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CONSERVATION GENETICS OF FRESHWATER MUSSELS
AND THE POTENTIAL FOR TRANSLOCATION INTO THE LAKE ERIE WATERSHED

A Final Report to the Lake Erie Protection Fund (project LEPF 00-01)

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Freshwater mussels of the family Unionidae form a diverse and conspicuous element of North American riverine and lake communities. Approximately three hundred taxa have been described from North America, with a large number of them endemic to the Mississippi basin, especially the watershed of the Ohio river (Williams et al. 1993). The Lake Erie drainage is also diverse, with 36 species native to the lake itself (Clark and Stansbery 1988) and additional species within tributary waters. In Canada, 75% of all species occur within the lower Great Lakes drainage (Metcalf-Smith et al. 1998). As the center of diversity of freshwater mussels, this region may be considered the unionid "equivalent" of the South American rain forests, with their immense diversity of insect species. Just as the Amazonian rain forests have seen an enormous loss of biodiversity, North American freshwater ecosystems have suffered precipitous declines in species richness and abundance of unionid communities. Currently, it is estimated that between 55% and 72% of North American unionid taxa are extinct, threatened or of special concern (Master 1990, Williams et al. 1993). Ohio is home to 79 described taxa.

Development of conservation strategies for mussel taxa often involve translocation of individuals from large, healthy populations. The purposes of such translocations include preservation of populations threatened by habitat modification (such as bridge construction), supplementing of small or threatened populations to aid in their recovery, establishment of populations for captive propagation, and reestablishment of populations previously extirpated (Neves 1997). Translocation has been considered as a management tool for restoring and augmenting populations of unionids by the Ohio Division of Wildlife (ODOW; G. T. Watters pers. comm.) and by other governmental agencies (National Native Mussel Conservation Committee 1998). Many of the large

populations capable of serving as sources for such translocations are located in other watersheds in either the Lake Erie or Ohio River basins. Concerns have been raised whether the use of such source populations will introduce significant amounts of genetic material into previously isolated populations (Storfer 1999) such as those across the Lake Erie / Ohio River drainage divide. The ultimate question is “Do Great Lakes and Ohio River mussel populations form discrete management units?” where a management unit is defined as a population with significantly different allele frequencies from other populations of the same species (Moritz 1994) and is roughly analogous to the stock concept used in fisheries biology. In addition, do individual river systems contain unique alleles – genetic material that is absent in other river basins? These questions must be addressed if translocation is to be an effective management tool for restoration of mussel populations.

Objectives

We proposed three objectives for this project.

- To quantify genetic variation within populations of freshwater mussels located in the Lake Erie watershed and the Ohio River watershed.
- To quantify among-population genetic variation at various spatial scales through the use of a hierarchical analysis.
- To develop guidelines for the maintenance of genetic identity of mussel populations within the Lake Erie watershed and among the Lake Erie and Ohio River watersheds.

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

Methods

Four common species of mussels were used in this study: *Actinonaias ligamentina* (mucket), *Elliptio dilatata* (spike), *Amblema plicata* (threeridge), and *Lasmigona costata* (fluted-shell). We designed a hierarchical analysis that included comparisons of genetic variation at four spatial scales: within population (8-12 populations total per species, Table 1), among populations within river (1-3 populations per river), among rivers within regions (2 or 3 rivers per region), and among regions (2 regions). Sampling localities are shown in Figure 1. Populations within a river were 4-150 river kilometers apart. Individuals were collected by hand with the aid of snorkel or SCUBA. At each site, most mussels were nondestructively sampled using a mantle biopsy (Berg et al. 1995) and tissues were flash-frozen in liquid nitrogen and stored at -70°C until analyzed.

Although we had originally proposed to use allozyme electrophoresis, we decided instead to utilize mtDNA sequencing of the cytochrome oxidase I (COI) gene because of its better resolving power. Given that the Lake Erie basin has only been ice-free since the Pleistocene glaciation, we felt that sequencing promised the best chance for

detecting geographic structure. We extracted DNA from approximately 25 mg of frozen tissue following the protocol for animal tissue of the DNeasy Tissue kit (Qiagen). Polymerase chain reaction amplification of a 631-682 base-pair region of the COI gene was performed using 22me (5'-GGTCAACAAATCATAAAGATATTGG-3') and 700dy (5'-TCAGGGTGACCAAAAATCA-3') primers modified from Folmer et al. (1994). Amplification was performed in a PTC-200 thermocycler (MJ Research) with an initial denaturation of 94°C for 120 seconds followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 42°C, 90 seconds at 72°C, and ended with 90 seconds at 72°C and an extension phase at 72°C for 210 seconds. In a final volume of 50µl, the PCR mix contained 10µl DNA (concentration 0.05µg/µl), 25µl Taq master mix, 2.5µl of each primer and 10µl ddH₂O. The PCR products were run on a 2% agarose gel and gel isolated using the QIAquick Spin Kit (Qiagen). All DNA samples were sequenced completely in both directions using PCR primers on a ABI Prism 3100 (Applied Biosystems). The sequences were aligned by eye and checked using MacClade vs. 4.06 (Sinauer Associate, Sunderland, MA). Population sample size ranged from 6-33 individuals.

The aligned mtDNA sequences were analyzed using Arlequin (Schneider et al. 2000), to calculate the amount of within-population variation, and provide an estimate of diversity (the number of haplotypes and number of unique haplotypes per population). Among population variation was quantified using the Analysis of Molecular Variation (AMOVA; Excoffier et al. 1992) procedure of Arlequin to partition total genetic variation within populations, among populations within rivers, among rivers within watersheds, and among watersheds. A mismatch distribution was created by comparing each individual to all other individuals and noting the number of base-pair differences. Parsimony networks showing relationships of haplotypes were constructed using TCS (Clement et al. 2000). Haplotypes were then grouped based on this network, and the frequencies of the groups were mapped.

Results

Lasmigona costata

Twenty-five haplotypes were identified among the 182 specimens examined. Sequences were 681 characters long with 27 polymorphic sites. Mean sample size was 15.2 individuals per population. An average of 3.9 haplotypes were found per population. A site on the Sandusky River had the largest number of haplotypes (7), while a Walhonding River population had the fewest (1). The largest number of haplotypes was found in the Sandusky River (9), and the fewest in the Sydenham River (4; Table 2). Seventy-five percent of the populations contained at least one unique haplotype and 41% of the populations had at least two. The WH1 population had the most unique haplotypes (4). Only two haplotypes were found in both the Lake Erie and Ohio River watersheds.

Of the total molecular variance determined by AMOVA, 33% was attributed to within population variation, 3% to variance among rivers within watersheds, and 63% to variance among watersheds. The mismatch distribution including all populations showed two modes, at 0 differences and at 9 differences. The highest number of differences between sequences was 11 nucleotides (Figure 2). The parsimony network showed two distinct clades (Figure 3). One clade corresponded to haplotypes that were common in the Ohio River basin but also found in the Lake Erie basin, while the other contained haplotypes found exclusively in the Lake Erie watershed (Figure 4). The two haplotypes found in both watersheds clustered with the Ohio River clade. This clade has two sub-clades, and in both cases, two haplotypes are only one base-pair different than the most frequent haplotype. Sandusky River haplotypes (7, 8, 13-19) are found in both clades. Haplotype 13 was found only in the Lake Erie basin, while haplotype 1 was limited to the Ohio River watershed. Haplotype 7 was seen in both the Ohio River watershed and the Sandusky River. A closer examination of the Sandusky River showed 44% of the haplotypes (4 haplotypes out of 9; haplotypes 13, 14, 15, and 18) resolved with the Lake Erie clade, and 56% resolved with the Ohio River clade (7, 9, 16, 17, and 19). Fifty-three percent of the individuals have haplotypes from the Lake Erie clade, so the Sandusky is almost equally composed of haplotypes from the two regions.

Elliptio dilatata

Twenty-three haplotypes were identified among the 134 specimens examined. Sequences were 682 characters long with 23 polymorphic sites. Mean sample size was 13.4 individuals per population. An average of 4.7 haplotypes were found per population. A site on the St. Joseph River had the largest number of haplotypes (8), while one population each from Darby Creek (but only 6 individuals were sampled) and the Little Miami River had the fewest (2). The largest number of haplotypes was found in the St. Joseph River (10), and the fewest in Darby Creek (4; Table 3). Seventeen of the 23 haplotypes (74%) were unique to a single population. All populations except DC1 contained at least one unique haplotype, while the mean number of unique haplotypes was 1.7 per population (± 0.4 s.e., $n = 10$). The LM2 population had the most unique haplotypes (4). Thirteen haplotypes were restricted to the Lake Erie watershed, six were found only in the Ohio River watershed, and four were found in both. The latter included two haplotypes that were common in all but one or two populations. One haplotype that was restricted to the Lake Erie watershed was common in all six of those populations.

Of the total molecular variance determined by AMOVA, 77.4% was attributed to within population variation, 15.3% to variance among populations within rivers, 0% to variation among rivers within regions, and 7.2% to variance among watersheds. The mismatch distribution including all populations showed a mode 1 difference and a very small mode at 9 differences; the latter was due to the presence of one very different haplotype in the Sydenham River. The highest number of differences between sequences was 10 nucleotides (Figure 5). The parsimony network showed one very large clade containing individuals from all populations and the one different haplotype mentioned above

(Figure 6). Haplotype 1 was found in nine populations and was the most common haplotype in all of them except one population from the Sydenham River (Figure 7). Haplotype 2 was found in all populations except one each from the Little Miami and St. Joseph rivers (Table 4). Haplotype 11 was found in the six populations from the Lake Erie watershed and was relatively common in all of these. All other haplotypes were restricted to three or fewer populations.

