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GENETIC DIVERSITY AMONG FOUR AMBLEMINI SPECIES (BIVALVIA: UNIONIDAE) IN THE CACHE AND WHITE RIVERS, ARKANSAS

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ABSTRACT—Allozymic analysis of 16 loci was utilized to determine the genetic diversity of four species of mussels in the Tribe Amblemini (*Amblema plicata plicata* (Say), *Plectomerus dombeyanus* (Valenciennes), *Quadrula pustulosa* (I. Lea), and *Q. quadrula* (Rafinesque)) in the Cache and White rivers of Arkansas. Mussel populations of both rivers have been subjected to frequent harvest, and White River populations have been exposed to periodic habitat destruction due to dredging. Ranges of polymorphism were from 0.572 for *A. plicata* to 0.360 for *Q. quadrula*; heterozygosity values ranged from 0.049 for *P. dombeyanus* to 0.144 for *Q. pustulosa*. With the exception of low heterozygosity for *Q. quadrula*, heterozygosity and polymorphism values were similar to previous studies involving Amblemini of other river drainages. Populations were characterized by heterozygote deficiencies at all loci. Several determinants of heterozygote deficiency were investigated, with selection and inbreeding posed as viable hypotheses. Bottlenecking may be occurring in *Q. quadrula*. Although no evidence of genetic decline associated with bottlenecking was identified for the other three Amblemini, mussel beds are on the decline in Arkansas, and loss of genetic diversity is detrimental to the temporal stability of populations.

RESUMEN—Un análisis alozimático de 16 loci se usó para determinar la diversidad genética de cuatro especies de mejillones de la Tribu Amblemini (*Amblema plicata plicata* (Say), *Plectomerus dombeyanus* (Valenciennes), *Quadrula pustulosa* (I. Lea), y *Q. quadrula* (Rafinesque)) en los ríos Cache y White de Arkansas. Las poblaciones de mejillones de los dos ríos se han cosechado con determinada frecuencia, y las poblaciones del White River han sido expuestas a una periódica destrucción del hábitat a causa del dragar del río. Los límites del polimorfismo fueron de 0.572 para *A. plicata* a 0.360 para *Q. quadrula*; los valores de la heterozigosidad se registraron de 0.049 para *P. dombeyanus* hasta 0.144 para *Q. pustulosa*. Con la excepción de la baja heterozigosidad de *Q. quadrula*, los valores de heterozigosidad y polimorfismo fueron similares a los estudios anteriores sobre Amblemini de cuencas fluviales de otros ríos. Las poblacionesse caracterizaron por una deficiencia de los heterozigotos en todos los loci. Se investigaron varios determinantes de la deficiencia de los heterozigotos, con la seleccion y cruzamiente consanguíneo señalados como hipótesis viables. Es posible que el embotellamiento sea el problema con *Q. quadrula*. Aunque no se identificó ninguna evidencia de una disminución genética asociada con dicho embotellamiento en las otras tres Amblemini, en Arkansas se está disminuyendo la cantidad de lechos de los mejillones, y la pérdida de diversidad genética es perjudicial para la estabilidad temporal de las poblaciones.

Arkansas is one of five major harvesting states for mussel shells in the United States. Mussel shells are exported and processed to produce seed nuclei for the pearl industry in Japan. As a result of the combined anthropogenic effects of over-harvesting, pollution, and destruction of habitat by impoundments, dredging, and bridge construction, the size and number of mussel beds have steadily decreased nationwide (Williams et al., 1993). Approximately one-third of the 300 North American mussel species are classified as endangered or possibly extinct (Williams et al., 1993). Researchers have reported dramatic, long-term decreases in mussel species diversity in various river systems for several states (Matteson and Dexter, 1966; Isom and Yokley, 1968; Starrett, 1971; Suloway, 1981; Vaughn, 1997). Commercially harvested mussel beds associated with Arkansas rivers recently have been described (Rust, 1993; Christian, 1995; Davidson, 1997; Posey, 1997). Christian, in a comprehensive survey during 1995, identified a 54% loss of historically documented mussel

beds in the White River of Arkansas. Catch per unit effort of shellers showed similar declines (Todd, 1994). Shellers have also indicated dramatic declines in sizes of mussel beds (K. C. Ward, E. Kohal, J. T. Easter, pers. comm.). Reduction in numbers of mussels in the White River also has been attributed partially to dredging, and the indirect effects of water regulation (Todd, 1993).

Reductions in genetic diversity of populations have been associated with population bottlenecks of species (Nei et al., 1975; Avise et al., 1988; Bernatchez et al., 1989). High genetic diversity provides species the ability to survive and adapt to changing environmental conditions (e.g., hypolimnetic release of impoundment waters in the White River), and may also provide a population the evolutionary flexibility to respond to temporally variable selection pressures (Hedrick et al., 1976; Gillespie and Guttman, 1989). Loss of genetic diversity may weaken the evolutionary stability of a species (Allendorf and Leary, 1986; Ehrlich, 1988). Management of freshwater mussels is a unique situation because it relies not only on an understanding of the genetic structure of these species but also the host-parasitic interactions of the glochidial stage. The effects of host fish migration during the glochidial stages greatly impacts gene flow. The additional consideration of facultative hermaphrodism in some mussel species provides another significant life history characteristic that complicates understanding of genetic diversity.

