertain taxonomic status of the Amblemini is that Davis and Fuller (1981) found a closer immunologic relationship between *Elliptio* (Tribe Pleurobemini) and *Amblema* than between *Amblema* and *Plectomerus.*

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