Amblema plicata

Fourteen haplotypes were identified among the 101 specimens examined. Sequences were 631 characters long with 14 polymorphic sites. Mean sample size was 12.6 individuals per population. An average of 4.3 haplotypes were found per population. A site on the Little Miami River had the largest number of haplotypes (7), while Sydenham River populations had the fewest (3). The largest number of haplotypes was found in the Little Miami River (8), and the fewest in the Sydenham River (3; Table 4). Nine of the 14 haplotypes (64%) were unique to a single population; five of these were found in the Ohio River watershed. The mean number of unique haplotypes was 1.1 per population (± 0.5 s.e., $n = 8$ populations), with no unique haplotypes in the Sydenham and Walhonding rivers. The LM2 population had the most unique haplotypes (4). Only three haplotypes were shared between the Lake Erie and Ohio River watersheds. None of the other haplotypes were shared between rivers.

Of the total molecular variance determined by AMOVA, 90.4% was attributed to within population variation, 0% to variance among populations within rivers, 9.6% to variation among rivers within regions, and 0% to variance among watersheds. The mismatch distribution including all populations showed two modes, at 0 differences and at 2 differences. The highest number of differences between sequences was 6 nucleotides (Figure 8). The parsimony network showed two common clades containing individuals from all populations (Figure 9). No geographic pattern was seen in the distribution of these clades (Figure 10) and the basal haplotypes for each clade (haplotypes 1 and 2) were only 2 base pairs different from one another. Haplotypes 1 and 2 were common in all populations (frequency ≥ 0.08 ; Table 4). Haplotype 3 was found in 5 populations. All other haplotypes were restricted to a single river and thus, one or two populations.

Actinonaias ligamentina

Fifty-two haplotypes were identified among the 181 specimens examined. Sequences were 681 characters long with 58 polymorphic sites. Mean sample size was 17.9 individuals per population. An average of 8.4 haplotypes were found per population. A site on the Licking River had the largest number of haplotypes (13), while one population each from the Thames and Sandusky Rivers had the fewest (6). The largest number of haplotypes was found in the Licking River (20), and the fewest in the Thames River (11; Table 5). Thirty-nine of the 52 haplotypes (75%) were unique to a single population; 19 of these were found in the Ohio River watershed. All populations contained at least one unique haplotype, while the mean number of unique haplotypes

was 3.9 per population (± 0.7 s.e., $n = 10$). The SY2 population had the most unique haplotypes (7). Only eight haplotypes were shared between the Lake Erie and Ohio River watersheds, but these included all three haplotypes that were present at frequencies ≥ 0.10 in at least one population.

Of the total molecular variance determined by AMOVA, 91.7% was attributed to within population variation, 6.7% to variance among populations within rivers, 0% to variation among rivers within regions, and 1.6% to variance among watersheds. The mismatch distribution including all populations showed two modes, at 0 differences and at 5 differences. A third very small mode occurred at 13 differences, due to the presence of one very different haplotype in the Licking River. The highest number of differences between sequences was 16 nucleotides (Figure 11). The parsimony network showed one very large clade containing individuals from all populations, a second clade with individuals from all rivers except the Thames, and several haplotypes that are very different from either clade (Figure 12). No geographic pattern was seen in the distribution of these clades (Figure 13). Haplotype 3 was found in all populations and was the most common haplotype in all of them except one population from the Walhonding River. Haplotype 11 was the most common haplotype in the latter and it was found in all other populations except those from the Sandusky River (Table 5). Haplotype 7 was found in six populations. All other haplotypes were restricted to four or fewer populations, but many times these were from multiple rivers.

Conclusions

We have been able to meet all of the objectives of this project. From our results, we can draw a number of conclusions regarding genetic structure of freshwater mussels and the importance of the Ohio River – Lake Erie drainage divide. These results also have significant management implications.

Objective 1: Genetic variation within populations

All four species of mussels contained significant levels of within-population genetic variation but the amount relative to total variation was highly variable. While almost all of the variation in *Amblema plicata* and *Actinonaias ligamentina* (> 90%) was found within populations, much lower amounts were found in *Elliptio dilatata* (77%) and *Lasmigona costata* (33%). This pattern of variation was not correlated with other measures of genetic diversity such as total number of haplotypes or average number of haplotypes per population. Unique haplotypes were quite common, although these were often only different from widespread haplotypes by 1-2 substitutions. Sample sizes may have played a role in determining total number of haplotypes found for each species, although the much larger number of haplotypes found in *A. ligamentina* is likely not due solely to sample sizes since a similar number of *L. costata* yielded half as many haplotypes. Rather, it seems that the former species has much more haplotype

diversity than do the other three species. Almost all of this higher diversity is found within populations as indicated by the large number of haplotypes per population in *A. ligamentina*. There is no pattern to the distribution of this variation among basins: while average number of haplotypes per population was the same for the Ohio River and Lake Erie watersheds for *L. costata* (4.0 for Ohio, 3.9 for Lake Erie), Ohio River populations contained more haplotypes for *A. plicata* (5.0 vs. 3.5) and *A. ligamentina* (9.3 vs. 7.8). Conversely, *E. dilatata* populations in the Lake Erie watershed contained more haplotypes on average than those from the Ohio River watershed (3.5 vs. 5.5).

Objective 2: Genetic variation among populations

Among-population genetic variation differed considerably among species and the spatial scale over which differentiation occurred was also quite variable. *Amblema plicata* and *Actinonaias ligamentina* showed little or no variation among watersheds, and a small amount of variation among populations within rivers (*A. ligamentina*) or among rivers (*A. plicata*). In both cases, haplotypes from throughout the parsimony networks were found in both the Lake Erie and Ohio River watersheds. In the case of *A. ligamentina*, both haplotypes 3 and 10 were found in both watersheds. Similarly, the two basal haplotypes for *A. plicata* (haplotypes 1 and 2) were common in both watersheds. Thus, no signal was found at the regional level in these species. This likely reflects the relatively recent movement of mussels into the Lake Erie watershed following the Pleistocene glaciation.

Both *Elliptio dilatata* and *Lasmigona costata* show greater amounts of variation among populations than did the other species. However, this variation is partitioned quite differently in the two species. In the former, much of the variation is between populations within rivers, indicating that the spatial scale at which structuring can occur is smaller for this species than for the others. This might reflect host-fish vagility if *E. dilatata* utilizes hosts that are not very mobile. The among-watershed variation in this species is likely due to the relatively high proportion of haplotype 11 individuals in the Lake Erie watershed and the complete absence of this haplotype in the Ohio River watershed. Interestingly, haplotype 10, which is very similar to haplotype 11 is found only in the Ohio River watershed. Very significant regional differentiation is seen in *L. costata*, with groups of haplotypes dominating one watershed or the other. All Ohio River populations are composed of haplotypes from one group. Lake Erie populations are a mixture of the Ohio River group and another group. Given that haplotypes in the latter group are closely related to a haplotype found in the St. Lawrence River basin (accessed via GenBank), it is possible that postglacial colonization of the Lake Erie basin (Ontario) was from two refugia, one in the Ohio River basin and the other in the St. Lawrence basin. This is consistent with proposed patterns of fish colonization following the melting of glaciers at the end of the Pleistocene (Mandrak and Crossman 1992). In both cases, *E. dilatata* and *L. costata*, significant unique genetic resources are found in both the Ohio River and Lake Erie watersheds.

Objective 3: Implications for conservation

Our results suggest that there are no readily identifiable overall patterns to the geographic structuring of genetic variation in freshwater mussels between the Lake Erie and Ohio River watersheds. This is likely due to several factors. The relatively young age of the Lake Erie watershed (about 10,000 years postglaciation) means that dispersal of freshwater mussels into the region has been quite recent, with little time for isolation and differentiation of populations. Time is likely a significant factor, since we see much greater total haplotype diversity and much greater variation among populations when we examine mussel populations from older ecosystems such as the interior highlands of Arkansas and Oklahoma (C. L. Elderkin et al. manuscript in prep., J. L. Hoisington et al. manuscript in prep.). Fish host vagility may be another important factor in determining mussel genetic structure. Mussels that utilize widespread, highly mobile fishes are likely to show less regional differentiation than are mussel species utilizing fishes with restricted ranges, narrow habitat requirements, or low vagility. In particular, headwater fishes may be restricted in their abilities to move from one headwater system to another. For instance, mussel populations in the Darby Creek ecosystem of central Ohio show differentiation in allele frequencies at allozyme loci over a range of about 100 km, while no differentiation is found among populations of other mussel species at distances of > 1000 km along the Ohio River (Berg et al. 1998) or in large rivers across the Mississippi basin (Berg and Elderkin, unpub. data). These differences (or lack thereof) may reflect differences in movement of small stream fishes that serve as hosts versus fishes of big rivers. Other factors that may also lead to differentiation might include local selection and small effective population sizes that increase the rate of genetic drift.

Regardless of the causes of differentiation, it is clear that unique genetic resources are present across the Ohio River / Lake Erie drainage divide in two of the four species we investigated. While maximum sequence similarity is relatively low (1-2.3% for all pairs of haplotypes within each species), geographic structure is beginning to appear in both *Lasmigona costata* and *Elliptio dilatata*. The precautionary principle would guide us to conclude that translocations across this drainage divide should only be undertaken after thorough study of geographic variation in genetic diversity for target species. In general, such translocations should occur across as short a distance as possible, using river distances rather than overland distances. In particular, cross-basin translocations should only be conducted as a measure of last resort.

Information Dissemination

We have given twelve presentations (five of these invited) at national or international scientific conferences including meetings of the North American Benthological Society, the National Shellfish Association, and the Freshwater Mollusk Conservation Society. We (DJB and CLE) have given invited seminars at the University of Windsor, Arkansas State University, Virginia Commonwealth University, Murray State University, Northern Michigan University, and the College of New Jersey. We currently have 3 manuscripts in preparation for submission to journals such as *Molecular Ecology* and *Conservation Genetics*. Two students produced undergraduate theses while working on this project.