This study utilized cellulose acetate electrophoresis and histochemical staining of allozymes to determine the genetic diversity of four mussel species within the tribe Amblemini common to Arkansas waters. *Amblema plicata plicata* (Say), *Plectomerus dombeyanus* (Valenciennes), *Quadrula pustulosa* (I. Lea), and *Q. quadrula* (Rafinesque) are dominant community members within the Cache and White rivers, which belong to the Mississippi River drainage (Christian, 1995). Each is classified currently as a stable species (Williams et al., 1993). Three of the four species range throughout the eastern United States reaching into Canada, although *Plectomerus* is restricted to the southeastern United States. Each is commercially important with the exception of *P. dombeyanus*, which possesses a commercially undesirable, heavily pigmented shell.

Both the Cache and White rivers represent significant sources of mussels to Arkansas shell takers. The White River has been the primary source of Arkansas mussels for the years 1991–1994, and the Cache River has progressively declined in importance from a second ranking in 1991 (Farwick, 1992; Todd, 1993, 1994, 1995). The rivers chosen for study were also coincidently being characterized for mussel density and diversity as part of a long term study funded by the Arkansas Game and Fish Commission, the United States Fish and Wildlife Service, and the Little Rock District of the Corps of Engineers. Maintenance dredging is performed annually in the White River to accommodate barge traffic, but only the upper reaches of the Cache River are channelized for flood control purposes (Tillman, United States Army Corps of Engineers, pers. comm.). Commercial gravel mining occurs in the White River.

There is an added urgency to gather information regarding population structure and systematics for unionid species because the decline in numbers of Arkansas mussels also possibly has reduced genetic diversity of existing populations. Few genetic studies have been performed on freshwater mussels, and none have been performed in Arkansas river drainages. The total absence of designated minimum viable population sizes for mussels suggests that conservation geneticists can only extrapolate such information from species possessing more simplistic life histories. A minimal threshold population of individuals is not utilized presently by fisheries managers and the application of minimum shell size to commercial shellers is the only means for maintaining current populations.

In addition to characterizing the genetic diversity of several mussel species in Arkansas, our primary question was whether mussel populations subjected to frequent harvest and habitat destruction have low genetic diversity relative to previously studied mussel species. Our second goal was to investigate whether differential heterozygosities for intraspecific populations were related to environmental variables.

METHODS—Genetic diversity was determined within populations for the lower regions of the Cache and White rivers. Twelve to 36 individuals of two species were collected from each of three sites from

both rivers, representing a total of 319 mussels. 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).

The Cache River basin drains southward along the western extent of the Mississippi Embayment and flows into the White River near Clarendon, Arkansas. Although the White River flows through the Ozark Highlands, the study region is best characterized by alluvial soil deposits of the Mississippi Delta. Both streams are characterized within the study region as having low stream gradient and high turbidity and dissolved solids.

Hookah rig diving was used to obtain mussels in the summer 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 (weight/volume) of Tris-HCl buffer (pH 7.0). Electrophoresis was performed at 200 volts for 15 min in TG buffer (0.025M Tris; 0.192M Glycine) at room temperature (2 mA/plate). Nine enzyme systems representing 16 loci were selected for analysis based on their expression in adductor muscle. Enzymatic loci were as follows: fumarase (FUM-1, FUM-2; Enzyme Commission (E.C.) 4.2.1.2); glutamate-oxaloacetate transferase (GOT-1, GOT-2; E.C. 2.6.1.1); isocitrate dehydrogenase (IDH-1, IDH-2; E.C. 1.1.1.42); lactate dehydrogenase (LDH-1, LDH-2; E.C. 1.1.1.27); malate dehydrogenase (MDH-1, MDH-2; E.C. 1.1.1.37); malic enzyme (ME-1, ME-2; E.C. 1.1.1.40); mannose phosphate isomerase (MPI-1; E.C. 5.3.1.8); phosphoglucose isomerase (PGI-1; E.C. 5.3.1.9); phosphoglucomutase (PGM-1, PGM-2; E.C. 2.7.5.1 IUBNC, 1984). The distance of migration for each specific enzyme was visualized by histochemical staining (Hebert and Beaton, 1989).

Eight of the nine enzyme systems used in the present study are integral within glycolysis or the citric acid cycle. All nine enzymes are categorized as group I enzymes, which process internally generated metabolites. The enzymes have high substrate specificity, reducing the potential for heterozygosity; group II enzymes process externally derived substances which are more variable, and thus favor heterozygosity (Gillespie and Langley, 1974). Values for genetic diversity obtained for the present and previous molluscan studies are conservative due to a reliance on group I enzymes (Davis and Fuller, 1981; Davis et al., 1981; Kat 1985*a*, 1985*b*; Davis, 1984; Stiven and Alderman, 1992).

