

Genetic Analysis of Freshwater Mussel Populations  
Across the Lake Erie / Ohio River Drainage Divide

A Final Report to the Lake Erie Protection Fund  
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Freshwater mussels (Unionidae) form the most diverse and endangered element of the North American benthic fauna (Bogan 1993). Many species have been extirpated from Lake Erie due to biofouling by zebra mussels (Nalepa et al. 1996). Other parts of Ohio have also seen drastic declines in numbers of mussels. Reintroduction of unionids has been proposed to restore extirpated populations. Large populations capable of serving as sources for reintroductions are found in Lake Erie tributaries and the Ohio River basin. However, it is not known whether these regions contain genetically distinct populations and no research has been conducted on the genetic or evolutionary implications of large-scale transport of mussels across drainage basins. This project compared genetic variation among freshwater mussel populations from these two regions. We used allozyme electrophoresis to determine genetic structure of large, stable populations from Lake Erie tributary streams, and compared genetic structure among populations of these species with results from Ohio River basin populations. Completion of this project provides the first step in assessing how reintroduction of unionids into Lake Erie and other parts of Ohio might best be accomplished.

### Objectives

We proposed three objectives for this project.

- To quantify genetic structure of populations of two freshwater mussel species, *Amblema plicata* and *Actinonaias ligamentina*, from Lake Erie and Ohio River basin populations. The latter is of particular interest because it has been extirpated from Ohio (Cummings and Mayer 1992).
- To examine patterns of variation among populations and among basins for each species and to compare the patterns between species.
- To provide preliminary assessment of the feasibility of using Ohio River basin populations as sources for reintroduction of mussels to Lake Erie.

All three objectives were met or exceeded over the course of the project.

## Methods

Two common species of mussels were used in this study. *Amblema plicata* (the threeridge) were collected from two populations each in the Sydenham and Au Sable Rivers of Ontario (Metcalf-Smith et al. 1999) and the St. Joseph River (Williams County, OH and Hillsdale County, MI). These sites are all located in the Lake Erie drainage basin. Ohio River basin samples consisted of single populations from the mainstem Ohio (RM 300), and the Muskingum (Washington County, OH), Licking (Bath County, KY), and Tennessee (Marshall County, KY) Rivers. *Actinonaias ligamentina* (the mucket) were collected from two populations each in the Sydenham and Thames Rivers of Ontario (Metcalf-Smith et al. 1998a, b) and from the Licking River (Bath County, KY). Individuals were collected by hand with the aid of snorkel or SCUBA. At each site, mussels were shucked and the mantle and adductor muscles were flash-frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  until analyzed or individuals were nondestructively sampled using a mantle biopsy (Berg et al. 1995). These latter tissue samples were stored as above.

Allozyme electrophoresis using starch gels was performed for seven (*Amblema plicata*) or ten (*Actinonaias ligamentina*) enzyme systems, using standard recipes and buffer systems (Harris and Hopkinson 1976). A total of nine loci were resolved from these systems for *A. plicata* and 12 loci for *A. ligamentina* (Table 1). The number of loci analyzed was limited by the small amounts of tissue available for many individuals. At least 20 individuals were analyzed for all loci for each population; additional individuals were analyzed for each variable locus. Loci were considered "polymorphic" when the most common allele was present at a frequency  $\leq 0.95$ . Because we were often limited in the amount of tissue available from each individual and occasionally gels did not produce results, sample size varied from one to 52 for each locus-by-population combination (Tables 2 and 4).

Electrophoretic results were analyzed using standard population genetic techniques contained in BIOSYS-1 (Swofford and Selander 1981). Descriptive statistics calculated for each population included percent polymorphic loci, mean number of alleles per locus, and average direct-count heterozygosity. Comparison of measured genotype frequencies with Hardy-Weinberg expectations were evaluated using the "Exact Probabilities" procedure of BIOSYS-1 with the "sharper" sequential-comparison Bonferroni technique to adjust significance levels described by Lessios (1992). Allele frequency differences were integrated across loci by calculating modified Rogers' genetic distances for all pairs of populations (Wright 1978). Genetic similarity of populations was determined by construction of dendrograms using genetic distance and the unweighted-pair-group method (UPGMA) to cluster populations (Sokal and Sneath 1963). Among-population genetic variation was further analyzed by calculating values of  $F_{ST}$  for polymorphic loci. The number of migrants per generation ( $Nm$ ) among populations was estimated from  $F_{ST}$ , assuming a stepping-stone model of dispersal (Slatkin and Barton 1989).