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Table 1. Collection locations for each species of mussel. Two sites were sampled for each species in each river, except for the Stillwater, where only site was sampled and the Grand, where three sites were sampled.

Species	Watersheds				
	Lake Erie, Ontario		Lake Erie, OH/IN	Ohio River	
<i>Lasmigona costata</i>	Sydenham	Grand	Sandusky	Licking Stillwater	Walhonding
<i>Elliptio dilatata</i>	Sydenham	Grand	St. Joseph	Darby	Little Miami
<i>Amblema plicata</i>	Sydenham	Ausable		Little Miami	Walhonding
<i>Actinonaias ligamentina</i>	Sydenham	Thames	Sandusky	Licking	Walhonding

Table 2. Haplotype frequencies for each population of *Lasmsgiona costata*

Haplotype	Population											
	LR1	LR2	ST1	WH1	WH2	SD1	SD2	SY1	SY11	GR18	GR21	GR24
1	0.250	0.200	0.467	0.750	1.000	-	-	-	-	-	-	-
2	-	-	-	0.063	-	-	-	-	-	-	-	-
3	-	-	-	0.063	-	-	-	-	-	-	-	-
4	-	-	-	0.063	-	-	-	-	-	-	-	-
5	-	-	-	0.063	-	-	-	-	-	-	-	-
6	-	-	0.133	-	-	-	-	-	-	-	-	-
7	0.625	0.667	0.200	-	-	-	0.125	-	-	-	-	-
8	-	-	0.067	-	-	-	-	-	-	-	-	-
9	-	0.067	0.067	-	-	0.375	0.188	0.143	0.154	-	-	0.063
10	-	0.067	0.067	-	-	-	-	-	-	-	-	-
11	0.063	-	-	-	-	-	-	-	-	-	-	-
12	0.063	-	-	-	-	0.438	0.438	0.786	0.769	0.938	0.800	0.875
13	-	-	-	-	-	0.063	-	-	-	-	-	-
14	-	-	-	-	-	0.063	-	-	-	-	-	-
15	-	-	-	-	-	0.063	0.063	-	-	-	-	-
16	-	-	-	-	-	0.063	0.063	-	-	-	-	-
17	-	-	-	-	-	-	0.063	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	0.071	0.077	-	-	-
21	-	-	-	-	-	-	-	-	-	0.063	-	-
22	-	-	-	-	-	-	-	-	-	-	0.067	-
23	-	-	-	-	-	-	-	-	-	-	0.067	0.063
24	-	-	-	-	-	-	-	-	-	-	0.067	-
25	-	-	-	-	-	-	-	-	-	-	0.067	-
Sample size	16	15	15	16	14	16	16	14	13	16	15	16
# haplotypes	4	4	6	5	1	5	7	3	3	2	4	3
# unique	2	0	2	4	0	2	3	1	1	1	2	0

LR = Licking River
 ST = Stillwater River
 WH = Walhonding River
 SD = Sandusky River
 SY = Sydenham River
 GR = Grand River

Table 3. Haplotype frequencies for each population of *Elipitio dilatata*

Haplotype	Population												
	DC1	DC2	LM1	LM2	GR1	GR2	SJ1	SJ2	SY1	SY2			
ED1	0.833	0.5	0	0.333	0.615	0.727	0.4	0.412	0.375	0.158			
ED2	0.167	0.357	0.857	0	0.0769	0.0909	0	0.118	0.188	0.211			
ED3	0	0.0714	0	0	0	0	0	0	0	0			
ED4	0	0.0714	0	0	0	0	0.1	0	0	0			
ED5	0	0	0.143	0	0	0	0	0	0	0			
ED6	0	0	0	0.133	0	0	0	0	0	0			
ED7	0	0	0	0.0667	0	0	0	0	0	0			
ED8	0	0	0	0.333	0	0	0	0	0	0			
ED9	0	0	0	0.0667	0	0	0.2	0.0588	0	0			
ED10	0	0	0	0.0667	0	0	0	0	0	0			
ED11	0	0	0	0	0.154	0.0909	0.2	0.176	0.25	0.368			
ED12	0	0	0	0	0.0769	0	0	0	0	0			
ED13	0	0	0	0	0.0769	0	0	0	0	0			
ED14	0	0	0	0	0	0.0909	0	0	0	0			
ED15	0	0	0	0	0	0	0.1	0	0	0			
ED16	0	0	0	0	0	0	0	0.0588	0	0			
ED17	0	0	0	0	0	0	0	0.0588	0	0			
ED18	0	0	0	0	0	0	0	0.0588	0	0.0526			
ED19	0	0	0	0	0	0	0	0.0588	0	0			
ED20	0	0	0	0	0	0	0	0	0.0625	0			
ED21	0	0	0	0	0	0	0	0	0.125	0			
ED22	0	0	0	0	0	0	0	0	0	0.0526			
ED23	0	0	0	0	0	0	0	0	0	0.158			
Sample size	6	14	13	15	13	11	10	17	16	19			
# haplotypes	2	4	2	6	5	4	5	8	5	6			
# unique	0	1	1	4	2	1	1	3	2	2			

DC = Darby Creek
 LM = Little Miami River
 GR = Grand River
 SJ = Saint Joseph River
 SY = Sydenham River

Table 4. Haplotype frequencies for each population of *Amblema plicata*

Haplotype	Population									
	SY1	SY2	AU1	AU2	WH1	WH2	LM1	LM2		
AP1	0.357	0.143	0.667	0.2	0.417	0.583	0.5	0.333		
AP2	0.5	0.714	0.2	0.5	0.167	0.0833	0.0833	0.0833		
AP3	0.143	0.143	0	0	0.0833	0	0.333	0.167		
AP4	0	0	0.0667	0	0	0	0	0		
AP5	0	0	0.0667	0	0	0	0	0		
AP6	0	0	0	0.1	0	0	0	0		
AP7	0	0	0	0.2	0	0	0	0		
AP8	0	0	0	0	0.167	0.167	0	0		
AP9	0	0	0	0	0.167	0.167	0	0		
AP10	0	0	0	0	0	0	0.0833	0		
AP11	0	0	0	0	0	0	0	0.167		
AP12	0	0	0	0	0	0	0	0.0833		
AP13	0	0	0	0	0	0	0	0.0833		
AP14	0	0	0	0	0	0	0	0.0833		
Sample size	14	14	15	10	12	12	12	12	12	12
# haplotypes	3	3	4	4	5	4	4	4	7	7
# unique	0	0	2	2	0	0	1	1	4	4

SY = Sydenham River
 AU = Au Sable River
 WH = Walhonding River
 LM = Little Miami River

Table 5. Haplotype frequencies for each population of *Actinonaias ligamentina*.

Haplotype	Population									
	LR1	LR2	TR2	TR6	SY1	SY2	WH1	WH2	SD1	SD2
ACT1	0.0303	0	0	0	0	0	0	0	0	0
ACT2	0.0303	0	0	0	0	0	0	0	0	0
ACT3	0.394	0.467	0.357	0.571	0.611	0.267	0.667	0.294	0.667	0.588
ACT4	0.0606	0	0	0	0	0	0.0476	0	0	0
ACT5	0.0303	0	0	0	0	0	0	0	0	0
ACT6	0.0303	0	0	0	0	0	0	0	0	0
ACT7	0.0303	0	0.143	0.0714	0.0556	0	0.0476	0	0	0.0588
ACT8	0.0606	0	0	0	0.0556	0	0	0	0	0
ACT9	0.0606	0	0	0	0	0.0667	0	0	0	0
ACT10	0.0303	0	0	0	0	0	0	0	0	0
ACT11	0.152	0.0667	0.0714	0.143	0.111	0.133	0.0476	0.412	0	0
ACT12	0.0606	0	0.0714	0	0	0	0	0.0588	0	0
ACT13	0.0303	0	0	0	0	0	0	0	0	0
ACT14	0	0.0667	0	0	0	0	0	0	0	0
ACT15	0	0.0667	0	0	0	0	0	0	0	0.0588
ACT16	0	0.0667	0	0	0	0	0	0	0	0
ACT17	0	0.0667	0	0	0	0	0	0	0	0
ACT18	0	0.0667	0	0	0	0	0	0	0	0
ACT19	0	0.0667	0	0	0	0	0	0	0	0
ACT20	0	0.0667	0	0	0	0	0	0	0	0
ACT21	0	0	0.0714	0	0	0	0	0	0	0.0588
ACT22	0	0	0.0714	0	0.0556	0	0	0	0	0
ACT23	0	0	0.0714	0.0714	0	0	0	0	0	0
ACT24	0	0	0.0714	0	0	0	0	0	0	0
ACT25	0	0	0.0714	0	0	0	0	0	0	0
ACT26	0	0	0	0.0714	0	0.0667	0	0	0	0
ACT27	0	0	0	0.0714	0	0	0	0	0	0
ACT28	0	0	0	0	0.0556	0	0	0	0	0
ACT29	0	0	0	0	0.0556	0	0.0476	0	0	0
ACT30	0	0	0	0	0	0.0667	0	0	0	0
ACT31	0	0	0	0	0	0.0667	0	0	0	0
ACT32	0	0	0	0	0	0.0667	0	0	0	0
ACT33	0	0	0	0	0	0.0667	0	0	0	0
ACT34	0	0	0	0	0	0.0667	0	0	0	0
ACT35	0	0	0	0	0	0.0667	0	0	0	0
ACT36	0	0	0	0	0	0.0667	0	0	0	0
ACT37	0	0	0	0	0	0	0.0476	0	0	0
ACT38	0	0	0	0	0	0	0.0476	0	0	0
ACT39	0	0	0	0	0	0	0.0476	0	0	0
ACT40	0	0	0	0	0	0	0	0.0588	0	0
ACT41	0	0	0	0	0	0	0	0.0588	0	0
ACT42	0	0	0	0	0	0	0	0.0588	0	0
ACT43	0	0	0	0	0	0	0	0.0588	0	0
ACT44	0	0	0	0	0	0	0	0	0.0667	0
ACT45	0	0	0	0	0	0	0	0	0.0667	0
ACT46	0	0	0	0	0	0	0	0	0.0667	0
ACT47	0	0	0	0	0	0	0	0	0.0667	0
ACT48	0	0	0	0	0	0	0	0	0.0667	0
ACT49	0	0	0	0	0	0	0	0	0	0.0588
ACT50	0	0	0	0	0	0	0	0	0	0.0588
ACT51	0	0	0	0	0	0	0	0	0	0.0588
ACT52	0	0	0	0	0	0	0	0	0	0.0588
Sample size	33	15	14	14	18	15	21	17	15	17
# haplotypes	13	9	9	6	7	11	8	7	6	8
# unique	6	6	2	1	1	7	3	4	5	4