Individual genotypes were used as original data with mean sample size per locus, mean number of alleles per locus, polymorphism, direct-count heterozygosity, Hardy-Weinberg equilibrium (D statistics), contingency chi-square analysis and Wright's statistics (F_{IS}, F_{IT}, F_{ST}) determined using the program BIOSYS-1 (Swofford and Selander, 1989).

RESULTS—Electrophoretic analysis of the nine enzyme systems revealed varying numbers of polymorphic (a dominant allele frequency less than 95 percent) loci for the four species studied (Table 1). *Amblema plicata* had the greatest number of polymorphic loci (12 of 14), followed by *P. dombeyanus* (10 of 13). *Quadrula* exhibited a lower degree of polymorphism than did the other two genera, with 7 and 10 of 16 loci polymorphic for *Q. quadrula* and *Q. pustulosa*, respectively. Sixty-six percent of all loci analyzed were polymorphic for the 12 populations studied.

The greatest polymorphism (P) for individual populations occurred in *A. plicata* (0.572) and *P. dombeyanus* (0.461) of the Cache River (Table 1). Cache River populations of *A. plicata* and *P. dombeyanus* exhibited downstream decreases in P, although P values in *Q. pustulosa* and *Q. quadrula* of the White River did not change (Fig. 1).

Average number of alleles per locus in the four species studied varied from 1.7 for both *Quadrula* species to 2.2 for *A. plicata* (Table 1). The GOT-2 and PGM-2 loci exhibited the greatest number of alleles, with five for *A. plicata* and *Q. pustulosa*, respectively. Downstream reductions in number of alleles per locus were identified for populations of *P. dombeyanus* and *A. plicata* from the Cache River, with no trends identified for *Quadrula* populations for either species.

Genotype frequencies of the polymorphic loci were analyzed to determine which loci were in Hardy-Weinberg equilibrium (Table 1). Most loci (0.74) were not in equilibrium with most deviations (0.94) from expected due to heterozygote deficiencies. Heterozygote deficiencies were found in all populations studied with the exception of a single *P. dombeyanus* population which possessed one polymorphic locus out of 13 loci analyzed. All polymorphic loci possessed heterozygote deficiencies in at least one population with LDH-1 exhibiting the least deviance from that expected.

Levels of direct-count heterozygosity (H) for the various populations were determined (Table 1). *Quadrula pustulosa* exhibited the highest

TABLE 1—Allele frequencies and heterozygote deficiency (−) or excess (HW) of *A. plicata* (AP), *P. dombeyanus* (PD), *Q. pustulosa* (QP), and *Q. quadrula* (QQ) from three sites each in the Cache (A, B, C) and White rivers (D, E, F), Arkansas. Mean direct-count heterozygosity (*H*), polymorphism (*P*), and alleles per locus are summarized for each population.

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-G
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
HW	0.971*	−0.112	−0.481*	—	—	—	—	—	—	—	—	—
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
HW	−1.000**	—	−1.000**	—	—	0.917**	0.000	−1.000**	—	−0.656**	—	—
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
HW	−0.829**	—	−0.551**	−0.885**	—	—	0.026	−1.000**	—	−1.000	−0.861	—
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
HW	−0.022	—	−1.000**	0.000	—	—	—	—	—	—	—	—

TABLE 1—Continued.

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
HDH-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
HW	—	—	—	—	—	−1.000**	0.000	—	—	—	—	—
HDH-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
HW	−1.000**	—	−1.000	—	—	—	—	—	—	—	0.000	—
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
HW	—	—	—	—	—	0.500	0.662	0.611*	0.246	0.062	−0.638*	0.213
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
HW	—	—	—	—	—	—	—	—	—	—	—	—

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-G
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
HW	0.087*	−0.101*	−1.000*	−0.319*	—	—	−1.000*	—	—	—	—	—
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
HW	−1.000*	−1.000*	−1.000*	−1.000*	—	—	—	0.036	—	—	—	—
ME-1												
n	34	23	27	18	36	12	20	15	23	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
HW	−0.957*	−1.000*	−1.000*	−1.000*	—	—	−0.655*	0.000	0.000	−1.000*	−0.223	−0.771*
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
HW	−1.000*	−1.000*	−1.000*	−1.000*	—	0.917*	−0.128*	−1.000*	—	—	—	—
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
HW	−0.936*	—	−0.920*	−0.514*	—	—	—	0.000	—	—	−0.732*	−0.649*

TABLE 1—Continued.

