Population genetics and conservation of the freshwater mussel *Margaritifera falcata* from the northwestern United States

Lindsay P. Anderson

*The University of Montana*

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Population genetics and conservation of the freshwater mussel

*Margaritifera falcata* from the Northwestern United States

By

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B.A. Macalester College, Minnesota, 1992

Presented in partial fulfillment of the requirements

for the degree of

Master of Science

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Approved by:

*Chairperson*

*Dean, Graduate School*

4-17-02

Date
Freshwater mussels are at great risk for losing genetic variability due to declining and increasingly fragmented habitat for themselves and the fish they depend upon for development and dispersal. Today margaritiferids are declining globally. *Margaritifera falcata* is currently listed as having undetermined conservation status due to the lack of knowledge of this species (Williams *et al.* 1993). Allozyme analysis was used to describe the genetic population structure of the freshwater mussel *Margaritifera falcata* from nine rivers of the Northwestern United States. Of twelve loci examined, six were found to be polymorphic. The mean heterozygosity in populations was 4.2%. Within-population genetic variation accounted for 57% of the total variation observed. Only 2% of the observed genetic differentiation is attributable to differences among populations within a minor drainage. Among-population variation ($F_{ST}$) accounted for 41% of the total variation. Seven of 13 populations had at least one locus that did not meet Hardy-Weinberg expectations and in all cases these loci represented a deficit of heterozygotes. The prevalence of heterozygote deficiency provides evidence for some self-fertilization in these populations. There is very little difference in genetic distances among populations but populations generally appear to fall into three geographical groupings: coastal, mid-basin, and headwaters. Conservation efforts should focus on preserving several different populations due to the relatively high among-population genetic variation.
Acknowledgements

I would like to thank Fred Allendorf, Robb Leary, Kevin Sage, David Tallmon and Kathy Knudsen for their technical and moral support. I would also like to thank Mark and Nancy for making finishing a reality. This project was funded by the Montana Organization for Research in Energy/Department of Energy Fellowship and the generosity of Fred Allendorf.
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INTRODUCTION

Freshwater mussels are at great risk for losing genetic variability due to declining and increasingly fragmented habitat for themselves and the fish they depend upon for development and dispersal. Currently more than 71% of the North American freshwater mussel fauna is considered endangered, threatened or of special concern, while less than 25% are considered stable in their current state (Williams et al. 1993).

The freshwater pearl mussel *Margaritifera falcata* currently has undetermined conservation status (Williams et al. 1993). This species has a broad distribution in the Pacific Northwestern United States (Clarke 1981, Toy 1998, Craig 1994, Stock 1996). Although *Margaritifera falcata* is widely distributed throughout the cold and clear rivers and streams of the west, it appears to have limited genetic diversity overall.

Extinction of unionoids in the United States has been mainly attributed to the impoundment of major rivers (Bogan 1993, Vaughn and Taylor 1999, Williams et al. 1993, Watters 1996, Richter et al. 1997). The presence of dams is a great threat to the long-term proliferation of freshwater mussels (Figure 1). The negative impact of dams in the Pacific Northwest has been clearly demonstrated by the declining populations of
salmonids. This likely affects *Margaritifera falcata* through its dependence on salmonids as hosts during their obligatory parasitic glochidial stage.

Dams create particular hardships for freshwater mussels for several reasons. For lotic species, they impound flowing habitat, greatly reducing water velocities as well as inundating diverse substrates with fine sediments (Bogan 1993). This is not only detrimental for the mussels but also for the lotic (and sometimes anadromous) fish species that they rely upon as hosts (Watters 1996). The fish are unable to move freely within the river system providing dissemination (gene flow through migration) of the mussels. Appropriate spawning habitat for the lotic fish is also lost or impaired. Mussels downstream of the dam are subjected to scouring effects from the outflow, which can create unstable substrates as well as inundation of fine sediments further downstream. Unnatural flow regimes in the dam outflow are also detrimental to mussels, as their life history strategies are adapted to a natural hydrograph (Payne and Miller 2000).