LR = Licking River
TR = Thames River
SY = Sydenham River
WH = Walhonding River
SD = Sandusky River

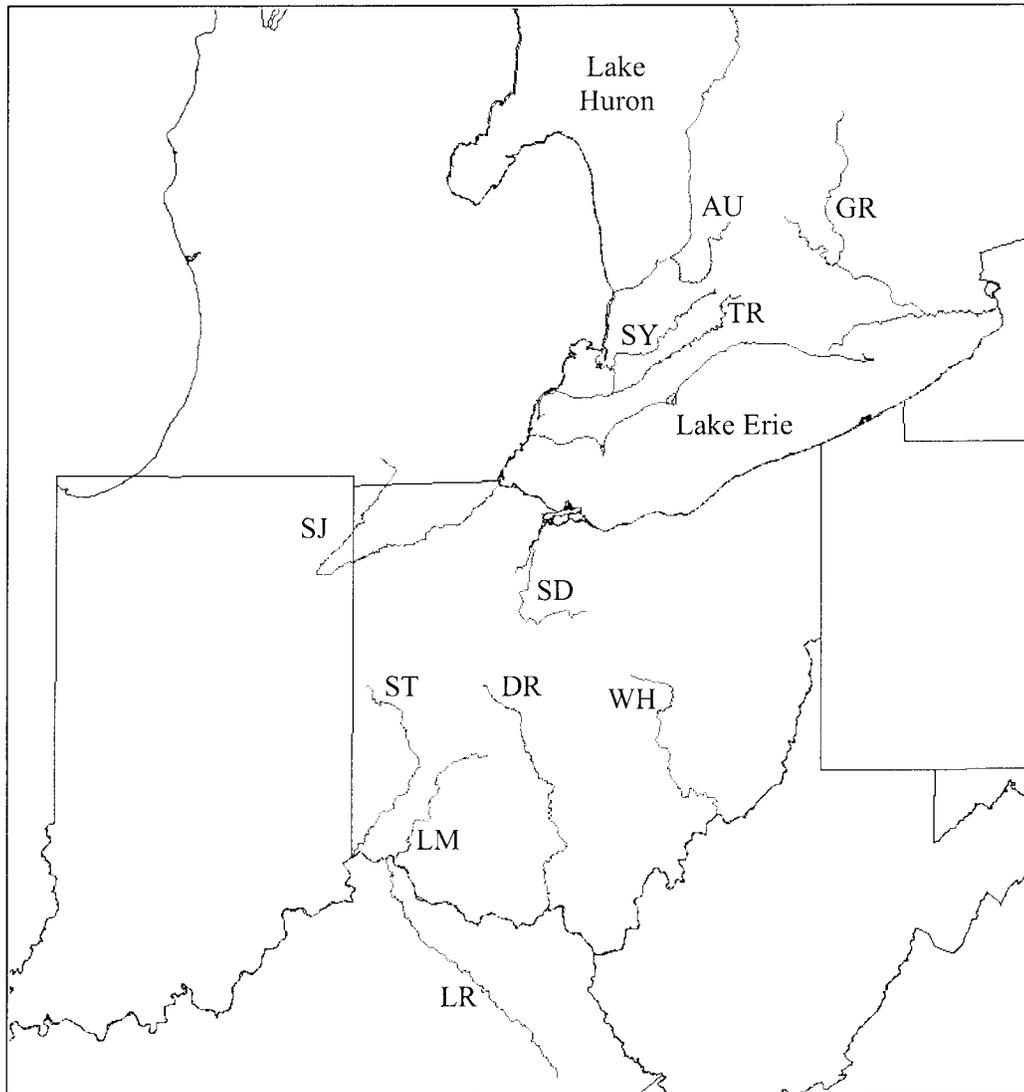


Figure 1. Rivers sampled for this project. River abbreviations are: LR = Licking, LM = Little Miami, ST = Stillwater, DC = Darby Creek, WH = Walhonding, SD = Sandusky, SJ = St. Joseph, SY = Sydenham, TH = Thames, AU = AuSable, GR = Grand.

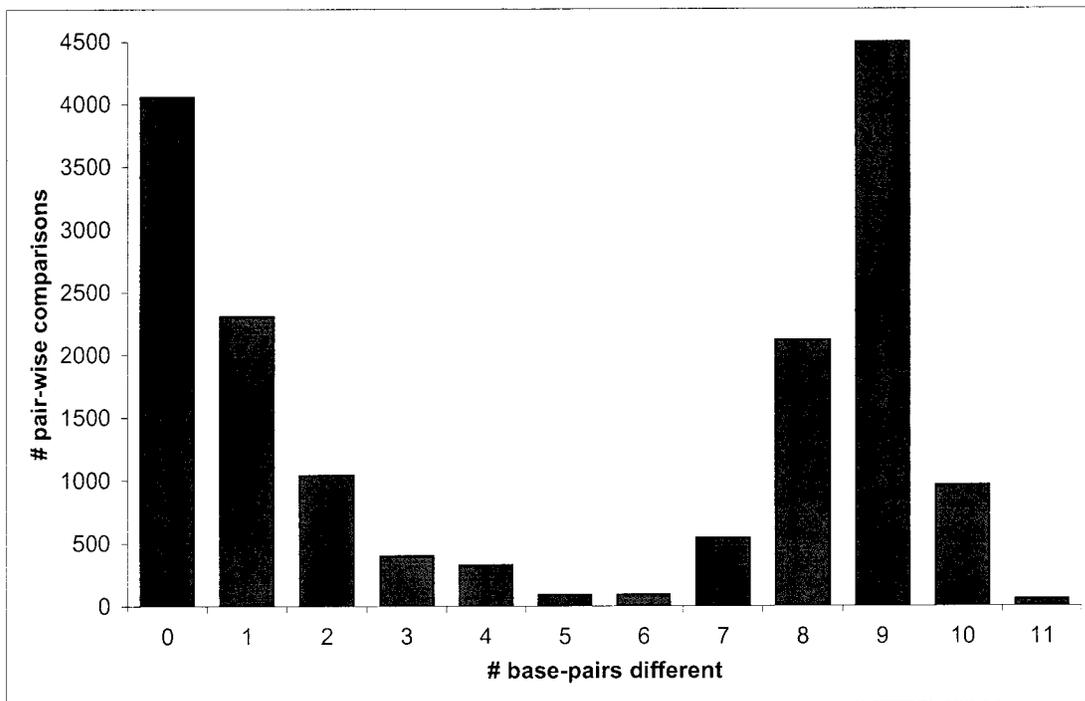


Figure 2. Mismatch distribution for *Lasmigona costata* for all pairwise combinations of individuals.