Population

C	0.000	0.000	0.429	0.000	0.000	0.000	0.000	0.033	1.000	0.354	0.917	1.000
HW	−0.936*	—	−0.920*	−0.514*	—	—	—	0.000	0.000	0.000	0.056	0.000
								0.000	—	—	−0.732*	−0.649*

TABLE 1—Continued.

	Population											
Locus	AP-A	AP-B	PD-A	PD-B	PD-C	QP-D	QP-E	QP-F	QQ-D	QQ-E	QQ-F	QQ-C
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
HW	—	—	−1.000*	—	—	−1.000*	−0.900*	—	−0.727*	−0.826*	−0.753*	−0.825*
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
HW	−0.256	0.000	−0.525*	−0.462*	−0.274	—	0.114	0.160	−0.945*	−0.941*	−0.281*	−0.918*
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	0.000	0.000	0.000	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
HW	−1.000*	—	—	—	—	−0.479	−0.174	−0.345*	−0.900*	−0.624*	−0.760*	−0.459*
H	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
Alleles/Locus	2.6	1.7	2.5	1.8	1.1	1.4	1.8	1.8	1.6	1.9	1.7	1.4
(*SD*)	(0.3)	(0.3)	(0.2)	(0.2)	(0.1)	(0.1)	(0.2)	(0.3)	(0.2)	(0.3)	(0.3)	(0.2)

* Significant departure from Hardy-Weinberg expectation.

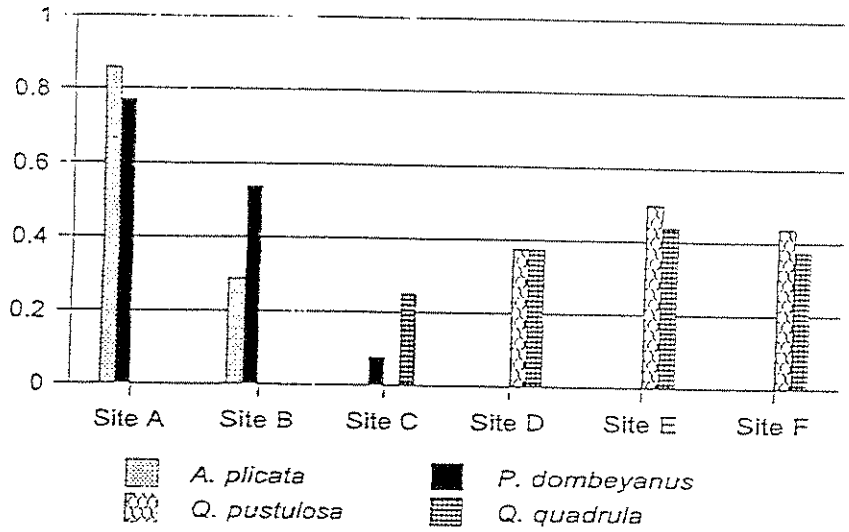

FIG. 1—Polymorphism of unionid mussel populations in the Cache (Sites A, B, and C) and White (Sites D, E, and F) rivers, Arkansas.

mean heterozygosity (H = 0.144), followed successively by *A. plicata* (H = 0.116), *Q. quadrula* (H = 0.058), and *P. dombeyanus* (H = 0.049). *Quadrula pustulosa* had significantly greater heterozygosity than did *Q. quadrula* or *P. dombeyanus* ($P < 0.01$). Heterozygosity for each population of *Q. quadrula* and *P. dombeyanus* was similar within their species, while *A. plicata* and *Q. pustulosa* populations exhibited significant decreases in H for downstream populations (X^2 = 6.752, $P < 0.01$; X^2 = 8.711, $P < 0.01$, respectively). Mean heterozygosity for the Amblemini studied was 0.087.

Wright's weighted F-statistics were utilized to analyze the genetic structure of these populations (Table 2). The mean F_{ST} for pooled loci was significant for each of the four species. F_{ST} values showed moderate to high differentiation as defined by Hartl (1980), who described F_{ST} values of 0.05 to 0.15 as representative of moderately differentiated populations and F_{ST} values of 0.15 to 0.25 for highly differentiated populations. Number of loci demonstrating significant differentiation ranged from five for *Q. pustulosa* to nine for *P. dombeyanus*. The greater number of loci demonstrating significant difference for *P. dombeyanus* is due in part to a single population (Site C) exhibiting a high degree of monomorphism (12 of 13 loci).

DISCUSSION—*Genetic Diversity*—We predicted that H and P values would be particularly low due to the dramatic reduction in numbers associated with shell harvest (and habitat modification for the White River). Genetic diversity of mussels has been studied in other river drainages (Hornbach et al., 1980; Davis and Fuller, 1981; Davis et al., 1981; Kat, 1985*a*, 1985*b*; Davis, 1984; Stiven and Alderman, 1992; Berg et al., pers. comm.), and these were used for comparative purposes. Although shell harvesting has been heavy throughout the Mississippi River drainage, habitat modification and water quality changes have been greatest in the lower region.