*Margaritifera falcata* (Western pearlshell) is a member of the Family Margaritiferidae, which is represented by 5 North American species (Turgeon et al. 1998). Unlike many other unionoid genera, *Margaritifera*...
has a holarctic representation. Most of our knowledge of the family comes from Europe where *Margaritifera margaritifera* was once very abundant (Beasley and Roberts 1999, Buddensiek 1995, Bauer 1987, Araujo and Ramos 2000). Today margaritiferids are declining globally. *Margaritifera falcata* is currently listed as having undetermined conservation status due to the lack of knowledge of this species (Williams *et al.* 1993).

The objectives of this study are to:

1. describe the population genetic structure of *Margaritifera falcata* from the Columbia River basin and 2 adjacent basins;

2. Determine the spatial relationship of the genetic variation between populations.

Through a better understanding of the population genetic structure of this species, we can begin to manage *Margaritifera falcata* in a way that will preserve its genetic diversity.

**Distribution of *Margaritifera falcata***

The range of *Margaritifera falcata* is from California and New Mexico to the southern interior of British Columbia to Revillagigedo Island, Alaska.
(Clarke 1981) (Figure 1). The eastern extent of the range is primarily contained by the Rocky Mountain Range. However, there are limited populations recorded from the Upper Missouri River (Stober 1972), most likely introduced through fish stocking.

Habitat

*Margaritifera falcata* is typically found in cold, clear and highly oxygenated streams and rivers (Roscoe and Redelings 1964, Stock 1996). Because of their dependence on their salmonid hosts, they are often found in trout and salmon habitats. Substrate stability is essential for long-term viability of mussel beds in these high gradient systems (Vannote and Minshall 1982, Stock 1996). Stock (1996) also noted the abundance of juvenile salmonids in and around the mussel beds.

Adult *Margaritifera falcata* are unable to escape any inundation with sediments (Vannote and Minshall 1982). The authors found several relic (subfossil) populations in the Snake River of Idaho where entire beds of *M. falcata* had been buried under several centimeters of sediment and died with no evidence of vertical movement. Vannote and Minshall (1982) found that under lab conditions, adult *M. falcata* would not vertically migrate to avoid inundation with sediments, whereas, juveniles would slowly migrate (0.2 cm·hr⁻¹) vertically.
Stock (1996) clearly discusses the similarities between juvenile salmonid habitat and *M. falcata* habitat. Where juvenile salmonids use cobbles, boulders and logs for in-stream cover, mussels need them for substrate stability. Both groups prefer slower water velocities than are found in the thalweg. These strong associations would be expected due to the host-parasite relationship between the salmonids and *M. falcata*. It appears that the habitat of *M. falcata* is most likely dictated by its host’s habitat preference (Stock 1996).

**Life History**

Most freshwater mussels are dioecious and become sexually mature at one to eight years of age (McMahon 1991). However, sexual maturity in *margaritifera* species has been estimated at anywhere from 9 to 20 years depending on their growth rate (Smith 1976; Young and Williams 1984; Bauer 1987; Toy 1998). *Margaritifera margaritifera* has been characterized as highly fecund and long-lived resulting in high reproductive potential during its lifetime (Bauer 1998). Toy (1998) found that *Margaritifera falcata* from a Western Washington stream, reached 100% reproductive success when densities reached 40 mussels/m² or greater. None of the populations in the current study had population densities this high.
Margaritifera falcata is capable of simultaneous hermaphroditism (Heard 1970, Van Der Schalie 1970) although the occurrence has generally been believed to be rare (Smith 1979). Bauer (1987) has demonstrated in the closely related Margaritifera margaritifera that mode of reproduction (dioecious vs. hermaphroditic) is density dependent. Under low densities Bauer (1987) found that female Margaritifera margaritifera will become hermaphrodites. Nearly all of the hermaphroditic specimens that Bauer described had predominantly female gonadal tissue and most likely used the limited sperm produced for self-fertilization. However, even in very low-density populations, some pure males still existed. Bauer points out that the presence of some obligately cross-fertilizing males in the population will reduce the homogenizing effects of self-fertilization. Smith (1979) examined several populations of M. margaritifera from New England and found no evidence of hermaphroditism.