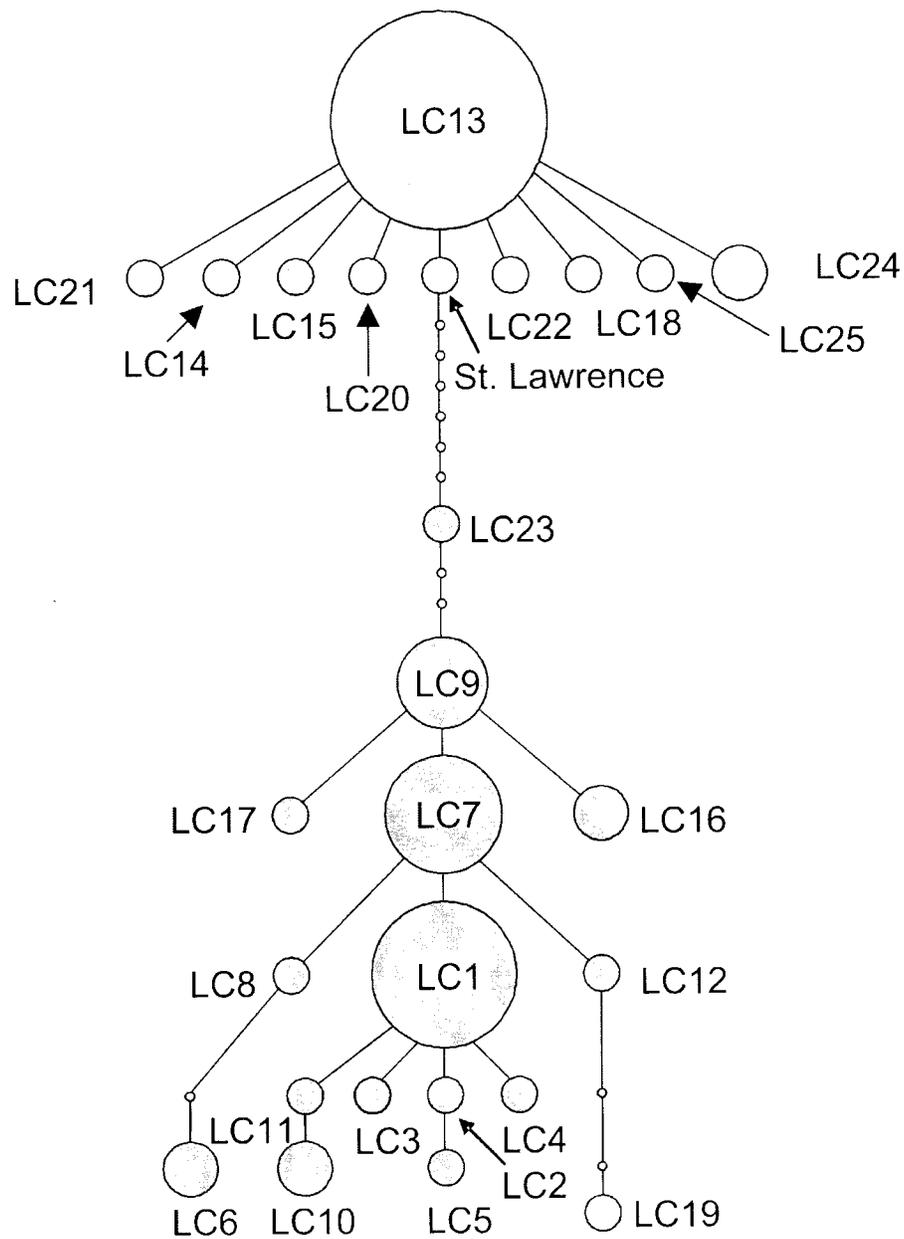


Figure 3. Parsimony network for haplotypes of *Lasmigona costata*. Circles are identified by haplotype name. Each node represents one base-pair difference. Green circles are haplotypes found only in the Lake Erie watershed.

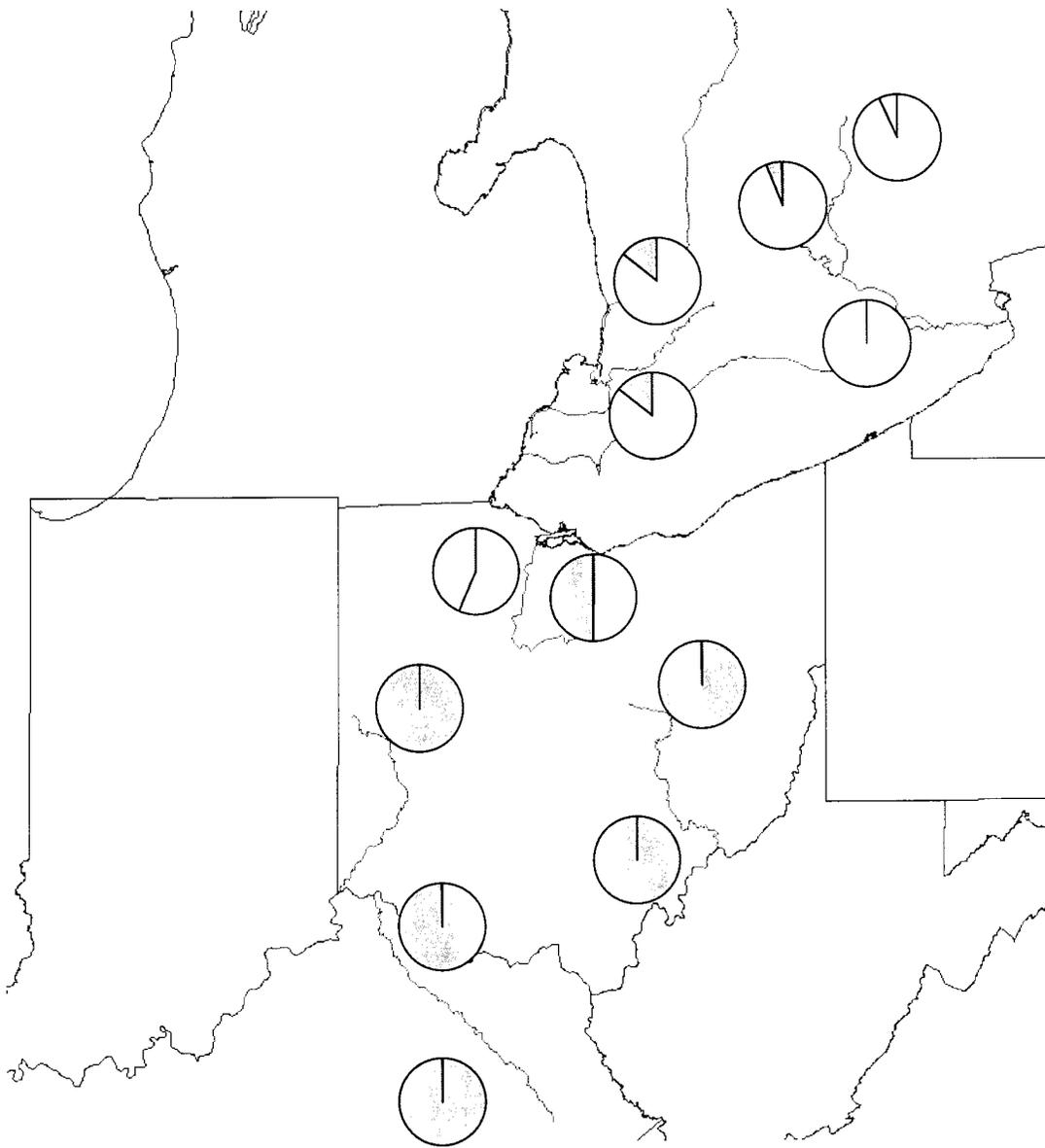


Figure 4. Frequencies of haplotype groups for *Lasmigona costata*. Group colors correspond to those in Figure 2.

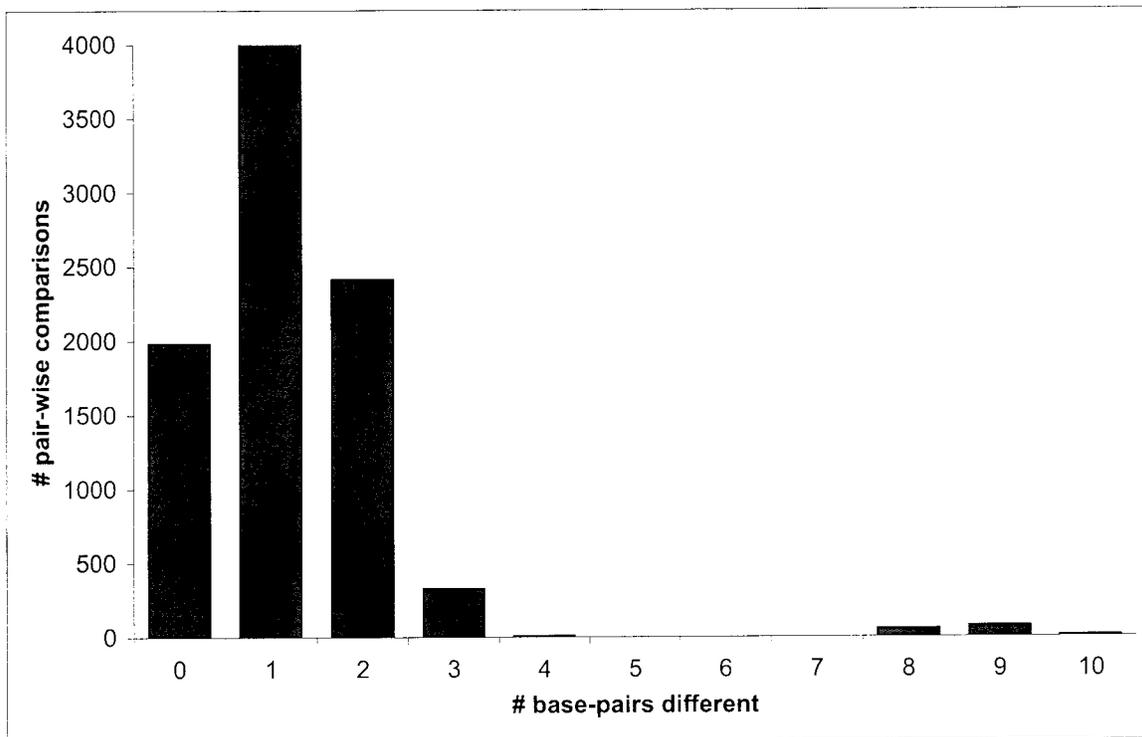


Figure 5. Mismatch distribution for *Elliptio dilatata* for all pairwise combinations of individuals.