Davis (1984) determined polymorphism for one population of *Q. quadrula* in the upper Mississippi River in Wisconsin to be 0.357, almost identical to the present study P value of 0.360. These P values are lower than those identified for seven populations of *Q. quadrula* within the Ohio and lower Mississippi river drainages (P = 0.614). Values obtained by other researchers are largely consistent within taxa, ranging from 0.142 for one species of Margaritiferinae (Davis et al., 1981), 0.298 for five species of Anodontinae (Davis et al., 1981; Kat, 1985*a*), 0.427 for seven species of Pleurobemini, to 0.460 for nine species of Lampsilini (Davis, 1984; Kat, 1985*b*; Stiven and Alderman, 1992). Mean P values of the Amblemini of past studies is 0.32 ± 0.08 (Davis, 1984; Berg et al.,

TABLE 2—Summary of Wright's F_{ST} for each polymorphic locus for populations of *A. p. plicata*, *P. dombeyanus*, *Q. pustulosa* and *Q. quadrula* in the Cache and White rivers, Arkansas. Significance of F_{ST} given by the Chi-Square value.

	A. p. plicata		*P. dombeyanus*		*Q. pustulosa*		*Q. quadrula*	
Locus	F_{ST}	Chi-Square (*df*)	F_{ST}	Chi-Square (*df*)	F_{ST}	Chi-Square (*df*)	F_{ST}	Chi-Square (*df*)
FUM-1	0.119	12.653 (1)*	0.036	6.354 (4)	—	—	—	—
FUM-2	0.096	0.588 (2)	0.022	3.525 (3)	0.241	23.517 (2)*	0.036	9.107 (3)*
GOT-1	0.219	24.008 (2)*	0.082	22.360 (3)*	0.031	7.033 (4)	0.336	116.180 (6)*
GOT-2	0.073	10.892 (4)*	0.084	15.349 (2)*	—	—	—	—
IDH-1	—	—	—	—	0.218	20.522 (2)*	—	—
IDH-2	0.029	2.722 (1)	0.022	3.525 (2)	—	—	0.016	4.350 (3)
LDH-1	—	—	—	—	0.009	1.549 (2)	0.036	18.925 (6)*
MDH-1	0.002	1.295 (3)	0.280	84.818 (4)*	—	—	—	—
MDH-2	0.023	7.090 (2)*	0.238	41.482 (2)*	0.021	0.825 (2)	—	—
ME-1	0.003	1.162 (3)	0.090	25.985 (3)*	0.022	2.620 (4)	0.141	85.014 (6)*
ME-2	0.000	0.031 (1)	0.059	15.660 (4)*	0.230	27.218 (4)*	—	—
MPI-1	0.197	19.590 (1)*	0.455	112.668 (3)*	0.022	2.156 (2)	0.494	150.703 (6)*
PGI-1	—	—	0.044	7.136 (2)*	0.193	18.445 (2)*	0.217	134.936 (6)*
PGM-1	0.055	5.183 (1)	0.057	18.052 (3)*	0.057	4.150 (2)	0.093	67.808 (9)*
PGM-2	0.171	18.590 (2)*	—	—	0.146	45.679 (8)*	0.074	38.059 (9)*
MEAN	0.082	8.639 (2)*	0.121	29.723 (3)*	0.108	13.974 (3)*	0.160	69.453 (5)*

* $P < 0.05$.

pers. comm.), which is insignificantly lower than the combined P value of 0.46 ± 0.20 for the present study. Our P values were at the upper range of tribal and familial means.

Direct-count heterozygosity values were largely consistent with those for polymorphism. *H* values of previous studies were greatest for species of the tribe Pleurobemini (mean = 0.113; range = 0.081–0.146—Kat, 1983*b*), intermediate for Amblemini and Anodontinae (mean = 0.108, range = 0.022–0.240—Davis, 1984; Berg et al., pers. comm.; mean = 0.094, range = 0.028–0.192—Kat, 1983*a*; Davis et al., 1981, respectively), and least for one species of Margaritiferinae (0.030—Davis et al., 1981). Similar to those studies cited above, *H* values for the present study were quite variable between species, ranging from 0.049 for *P. dombeyanus* to 0.144 for *Quadrula pustulosa*. Heterozygosity for *Q. quadrula* in the present study was 0.058, half (0.112) that obtained by Davis (1984) and one-fourth that found by Berg et al. (pers. comm.). In a comparison of Amblemini, the four species of the present study had a mean *H* value of 0.087 ± 0.048, slightly lower, but not statistically different from results of previous investigations (mean = 0.108 ± 0.033—Davis et al., 1981; Davis, 1984; Berg et al., pers. comm.). Mean *H* was intermediate for the tribes previously studied.