## Results

### *Amblema plicata*

Four of the nine loci sampled (PGM-1, PGM-2, GPI, and MDH-2) were polymorphic in at least one population (Table 2). One other locus (GOT) was variable but not polymorphic according to the 95% criterion (see above), while four loci (EST, IDH, MDH-1, and SOD) were fixed for a single allele in all populations. Within-population genetic variation was similar for all populations except for Au Sable River population 5 in which no variation was detected. Results for this population are suspect and we plan to reanalyze these samples in the near future. Twenty-nine of 32 polymorphic locus-by-population combinations had genotype frequencies that were not significantly different from Hardy-Weinberg expectation at an experiment-wise error rate of  $\alpha = 0.05$ .

Genetic distances varied considerably among populations, ranging from 0.062 to 0.519 (Table 3). Average distance among Ohio River basin populations was 0.155 (range = 0.062 to 0.210). Lake Erie populations showed considerably greater differentiation, with an average distance of 0.328 (0.128 to 0.519). In fact, average distance within the Lake Erie basin was very similar to average distance among basins (0.312 with range = 0.086 to 0.457). Cluster analysis shows that populations from Canada cluster together, while the St. Joseph River populations cluster with the Ohio River basin populations (Figure 1). Average  $F_{ST}$  was high (0.442, range = 0.84 to 0.687) and indicated significant variation among populations. Number of migrants per generation was correspondingly low (0.32), indicating that gene flow was low enough to allow differentiation of populations via genetic drift.

### *Actinonaias ligamentina*

Eight of the 12 loci sampled (EST-UV, IDH, PGM-1, PGM-2, GPI, MDH-2, LAP, SOD) were polymorphic in at least one population (Table 4). One other locus (MDH-1) was variable but not polymorphic according to the 95% criterion (see above), while three loci (EST, GOT, and MPI) were fixed for a single allele in all populations. Within-population genetic variation was similar for all populations, and considerably higher than for *Amblema plicata*. Twenty-eight of 35 polymorphic locus-by-population combinations had genotype frequencies that were not significantly different from Hardy-Weinberg expectation at an experiment-wise error rate of  $\alpha = 0.05$ .

Genetic distances were less variable than for *Amblema plicata*, ranging from 0.095 to 0.336 (Table 5). Average distance among Lake Erie populations was 0.248 (range = 0.155 to 0.336) and very similar to the average distance between the Licking River and Lake Erie populations (0.209 with range = 0.095 to 0.278). Cluster analysis shows that the Licking River population clusters among the Lake Erie populations (Figure 2). Average  $F_{ST}$  was much lower than for *A. plicata* (0.130, range = 0.018 to 0.541) but did indicate significant variation among populations. Number of migrants per generation (1.67) was high enough that significant gene flow likely prevents differentiation of populations via genetic drift.

## Discussion

Both species contain significant levels of variation within populations. For *Amblema plicata*, within-population variation was similar to that found in a previous study (Berg and Berg, manuscript in review, Berg and Guttman, unpub. data) but considerably lower than in *Actinonaias ligamentina*. In fact, the latter species had levels of within population variation comparable to that found in *Quadrula quadrula* (Berg et al. 1998). These levels are among the highest reported for freshwater mussels.

The most striking result of this study is the different patterns of among-population variation shown by these two species. *Amblema plicata* shows considerable distance among populations, with evidence of little gene flow. Canadian populations are differentiated from more southerly populations. Surprisingly, the St. Joseph River populations, even though located within the Lake Erie basin, are more similar to the Ohio basin populations. One potential explanation for this pattern is that it might represent a relict of the Fort Wayne Outlet that connected the Maumee River with the Wabash River in northeastern Indiana following the initial retreat of the Wisconsin glacier (Trautman 1981). Support for such an explanation will require additional research on mussel populations of this region.

Our initial results indicate little, if any, differentiation among Ohio River basin and Lake Erie basin *Actinonaias ligamentina*. Genetic structure of this species appears to be similar to that of *Quadrula quadrula* (Berg et al. 1998), with high levels of variation within populations and relatively little variation among populations. However, we did sample only a single population outside of the Lake Erie basin. Additional Ohio basin populations should be sampled and compared to Lake Erie basin populations to verify this pattern. Thus, our two subject species show considerable difference in genetic structure, and suggest that conservation strategies for managing these species must account for such differences.

There has been considerable discussion of translocation as a management tool for restoration of freshwater mussel populations (Neves 1997). One of the principle concerns when considering such a tool is the evolutionary implications of reintroducing populations across drainage divides. Our results are unclear on this matter and will require further research. While it seems possible that Ohio River basin and/or Canadian populations of *Actinonaias ligamentina* might be suitable source populations for restoration of this species to Ohio streams, further populations will need to be studied to determine whether there is actually little differentiation among populations from these basins. In the case of *Amblema plicata*, it appears that significant differentiation has occurred between the Canadian and Ohio River basin populations. The situation is further complicated by the relationship between the St. Joseph River and Ohio basin populations.