Freshwater mussels can exhibit two different reproductive strategies, tachytictia or bradytictia. Bradytictia is a long term breeding strategy where the females hold the fertilized eggs within the gill marsupium over the winter and release the glochidia in the spring. Tachytictia is a short term breeding strategy well adapted to a longer growing season and
warmer water temperatures (Graf 1997, Kat 1984). Tachytictic mussels typically breed in the spring and/or early summer and release the mature glochidia in late summer/fall for attachment on the host. *Margaritifera falcata* is a tachytictic breeder. Although the rivers inhabited by *Margaritifera falcata* are not warm water streams, there is likely another strategy involved. It is most advantageous to the mussels to be ready to release their glochidia when the host fish are present in the greatest numbers, and are most susceptible to infection. In the Pacific Northwest, one might expect that this would coincide with spawning migrations or hatching of salmonids.

The young mussels are dependent upon the fish host not only for development but also for dispersal. The reliance upon the host fish is necessary if the mussels are to be dispersed upstream. The host fish relationship poses many interesting questions regarding the distribution of mussels and the genetic implications therein. Due to the complexity of the reproductive strategies of freshwater mussels there are many points at which the process is vulnerable to failure.

There are several mechanisms within this system that can cause reproductive isolation. First they must be in close proximity and in great enough densities to successfully sexually reproduce. Second, there must
be host fish available for parasitism to occur. Finally, the parent must release the glochidia in the presence of the appropriate host fish.

**Genetics**

Previous work by several authors has shown that freshwater mussels can exhibit reduced genetic diversity, especially in peripheral populations. It has been reported by Davis *et al.* (1981), that *Margaritifera margaritifera* is highly monomorphic with heterozygosity ($H$) values as low as 0.03 and proportion of polymorphic loci ($P$) around 0.14. Kat (1982) examined populations, peripheral to their range, of two species of Unionidae and discovered reduced heterozygosity in these populations compared to populations more centrally located within their range. Kat (1982) attributed this finding to the loss of alternative alleles in peripheral populations. The low levels of heterozygosity in peripheral populations would be expected due to the effects of genetic drift and natural selection (Lesica and Allendorf 1995). The combined effect of drift and selection can further increase divergence from centrally located populations. Lesica and Allendorf (1995) also predict that peripheral populations that experience self-fertilization will have reduced gene flow among populations causing increased among-population differentiation.
Kat (1983) found that many typically dioecious species (30 of 101 species examined) occasionally have some simultaneous hermaphrodites. He attributes this phenomenon to a few potential causes; the presence of parasitic trematodes which alter the mussels’ response to sexual hormones, actual hormone levels or a developmental disorder. Kat did find a strong correlation between mussel density and male: female gonad ratios, where male gonadal allocation was significantly higher in low-density populations.

Berg and others (1998) examined the population genetic structure of several populations of Quadrula quadrula from the central United States. They found within-population genetic variation to be relatively high compared to other unionoids ($P=0.61, H=0.24$), whereas among-population genetic variation was quite low. Berg et al. (1998) did find a significant positive correlation between genetic distance and geographic distance.

Nagel (2000) examined European populations of Unio pictorum and found high among-population differentiation overall. The population genetic structure of the central European populations studied reflected paleogeographical river relationships. King and others (1999) described the phylogeography of Lasmigona species from the Atlantic slope. They
found a substantial discontinuity between northern and southern populations and concluded that they should be treated as Evolutionarily Significant Units (ESU).

Hoeh and others (1998) compared the population genetic structure of several species of *Utterbackia* believed to exhibit varying reproductive strategies. Two of the species they examined were simultaneous hermaphrodites while the other two congeners were not. Low levels of heterozygosity and severe heterozygote deficiency in comparison to the outcrossing species characterized the hermaphroditic species. Several of the populations of the hermaphroditic species showed no genetic variation. The widespread occurrence of heterozygote deficiency among hermaphroditic populations and its absence in outcrossing populations suggests that it is a result of self-fertilization and not of sampling error (Wahlund effect). A small effective population size \((N_e)\) would result in reduced heterozygosity in a population and under specific conditions such as a population being founded by self-fertilization of a single homozygous individual (an extreme case), would lead to complete monomorphism. The variance in fecundity of freshwater mussels likely plays a major role in the estimation of effective population size. Individuals can produce millions of eggs at a time and the chance involved in cross-fertilization could cause uneven distribution of
reproductive success. If populations are self-fertilizing, the variance in fecundity is almost certainly much greater than a cross-fertilizing population. A single individual undergoing self-fertilization could be the sole contributor to the next generation. Further, the long life span and high fecundity of individuals can lead to a reduction in \( N_e \) due to uneven contribution among individuals and sexes.