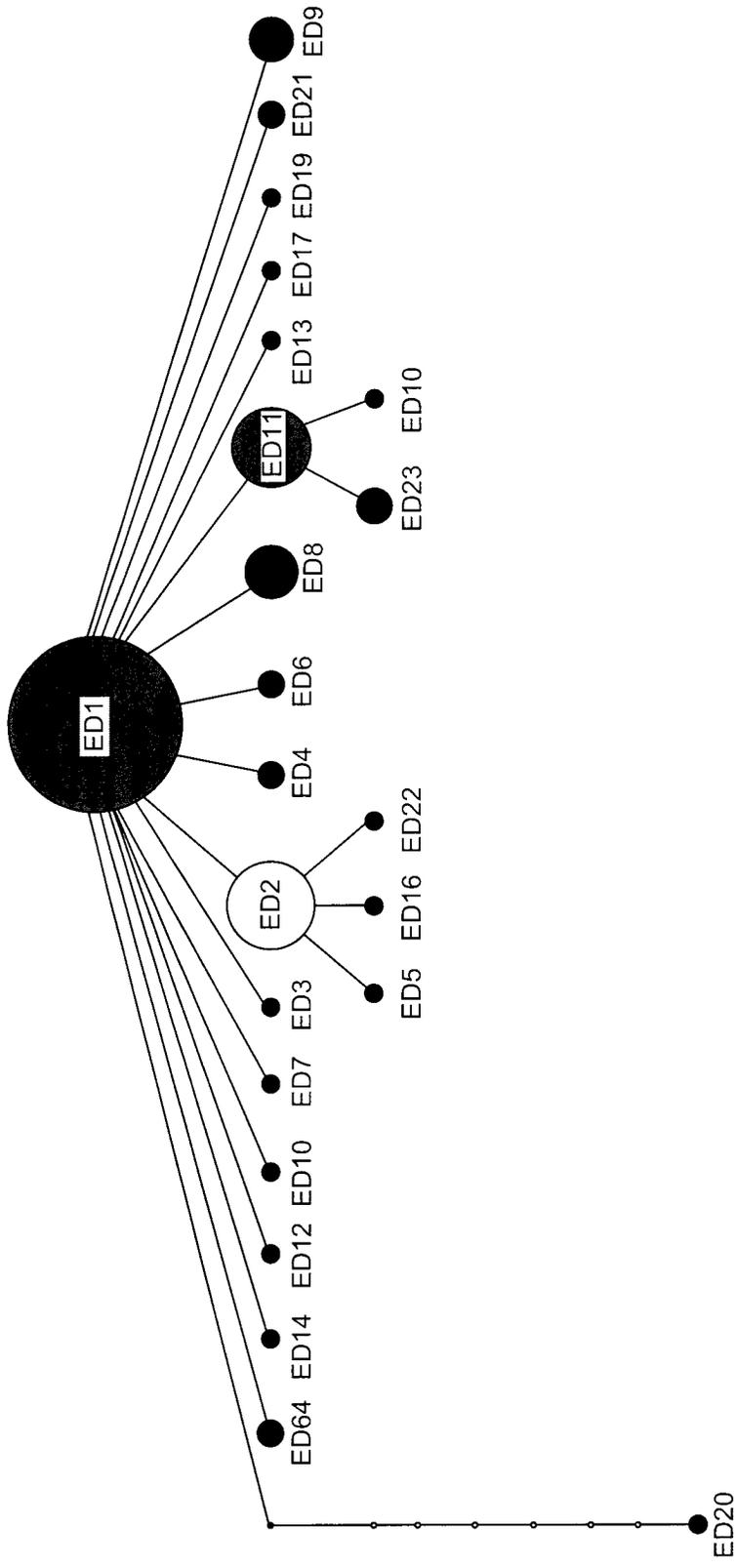


Figure 6. Parsimony network for haplotypes of *Elliptio dilatata*. Circles are identified by haplotype name. Each node represents one base-pair difference.

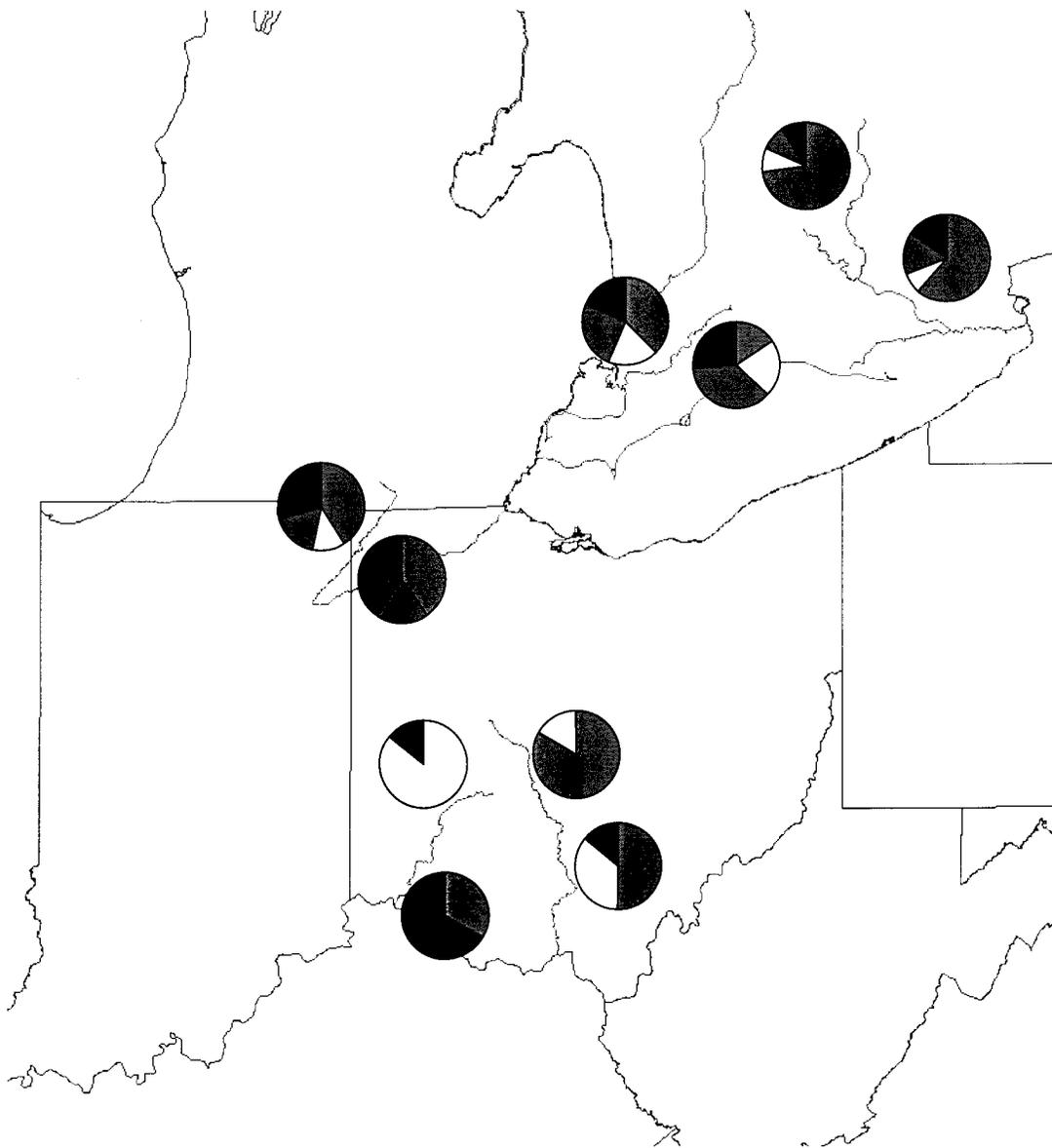


Figure 7. Frequencies of haplotypes for *Elliptio dilatata*. Colors correspond to those in Figure 5.

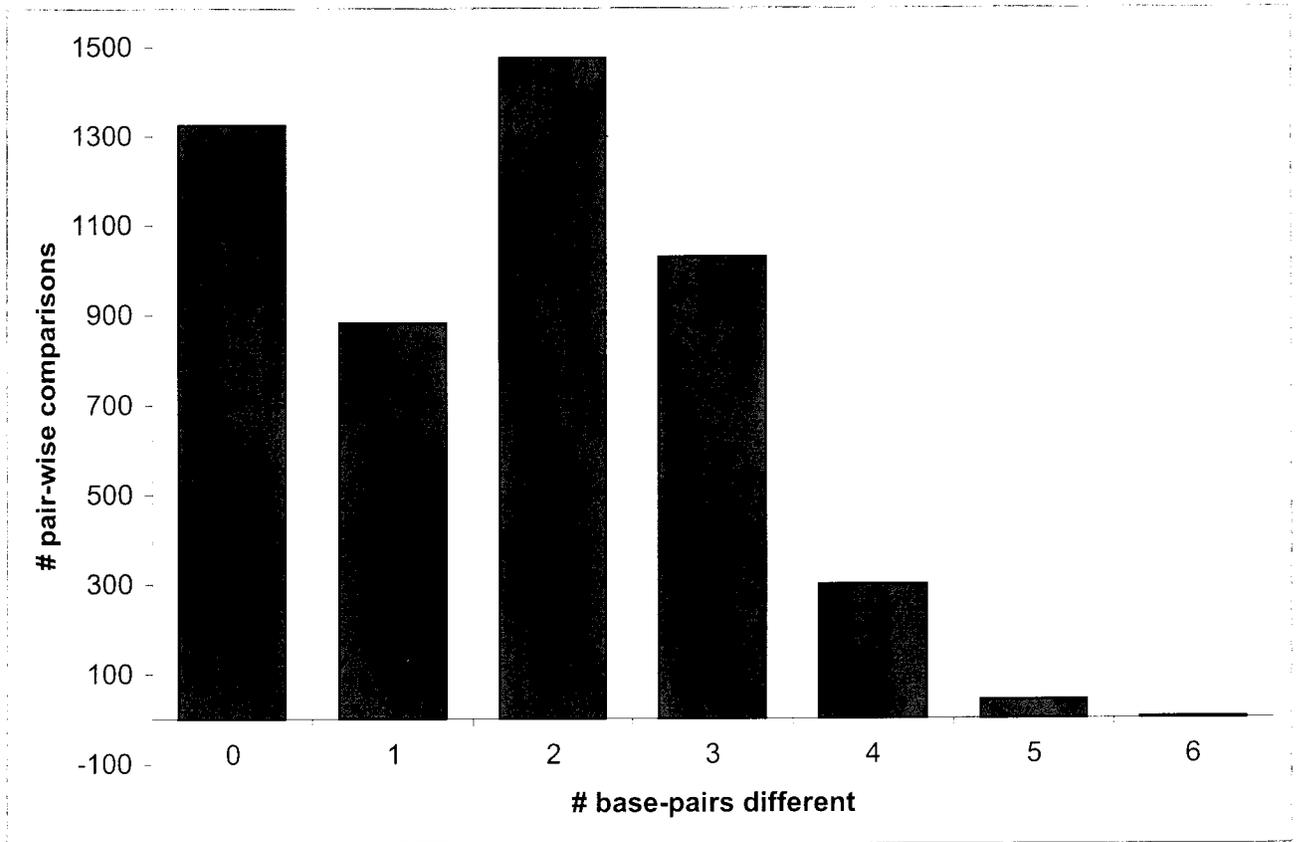


Figure 8. Mismatch distribution for *Amblema plicata* for all pairwise combinations of individuals.