Given that we have seen two different patterns with our limited sampling of two subject species, we can only recommend that further research be undertaken before translocations be made across the Lake Erie / Ohio River drainage divide. Our results are very intriguing and suggest that additional study might yield insight into the design of management strategies for this highly endangered element of the North American freshwater fauna. We envision this project as providing preliminary results to support

development of a larger proposal to comprehensively investigate the genetic relationships of mussels across the Lake Erie / Ohio River drainage divide. Such a proposal will be submitted to the LEPF in the next research grant cycle.

### Project Outputs

Results generated by this project were presented as part of an invited symposium at the 1999 annual meeting of the North American Benthological Society. In addition, results were also presented at the 1999 annual meeting of the Freshwater Mollusk Conservation Society. Another presentation will be given at the 2000 annual meeting of the North American Benthological Society. Our results have also been included in a manuscript that is currently in final review for the peer-reviewed journal *Conservation Biology*. The funding of this project was instrumental in generating an additional \$20,000 in funding from the Ohio Biological Survey to perform both allozyme and mitochondrial DNA analyses on additional populations. Completion of this project has provided preliminary data for a larger proposal that will be submitted to the Lake Erie Protection Fund during the 2000 funding cycle.

### Citations

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Table 1. Enzyme systems used and their abbreviations.

Enzyme System	Abbreviation	<i>Amblema plicata</i>	<i>Actinonaias ligamentina</i>
Esterase	EST	*	*
Esterase - ultraviolet locus	EST-UV		*
Glutamate Oxaloacetate Transaminase	GOT	*	*
Glucosephosphate Isomerase	GPI	*	*
Isocitrate Dehydrogenase	IDH	*	*
Leucine Aminopeptidase	LAP		*
Malate Dehydrogenase	MDH-1	*	*
	MDH-2	*	*
Mannose-6-phosphate Isomerase	MPI		*
Phosphoglucomutase	PGM-1	*	*
	PGM-2	*	*
Superoxide dismutase	SOD	*	*



Table 2 (con'd).

MDH-2										
(N)	42	33	1	5	1	7	27	6	26	6
1	.345	.485	.000	.000	.000	.000	.000	.000	.038	.250
2	.655	.515	1.000	.300	.000	.000	.130	.000	.750	.667
3	.000	.000	.000	.500	1.000	1.000	.685	.833	.212	.083
4	.000	.000	.000	.200	.000	.000	.185	.167	.000	.000
SOD										
(N)	42	33	52	36	20	1	1	1	26	29
1	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mean # of alleles per locus										
	1.9	1.7	1.7	1.8	1.0	1.2	1.7	1.4	1.7	1.8
	(0.4)	(0.3)	(0.3)	(0.3)	(0.0)	(0.1)	(0.3)	(0.2)	(0.3)	
(0.3)										
% P	44.4	44.4	22.2	44.4	0.0	22.2	44.4	44.4	44.4	44.4
avg. H	0.13	0.17	0.08	0.14	0.0	0.10	0.09	0.05	0.08	0.21
	(0.06)	(0.08)	(0.05)	(0.08)	(0.0)	(0.07)	(0.05)	(0.03)	(0.03)	(0.10)

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 %P = percentage of polymorphic loci (95% criterion)  
 avg H = average direct-count heterozygosity  
 Standard errors are in parentheses below means.



Table 4. Allelic frequencies for each locus and descriptive statistics for *Actinonaias ligamentina*.

Population					
Locus	Licking	Sydenham site 5	Sydanham site 7	Thames site 2	Thames site 6
EST-UV					
(N)	47	21	23	25	26
1	.000	.071	.000	.040	.000
2	.064	.095	.152	.160	.038
3	.149	.286	.109	.280	.288
4	.574	.500	.565	.300	.423
5	.213	.000	.174	.220	.250
6	.000	.048	.000	.000	.000
EST					
(N)	37	24	25	26	19
1	1.000	1.000	1.000	1.000	1.000
GOT					
(N)	27	27	24	23	25
1	1.000	1.000	1.000	1.000	1.000
IDH					
(N)	48	27	24	24	26
1	.000	.111	.542	.000	.000
2	.688	.611	.438	.667	.712
3	.313	.278	.021	.313	.288
4	.000	.000	.000	.021	.000
PGM-1					
(N)	37	28	25	26	25
1	.095	.143	.120	.212	.080
2	.743	.446	.720	.692	.700
3	.095	.339	.140	.038	.140
4	.068	.018	.020	.058	.080
5	.000	.036	.000	.000	.000
6	.000	.018	.000	.000	.000
PGM-2					
(N)	47	28	26	26	26
1	.053	.571	.019	.038	.058
2	.777	.393	.288	.250	.538
3	.085	.018	.577	.635	.212
4	.085	.018	.115	.077	.192
MPI					
(N)	24	21	25	26	25
1	1.000	1.000	1.000	1.000	1.000