There was a low mean number of alleles (mean = 1.7; range = 1.1–2.6) observed per locus in the present study (Table 2). Means were consistently higher than those found for *Anodonta* (range = 1.1–1.4—Kat, 1983*a*), comparable to those of *Lampsilis* sp. (mean = 2.1) and *Leptodea* (mean = 1.8), yet lower than those obtained for *Elliptio* (mean = 3.5—Kat, 1983*b*; Stiven and Alderman, 1992).

Hardy-Weinberg Equilibrium—Hardy-Weinberg disequilibrium has been identified for loci in many bivalve populations (Milkman and Beaty, 1970; Hornbach et al., 1980; Adamkewicz et al., 1984; Stiven and Alderman, 1992), but not to the degree found in the present study. Conversely, no Hardy-Weinberg disequilibrium was identified for seven populations of *Q. quadrula* studied by Berg et al. (pers. comm.). The relatively high and mostly positive values of F_{IS} and F_{IT} for each species support high levels of homozygosity (F_{IS} and F_{IT} data available upon request).

Many explanations have been offered for heterozygote deficiencies, including bottleneck effects, Wahlund effect, presence of null alleles, linkage disequilibrium, genetic imprint-

ing, inbreeding, and selection (Singh and Green, 1984). The combined effects of harvesting and habitat modification could be producing a bottleneck effect, where genetic drift is serving as a dominant force in gene frequency changes of these populations (Nei et al., 1975). Bottleneck effects have been demonstrated to reduce heterozygosity for hundreds of thousands of years following population recovery (Nei et al., 1975). Historic quantitative data are not available, but Christian (1995) reported a dramatic reduction in numbers of mussel beds and anecdotal reports by shellers have described a precipitous decrease in numbers of mussels available for harvest (K.C. Ward, E. Kohal, J.T. Easter, pers. comm.). If bottlenecking were indeed occurring, H values should be reduced relative to other studies. Mean H for *Q. quadrula* was low compared to that obtained by Davis (1984), which lends support for bottlenecking. This does not appear to be the case for the other three species in comparisons within the family Amblemini.

Null alleles represent the loss of enzyme activity for multimeric enzymes (e.g., dimers, trimers, tetramers) in heterozygotes due to structural instability (Milkman and Beaty, 1970; Koehn and Eanes, 1978). Zouros and Foltz (1984) successfully argued against the presence of null alleles as determinants of heterozygote deficiency in bivalves. No relationship was found in the present study between the number of subunits in the functional enzyme and the degree of Hardy-Weinberg disequilibrium ($P < 0.75$). Zouros and Foltz (1984) considered the existence of independently reproducing subgroups (Wahlund effect) as a viable explanation for highly variable bivalve populations. Mussel bed sizes for the present study ranged from 200 to 780 m^2 (Christian, 1995); dispersal mechanisms should adequately prevent the formation of subpopulations within these moderately sized mussel beds. Inbreeding also has been proposed as an explanation for heterozygote deficiency (Hornbach et al., 1980). Koehn et al. (1971) stated that if inbreeding is indeed occurring, heterozygote deficiency should be consistent for all polymorphic loci. Inbreeding may be occurring, particularly for *P. dombeyanus*, as each locus exhibited heterozygote deficiency. Very few loci were not subject to heterozygote deficiency (e.g., GOT-2 and PGM-1 for *A. plicata*; MDH-2, MPI-1, PGM-2 in *Q. pustulosa*; IDH-2 for *Q. quadrula*). Nei et al. (1975) have previously identified significant positive correlations of population size to heterozygosity values, with inbreeding enhanced in smaller populations. No significant correlation was observed between effective population size as determined by Christian (1995) and heterozygosity ($r_s = 0.14$; $P < 0.62$).

Genetic Diversity and Selection—Low heterozygosity has been associated with a reduction in resistance to pollutants (Nevo et al., 1986), although contradictory data exist as to the presence of a selective effect of toxicants on heterozygotes (Nevo et al., 1981; Lavie and Nevo, 1986). Toxicants appear to exert a greater selective effect on specific loci as compared to overall heterozygosity (Diamond et al., 1991; Schleuter et al., 1995). Downstream populations of *A. plicata* and *P. dombeyanus* in the Cache River exhibited a consistent decrease in polymorphism and mean number of alleles per locus. This was particularly notable for *P. dombeyanus*, as mean number of alleles per locus declined from 2.5 at Site A to 1.1 at Site C. There was a downstream reduction in heterozygosity for site B for *A. plicata*, yet not for *P. dombeyanus*, while H, P and the number of alleles per locus were lower for *Q. quadrula* at Site C in the Cache River than for White River sites. Site B on the Cache River represents a low-lying catch basin for non-point agricultural runoff, which includes fertilizers, herbicides, pesticides, and defoliants. If a wide range of toxicants introduced into the Cache River were exerting a selective influence on specific genotypes, then a lower variability of genotypes would be predicted. Several alleles of mussel species at site B of the Cache River exhibited significant differences from those of other sites (e.g., FUM-1 B allele—$\chi^2 = 4.85$, $P < 0.05$ and PGM-2 B allele—$\chi^2 = 6.333$, $P < 0.05$ in *A. plicata* and the MDH-2 B allele—$\chi^2 = 21.353$, $P < 0.05$ and PGM-1 C allele—$\chi^2 = 13.937$, $P < 0.05$ in *P. dombeyanus*). Other researchers have documented that some alleles are differentially sensitive to specific toxicants in a laboratory setting (Lavie and Nevo, 1986; Lavie and Nevo, 1988; Gillespie and Guttman 1989; Diamond et al., 1991; Schleuter et al., 1995). However, this differential sensitivity is not consistent for all enzyme systems (Diamond et al., 1991). Schleuter et al. (1995) proposed that different environmental stressors have differ-