The ability of *Margaritifera falcata* to self-fertilize could potentially lead to reduced genetic diversity, especially in isolated sub-populations. Genetic theory predicts that long-term self-fertilization would lead to the decay of genetic diversity within a population, but could result in increased among population variation due to the presence of rare alleles in sub-populations. Self-fertilization may be an effective strategy for range expansion and may explain the holarctic distribution of Margaritiferids. The resulting loss of genetic variation may lead to the demise of the species due to a lack of evolutionary options. That is, the reduction in genetic diversity may effect the ability of *Margaritifera falcata* to respond to environmental changes such as habitat degradation or ecological changes such as shifts in fish community structure.
I used starch gel electrophoresis to examine enzyme variation in 11 populations of *Margaritifera falcata* from the Columbia drainage basin, one population from the Klamath drainage basin and one population from the Malheur (Harney) drainage basin. Sampling sites were selected based on the following criteria: presence of *Margaritifera falcata*, geographical distribution of site within (or in relation to) the Columbia watershed (Figure 2), access to sampling site, and depth of water. Upon locating a potential sampling site, a general survey was conducted to assess local population size and mussel size (a general proxy for age) distribution within the local population.

Population number, sample size, location, individual size range, mean individual size and collection dates are provided in Table 1. All populations are lotic environments.

**Sample Collection**

Samples were collected by hand while wading and snorkeling. Individuals were chosen to represent the size distribution within the sampling area. Samples were measured (shell length) then dissected streamside and the mantle tissue was removed, rinsed with distilled water and frozen on dry
ice. All samples were stored at -40 degrees Celsius in the lab. All samples were sub-sampled (mantle tissue), thawed, homogenized and run on horizontal starch gels.

**Electrophoresis**

I compared the protein products of 12 gene loci using horizontal starch gel electrophoresis in accordance with the methods described by Leary and Book (1990) and Aebersold *et al.* (1987). The buffer and staining systems were those of Allendorf *et al.* (1977) with the exception of the SR gel buffer, which was prepared without the addition of the 1% RW tray buffer. Gel buffers were: AC (Clayton and Tretiak 1972) pH 6.5-6.7; RW (Ridgway *et al.* 1970) pH 8.0-8.3; SR (Gall and Bentley 1981) pH 8.1; MF (Markert and Faulhaber 1965) pH 8.7. An electrophoretic survey of 27 enzymes and 4 buffer combinations was conducted to identify enzyme-coding loci. Twelve loci (relative mobilities of alleles in parentheses) provided consistent resolution and were included in analysis: *AAT* (100), *FUM* (100, 110), *G3PDH* (100), *HEX* (100), *HK* (100, 125), *LGG* (100, 120), *MDH* (100, 50), *PGK* (100), *PGM* (100, 80), *PK* (100), *6PGDH* (-100, -87) and *SOD* (100, 125) (Table 2).
The Blackfoot River (ID) population was used to determine which tissue gave the most consistent results. I determined that the mantle tissue was the most dependable as well as being readily accessible.

Enzyme mobilities were measured by comparison to the most common allele across all populations. A standard was run from the same individual (from the St. Joe River Midstream population) on all gels for comparison.