Table 4 (con'd)

MDH-1					
(N)	48	28	26	26	26
1	1.000	1.000	1.000	.962	1.000
2	.000	.000	.000	.038	.000
MDH-2					
(N)	48	13	26	7	26
1	.938	.231	1.000	1.000	1.000
2	.000	.615	.000	.000	.000
3	.063	.154	.000	.000	.000
GPI					
(N)	40	28	25	23	26
1	.000	.000	.040	.043	.000
2	.025	.089	.420	.109	.154
3	.000	.054	.300	.097	.038
4	.613	.571	.180	.522	.462
5	.363	.286	.060	.239	.346
LAP					
(N)	48	28	26	24	22
1	.000	.125	.077	.292	.000
2	.000	.214	.019	.250	.068
3	.177	.411	.115	.125	.091
4	.125	.089	.077	.125	.182
5	.469	.089	.269	.146	.409
6	.052	.036	.096	.042	.136
7	.115	.000	.115	.021	.114
8	.063	.018	.231	.000	.000
9	.000	.018	.000	.000	.000
SOD-1					
(N)	48	28	26	14	19
1	.979	1.000	.942	.929	.947
2	.021	.000	.058	.071	.053
Mean number of alleles per locus					
	2.6(0.5)	3.2(0.7)	2.9(0.6)	3.0(0.6)	2.6(0.5)
% P					
	58.3	58.3	58.3	58.3	58.3
avg. H					
	0.21(0.07)	0.24(0.07)	0.22(0.07)	0.22(0.07)	0.25(0.08)

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 %P = percentage of polymorphic loci (95% criterion)

avg H = average direct-count heterozygosity

Standard errors are in parentheses following means.

Table 5. Modified Rogers' Distance among populations of *Actinonaias ligamentina*.

Population	1	2	3	4	5
1 LICKING RIVER	*****	.278	.255	.207	.095
2 SYDENHAM site 6		*****	.336	.295	.279
3 SYDENHAM site 7			*****	.208	.216
4 THAMES site 2				*****	.155
5 THAMES site 6					*****