ent effects on specific loci. Further study is needed to determine the effects of introduced toxicants on heterozygosity and allele frequency in downstream populations of mussels in the Cache River.

Davis (1984) and Nei (1987) previously identified significant correlations of sample size to heterozygosity values. We observed no significant correlation between sample size and heterozygosity ($r_s = -0.57$; $P < 0.20$).

Summary—Mean heterozygosity and polymorphism values of the Amblemini of the Cache and White Rivers were similar to those of previous studies. Populations were characterized by heterozygote deficiencies at all loci. Several determinants of heterozygote deficiency were investigated, with selection and inbreeding posed as viable hypotheses. Bottlenecking may be occurring in *Q. quadrula*. Although no evidence of genetic decline associated with bottlenecking was identified for the other three Amblemini, mussel beds are on the decline in Arkansas, and low genetic diversity is detrimental to the temporal stability of populations.

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Literature Cited

Adamkewicz, L., S. R. Taub, and J. R. Wall. 1984. Genetics of the clam *Mercenaria mercenaria*. II. size and genotype. Malacologia 25:525–533.

Allendorf, F. W., and R. F. Leary. 1986. Heterozygosity and fitness in natural populations of animals. In: Soule, M., editor. Conservation biology. Sinauer, Sunderland, Massachusetts. Pp. 57–76.

Avise, J. C., R. M. Ball, and J. Arnold. 1988. Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. Molecular Biology and Evolution 5:331–344.

Bernatchez, L., J. J. Dodson, and S. Boivin. 1989. Population bottlenecks: influence on mitochondrial DNA diversity and its effect on corregonine stock discrimination. Journal of Fisheries Biology (Supplement A) 34:239–244.

Christian, A. D. 1995. Major freshwater mussel beds in Arkansas: location, species composition and community estimates for the Cache and White Rivers. M.S. thesis, Arkansas State University, Jonesboro.

Davidson, C. L. 1997. Analysis of mussel beds in the Little Missouri and Saline rivers, Blue Mountain, Ozark and Dardanelle Lakes, Arkansas. M.S. thesis, Arkansas State University, Jonesboro.

Davis, G. M. 1984. Genetic relationships among some North American Unionidae (Bivalvia): sibling species, convergence, and cladistic relationships. Malacologia 25:629–648.

Davis, G. M., and S. L. Fuller. 1981. Genetic relationships among recent Unionacea (Bivalvia) of North America. Malacologia 20:217–253.

Davis, G. M., W. H. Heard, S. L. Fuller, and C. Hesterman. 1981. Molecular genetics and speciation in *Elliptio* and its relationships to other taxa of North American Unionidae (Bivalvia). Biological Journal of the Linnaean Society 15:131–150.

Diamond, S. A., M. C. Newman, M. Mulvey, and S. I. Guttman. 1991. Allozyme genotype and time to death of mosquitofish, *Gambusia affinis*, during acute inorganic mercury exposure: a comparison of populations. Aquatic Toxicology 21:119–134.

Ehrlich, P. R. 1988. The loss of diversity: causes and consequences. In: Wilson, E. O., editor. Biodiversity. National Academy Press, Washington. Pp. 21–27.

Farwick, J. 1992. 1991 mussel harvest report, Arkansas Game and Fish Commission. Little Rock, Arkansas. 4 pp.

Gillespie, J. H., and C. H. Langley. 1974. A general model to account for enzyme variation in natural populations. Genetics 76:837–848.

Gillespie, R. B., and S. I. Guttman. 1989. Effects of contaminants on the frequencies of allozymes in populations of the central stoneroller. Environmental Toxicology and Chemistry 8:309–317.

Hartl, D. L. 1980. Principles of population genetics. Sinauer Press, Sunderland, Massachusetts.

Hebert, P. D. N., and M. J. Beaton. 1989. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helena Laboratories, Beaumont, Texas.

Hedrick, P. W., M. E. Ginevan, and E. P. Ewing. 1976. Genetic polymorphism in heterogeneous environments. Annual Review of Ecology and Systematics 7:1–32.

Hornbach, D. J., M. J. McLeod, S. I. Guttman, and S. K. Seilkop. 1980. Genetic and morphological variation in the freshwater clam, *Sphaerium* (Bivalvia: Sphaeriidae). Journal of Molluscan Studies 46:158–170.