**Data Analysis**

Statistical analyses of the allozyme data were conducted using BIOSYS-1 (Swofford and Selander 1981), LINKDIS (Black 1997) and TFPGA (Tools For Population Genetics Analysis)(Miller 1997). The BIOSYS-1 statistical package was used to calculate mean number of alleles per locus, $F_{IS}$, Fisher’s exact test and Wright’s $F$ statistics. Miller’s TFPGA (1997) was used to calculate heterozygosities, proportion of polymorphic loci and Nei’s genetic distance. LINKDIS was used to calculate linkage disequilibrium. The Mantel tests were conducted using Miller’s TFPGA (1997). The Mantel test was performed to determine if there was a significant relationship between genetic distance (Nei’s D) and geographic distance (river kilometers separating populations). Estimation of out-
crossing rates (Weir 1990) were determined using: \[ t = \frac{(1-F_{is})}{(1+F_{is})} \]. It then follows that the self-fertilization rate is equal to 1-\( t \).

**RESULTS**

**Individual Size Structure of Populations**

None of the populations sampled in this study exhibited the high densities and large beds that margaritiferids are notorious for (Vannote and Minshall 1982, Bauer 1987, Young and Williams 1984). Although the populations were not quantitatively surveyed for age/size diversity, most of the populations in this study had low diversity in age/size of individuals (Table 1). Nearly all of the mussels found were probably of reproductive age (>5cm according to Toy 1998). It should be noted that the survey techniques employed in this study were biased toward adult mussels. This bias is due to the visual survey methods, which tend to overlook juveniles that may be buried in the sediments.

**Genetics**

Of the 12 loci analyzed, six were polymorphic: \textit{FUM}, \textit{HK}, \textit{LGG}, \textit{MDH}, \textit{PGM} and \textit{6PGDH}. Allelic frequencies of all loci are provided in Table 2.
Of the 13 populations sampled, three showed no genetic variability (Yaak River Downstream, Yaak River Midstream, and Yaak River Upstream) (Figure 4). The Blackfoot River and Monture Creek populations were variable only at the LGG locus; both of these populations had small sample sizes (n=10).

Mean observed heterozygosity ($H_o$) for all populations was 0.042 with values ranging from 0 to 0.105 (Table 1). Mean expected heterozygosity ($H_e$) for all populations was 0.071 with values ranging from 0 to 0.162 (Table 1). The mean number of alleles per locus ranged from 1.0 to 1.5. Seven of 13 populations had at least one locus that did not meet Hardy-Weinberg expectations (Fisher's Exact Test, $p<0.05$, Table 2). In all cases these loci represented a deficit of heterozygotes ($F_{IS}>0$, Table 2). Seven of 13 populations had self-fertilization rates greater than 0.2 (Table 3).

Within-population genetic variation accounted for 57% of the total variation observed. Only 2% of the observed genetic differentiation was attributable to differences among populations within a minor drainage. All of this variation is due to the St. Joe River populations since the Yaak River populations showed no genetic variation. Among-population
variation ($F_{ST}$) accounted for 41% of the total variation with mean $F_{ST}=0.414$ for all loci.

The results of a Mantel Test (Miller 1997) showed no significant correlation between genetic distance and geographic distance ($r=-0.169$, $n=36$, $p_u=0.852$, $p_l=0.149$). When the Myrtle Creek population, which resides in a closed basin, was eliminated from the analysis, the negative correlation improved only slightly ($r=-0.269$, $n=27$, $p_u=0.862$, $p_l=0.139$).

Results of a linkage disequilibrium test (LINKDIS Black 1997) showed no significant linkage disequilibrium in any of the samples.

Nei's genetic distances are low overall with values ranging from 0 to 0.160 (Table 6, Figure 5). The results of the Cluster Analysis using unweighted pair group methods sorts populations into 4 distinct subgroups (Figure 5): a coastal group (Wenatchee River, Canyon Creek and Williamson River), a central group (St. Joe River and Myrtle Creek), an eastern group (Yaak River, Monture Creek and Blackfoot River) and the West Fork Sanpoil River.
**DISCUSSION**

**Population Genetics**

Heterozygosities were quite low for *Margaritifera falcata* (*H*=0.042) compared to other freshwater mussels. This is not a surprise as Davis and others (1981) found *Margaritifera margaritifera*, a sister species, to be highly monomorphic with mean heterozygosity around 0.03. Low levels of heterozygosity are due to recent population bottlenecks, inbreeding or small *N_e*. As previously discussed, self-fertilizing populations are often characterized by small *N_e* due to a high variance in fecundity.