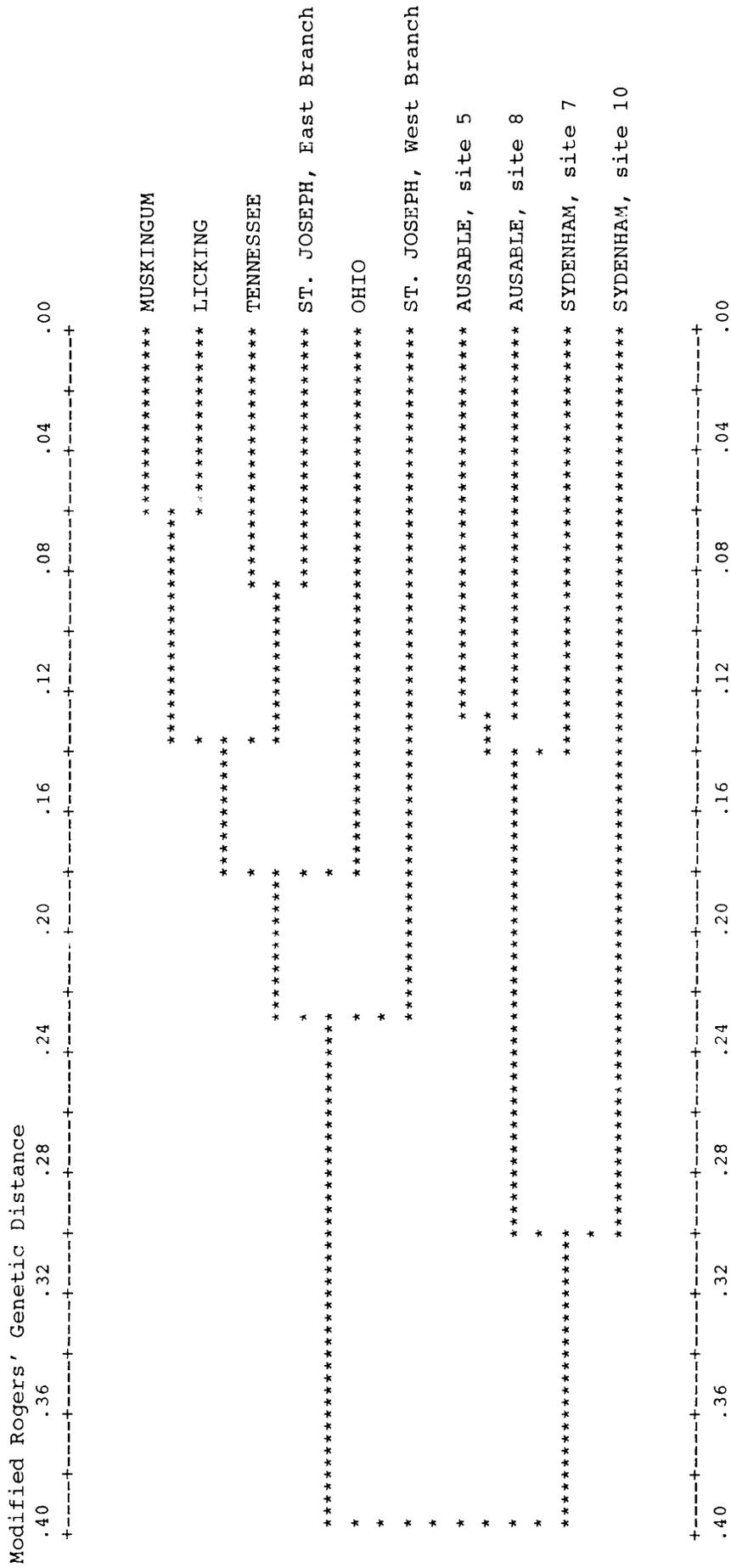


Figure 1. Modified Rogers' genetic distances (Wright 1978) for populations of *Amblyomma plicata*. Distances calculated using nine allozyme loci.

Modified Rogers' Genetic Distance

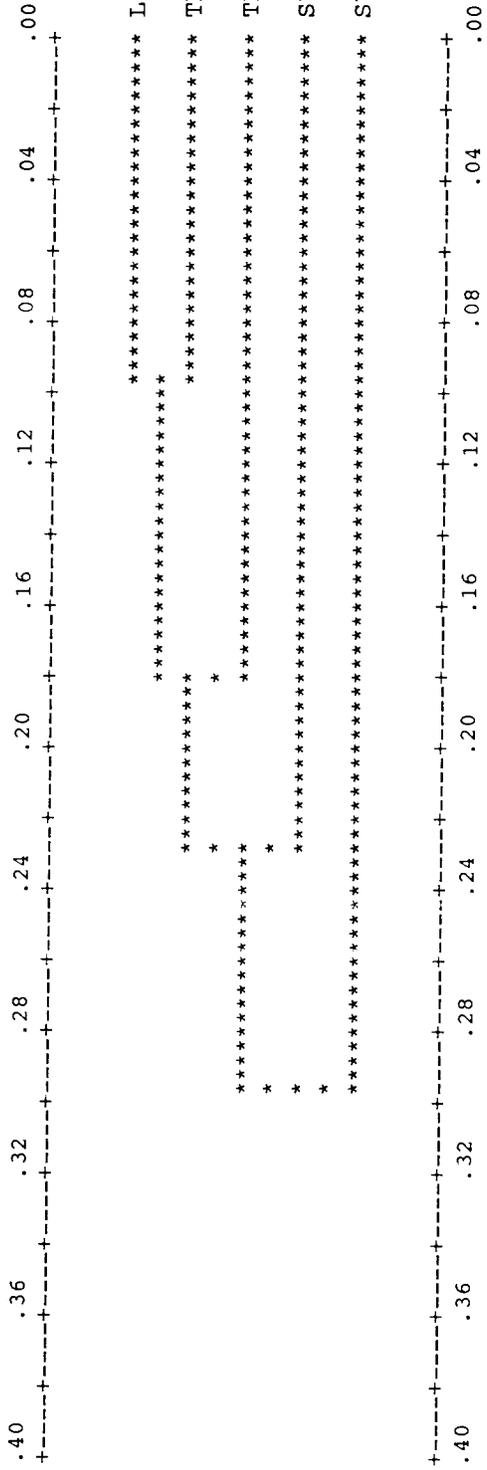


Figure 2. Modified Rogers' genetic distances (Wright 1978) for populations of *Actinonaias ligamentina*. Distances calculated using 12 allozyme loci.