Isom, B. G., and P. Yokley. 1968. The mussel fauna of Duck River in Tennessee, 1965. American Midland Naturalist 80:34–42.

IUBNC (International Union of Biochemistry, Nomenclature Committee). 1984. Enzyme no-

menclature 1984. Academic Press, San Diego, California.

KAT, P. W. 1983*a*. Genetic and morphological divergence among nominal species of North American *Anodonta* (Bivalvia: Unionidae). Malacologia 23:361–374.

KAT, P. W. 1983*b*. Morphological divergence, genetics, and speciation among *Lampsilis* (Bivalvia: Unionidae). Journal of Molluscan Studies 49: 133–145.

KOEHN, R. K., AND W. F. EANES. 1978. Molecular structure and protein variation within and among populations. Evolutionary Biology 11:39–100.

KOEHN, R. K., J. E. PEREZ, AND R. B. MERRITT. 1971. Esterase enzyme function and genetical structure of populations of the fresh water fish, *Notropis stramineus*. American Naturalist 105:51–69.

LAVIE, B., AND E. NEVO. 1986. Genetic selection of homozygote allozyme genotypes in marine gastropods exposed to cadmium pollution. Science of the Total Environment 57:91–98.

LAVIE, B., AND E. NEVO. 1988. Multilocus genetic resistance and susceptibility to mercury and cadmium pollution in the marine gastropod, *Cerithium scabridum*. Aquatic Toxicology 13:291–296.

MATTESON, M. R., AND R. W. DEXTER. 1966. Changes in pelecypod populations in Salt Fork of Big Vermilion River, Illinois, 1918–1962. Nautilus 79:96–101.

MILKMAN, R., AND L. D. BEATY. 1970. Large-scale electrophoretic studies of allelic variation in *Mytilus edulis*. Biological Bulletin 139:4–30.

NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

NEI, M., T. MARUYAMA, AND R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1–10.

NEVO, E., T. PERL, A. BEILES, AND D. WOOL. 1981. Mercury selection of allozyme genotypes in shrimps. Experientia 37:1152–1154.

NEVO, E., R. NOY, B. LAVIE, A. BEILES, AND S. MUCHTAR. 1986. Genetic diversity and resistance to marine pollution. Biological Journal of the Linnean Society 29:139–144.

POSEY II, W. R. 1997. Location, species composition and community estimates for mussel beds in the St. Francis and Ouachita rivers in Arkansas. M.S. thesis, Arkansas State University, Jonesboro.

RUST, P. J. 1993. Analysis of the commercial mussel beds in the Black, Spring, Strawberry and Current Rivers in Arkansas. M.S. thesis, Arkansas State University, Jonesboro.

SCHLEUTER, M. A., S. I. GUTTMAN, J. T. ORIS, AND A. J. BAILER. 1995. Survival of copper-exposed juvenile fathead minnows (*Pimephales promelas*) differs among allozyme genotypes. Environmental Toxicology and Chemistry 14:1727–1734.

SINGH, S. M., AND R. H. GREEN. 1984. Excess of allozyme homozygosity in marine molluscs and its possible biological significance. Malacologia 25: 569–581.

STARRETT, W. C. 1971. A survey of the mussels (Unionacea) of the Illinois River, a polluted stream. Bulletin of the Illinois Natural Historical Survey 30:266–403.

STIVEN, A. E., AND J. ALDERMAN. 1992. Genetic similarities among certain freshwater mussel populations of the *Lampsilis* genus in North Carolina. Malacologia 34:355–369.

SULOWAY, L. 1981. The Unionid (Mollusca: Bivalvia) fauna of the Kankakee River in Illinois. American Midland Naturalist 105:233–239.

SWOFFORD, D. L., AND R. B. SELANDER. 1989. BIOSYS-1, a computer program for the analysis of allelic variation in population genetics and biochemical systematics. Illinois Natural History Survey.

TODD, C. S. 1993. 1992 mussel harvest report, Arkansas Game and Fish Commission. Little Rock, Arkansas. 15 pp.

TODD, C. S. 1994. 1993 mussel harvest report, Arkansas Game and Fish Commission. Little Rock, Arkansas. 14 pp.

TODD, C. S. 1995. 1994 mussel harvest report, Arkansas Game and Fish Commission. Little Rock, Arkansas. 12 pp.

VAUGHN, C. C. 1997. Catastrophic decline of the mussel fauna of the Blue River, Oklahoma. Southwestern Naturalist 42:332–335.

WILLIAMS, J. D., M. L. WARREN, JR., K. S. CUMMINGS, J. L. HARRIS, AND R. J. NEVES. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18:6–22.

ZOUROS, E., AND D. W. FOLTZ. 1984. Possible explanations of heterozygote deficiency in bivalve molluscs. Malacologia 25:583–591.