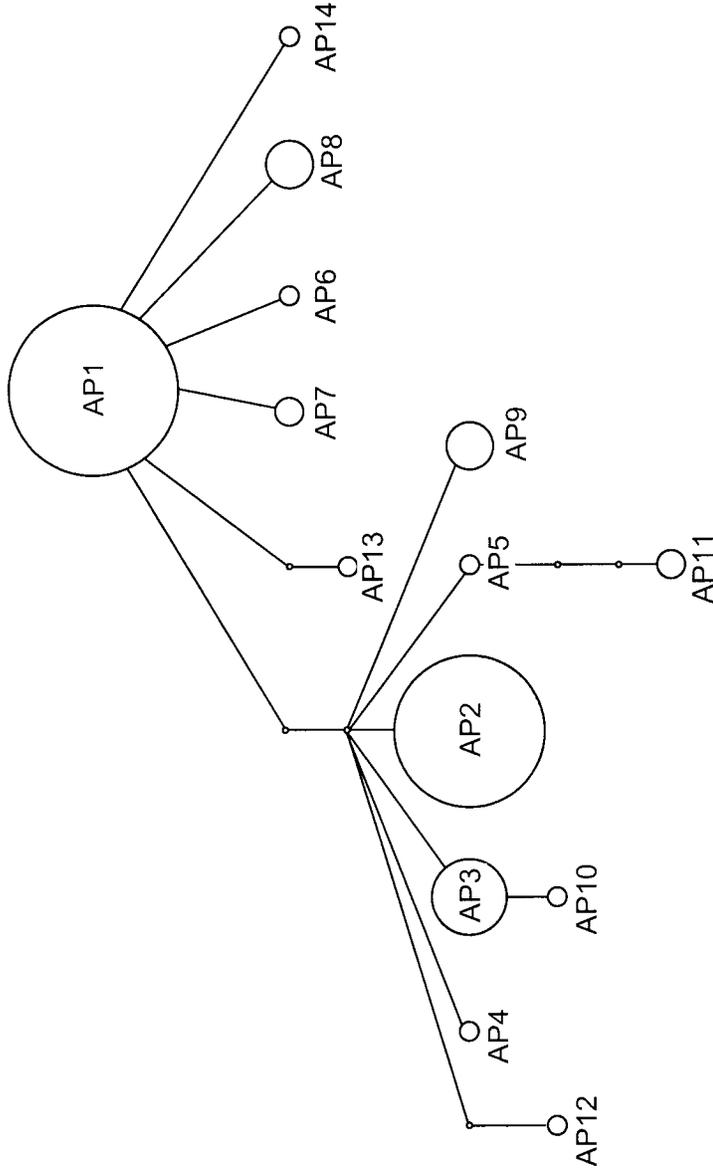


Figure 9. Parsimony network for haplotypes of *Amblema plicata*. Circles are identified by haplotype name. Each node represents one base-pair difference.

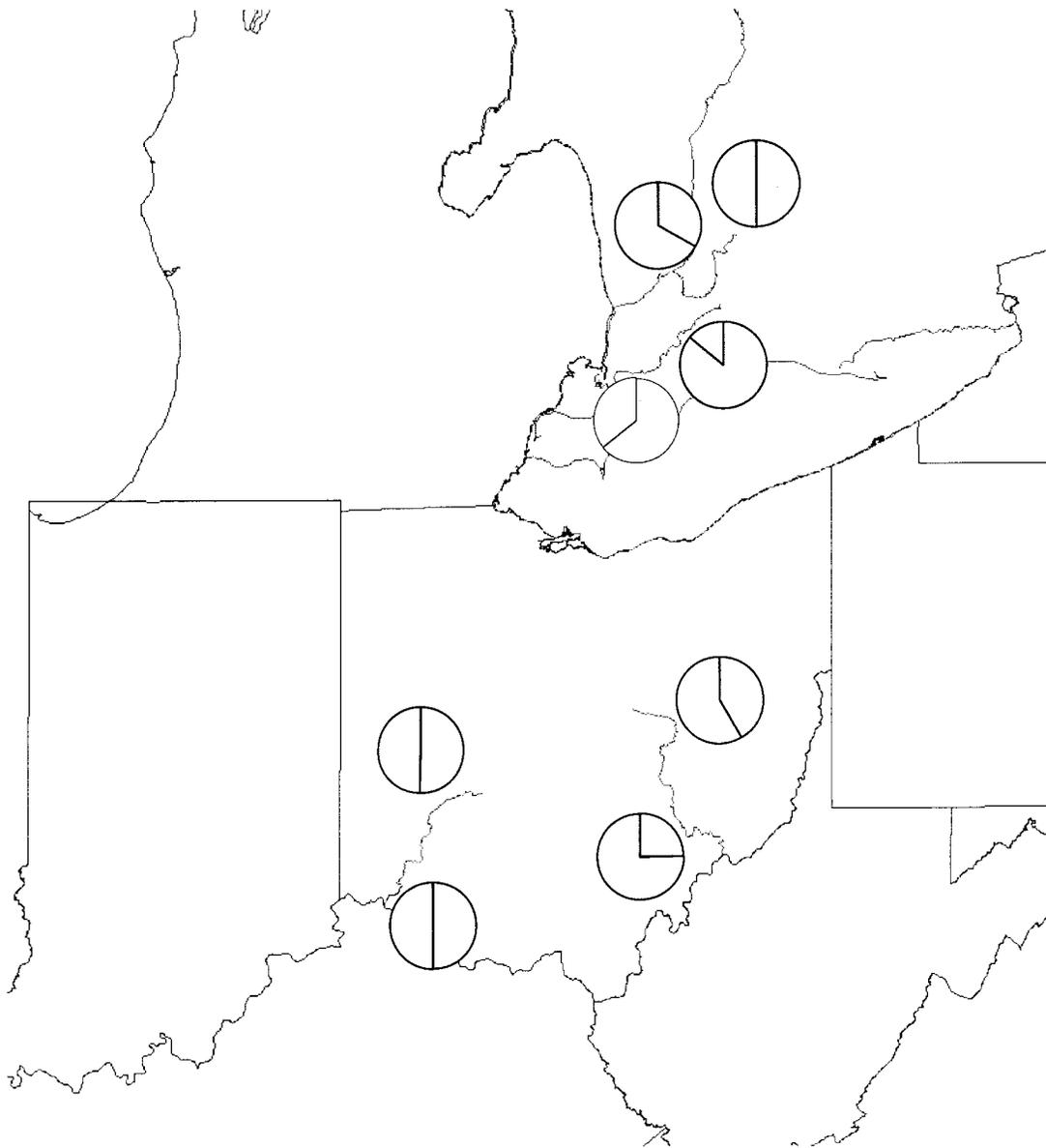


Figure 10. Frequencies of haplotype groups for *Amblema plicata*. Group colors correspond to those in Figure 8.

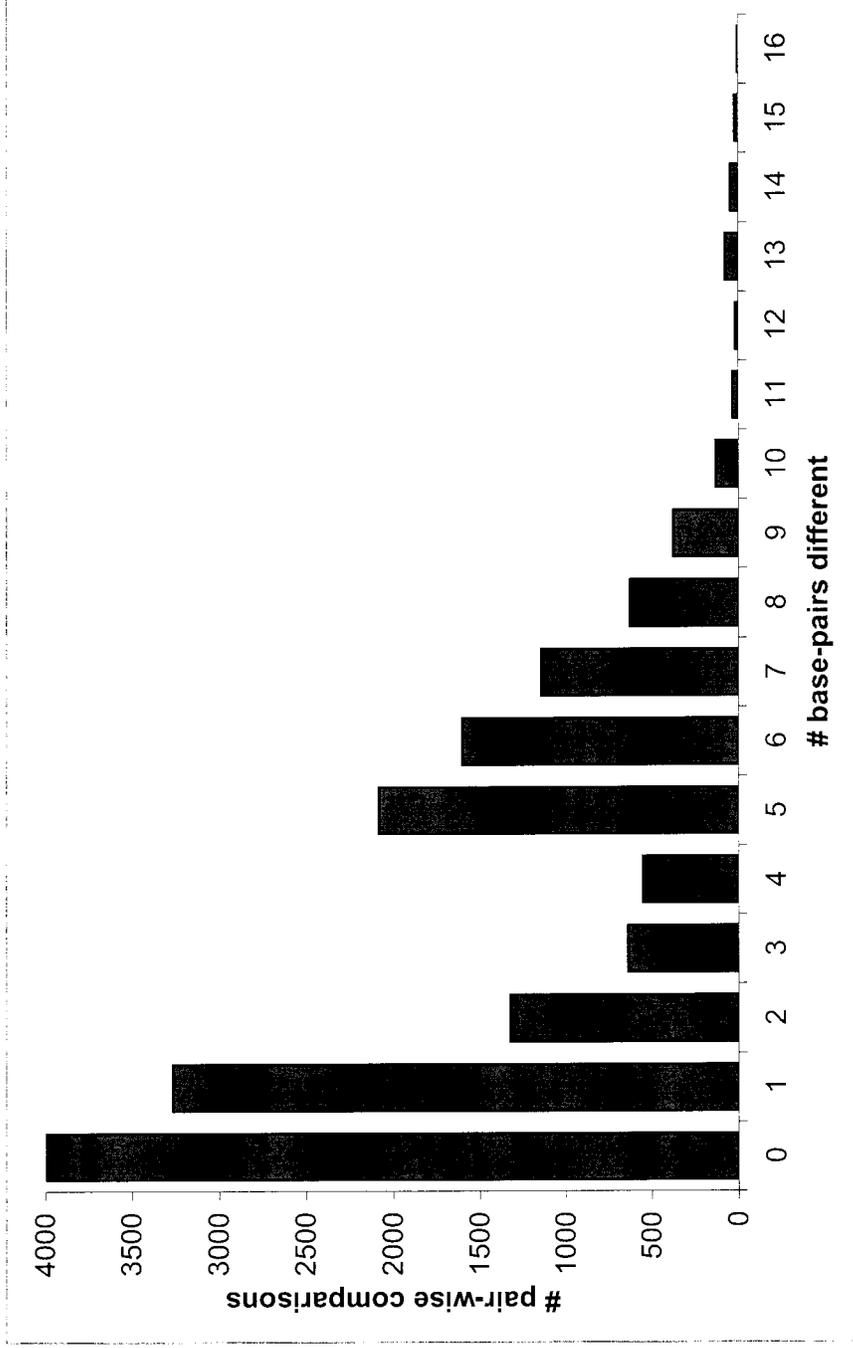


Figure 11. Mismatch distribution for *Actinonaias ligamentina* for all pairwise combinations of individuals.

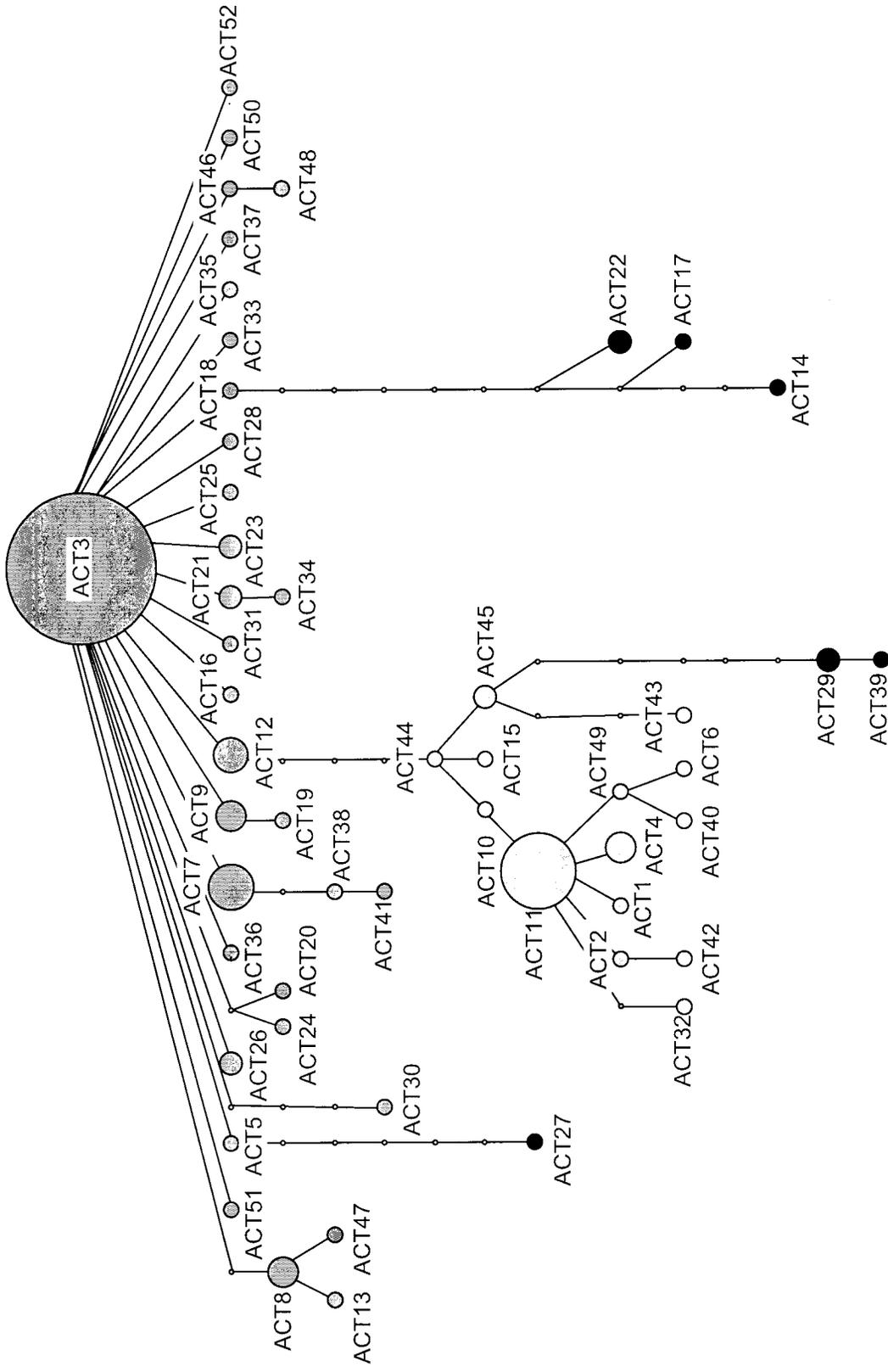


Figure 12. Parsimony network for haplotypes of *Actinonaias ligamentina*. Circles are identified by haplotype name. Each node represents one base-pair difference.

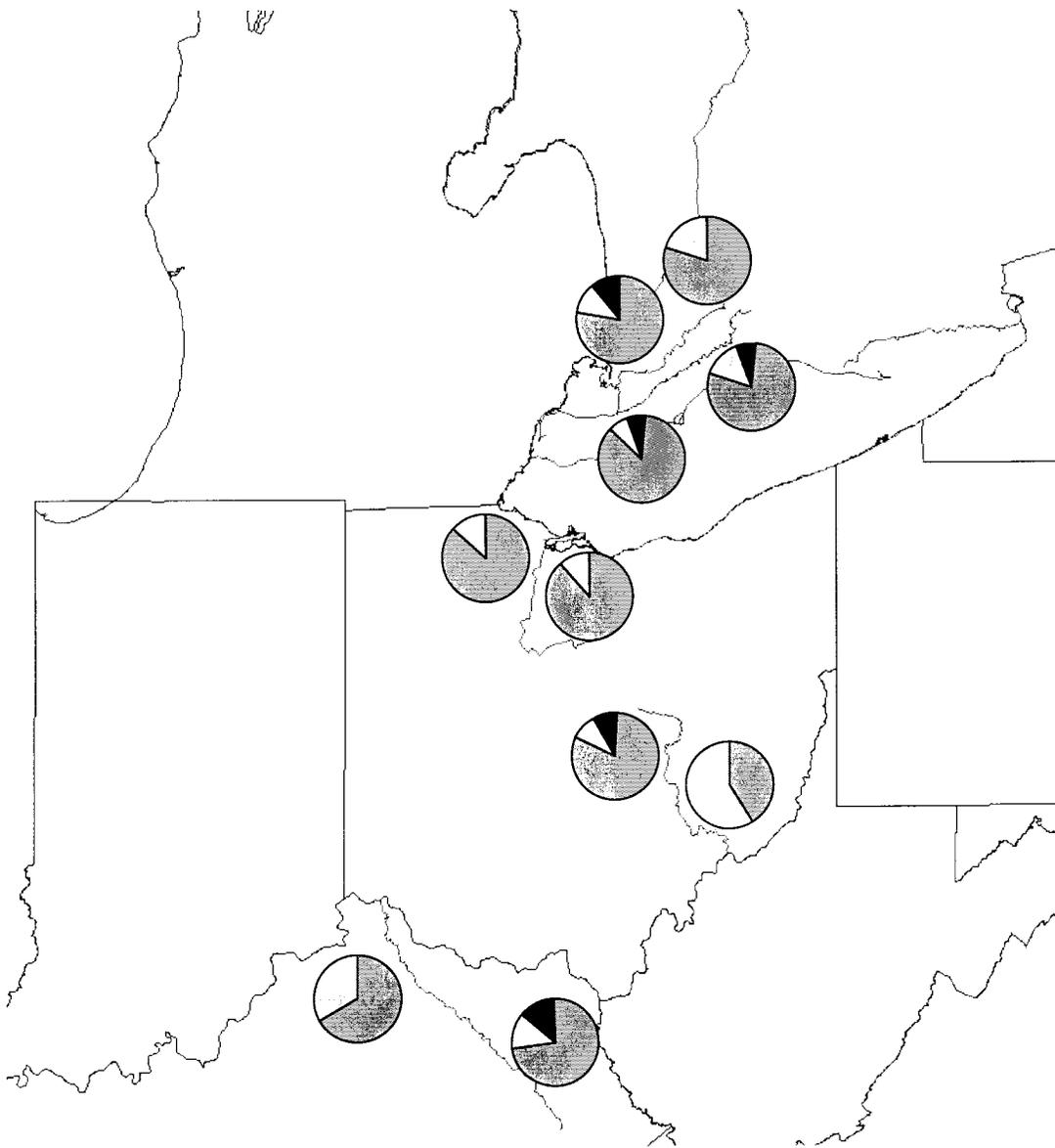


Figure 13. Frequencies of haplotype groups for *Actinonaias ligamentina*. Group colors correspond to those in Figure 11.