The life history strategy of *Margaritifera falcata* is likely to result in small *N_e* due to several factors. The dispersal mechanism of the host fish could potentially drop the glochidia of full siblings (or self-fertilized progeny) in an area forming a new population with a very small *N_e*. Unequal parental contribution due to poor reproductive success (especially among outcrossing individuals) could lead to a small *N_e*. There is still very little known about reproductive efficiency of mussels in rivers, that is how close together do mating individuals have to be and how long is sperm viable in the water column. These issues could result in very small neighborhood size and thus a small *N_e*.  

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The results of this study exhibit strong evidence that 8 of the 13 populations are undergoing some degree of self-fertilization (Table 3). The remaining 5 populations are either monomorphic (Yaak River) or exhibit very low levels of polymorphism (Monture Creek and Blackfoot River). The lack of variation in these remaining populations makes it impossible to discern whether it is due to self-fertilization, small $N_e$ or genetic drift.

The reduced heterozygosity in the peripheral populations (near the eastern extent of the range) of this study (Monture Creek, Blackfoot River, and Yaak River) corroborates Kat's (1982) findings in Nova Scotian Unionids, as well as predictions based on theory. The peripheral populations in this study do not appear to have any rare alleles, however all of the "peripheral" populations (all Yaak River, Monture Creek and Blackfoot River) are fixed for the alternative allele at the $6PGDH$ locus (Table 2).

Although the Monture Creek and Blackfoot River populations do not meet the criteria for self-fertilizing populations, I believe that their reduced genetic variability is indicative of self-fertilization. The Monture Creek population is a very small population with a small size range (7.9-
9.1cm corresponding to ~30-40 years old (Toy 1998)), indicating that it may be a remnant population that is not successfully reproducing.

It is not surprising that among-population variation is so high among these populations of *Margaritifera falcata*. Population genetics theory would predict that the regular practice of self-fertilization could propagate the spread of rare alleles within a population, causing increasing divergence among populations. This may explain the presence of alternative alleles in single populations (Table 1) such as the *PGM* locus in the Wenatchee River population and the *SOD* locus in the West Fork Sanpoil River population.

The act of self-fertilization is not without advantages. Simultaneous hermaphroditism facilitates range expansion and allows a population to persist and grow until the population is large enough to support cross-fertilization (Bauer 1987). The ability to self-fertilize may also facilitate post-disturbance recovery (McMahon 1991). The ability of *Margaritifera falcata* (and its congener *Margaritifera margaritifera*) to self-fertilize, has likely facilitated the holarctic distribution of the genus. Founder effects in self-fertilizing populations are great and can result in little or no variation within a population making the population highly susceptible to both natural and anthropogenic disturbance.
Conservation Strategies

Because of the relatively high among-population variation of *Margaritifera falcata* from the Columbia River Basin, it is important that any conservation strategy take into account this genetic population structure. Maintenance of several populations in different watersheds would be the best strategy for maintaining the genetic diversity of *Margaritifera falcata*. The goals of any conservation program need to address the maintenance of existing genetic diversity in order to avoid the extinction of locally adapted populations.

The removal of dams throughout the range of *Margaritifera falcata* would facilitate increased natural gene flow among populations and would allow re-colonization of habitats from which the species has been extirpated. The reliance of *Margaritifera falcata* upon anadromous salmonids as host fish makes them even more susceptible to the abundance of dams throughout their range.

The low levels of heterozygosity and high inbreeding coefficients lead me to believe that *Margaritifera falcata* is holding on in the Pacific northwest by its ability to reproduce hermaphroditically. The abundance of large
mussels in all of the populations surveyed indicates that the populations are relatively old and may be experiencing very low, if any, recruitment.
Literature Cited


Murphy, G. 1942. Relationship of the fresh-water mussel to trout in the Truckee River. California Fish and Game 28:89-102.


Table 1. Population reference number, name, major drainage basin, UTM coordinate, sample size, date of collection, individual size range and mean individual size.

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<th>Ref. Number</th>
<th>Population Name</th>
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<th>Mean Individual Size (cm)</th>
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</tr>
<tr>
<td>12</td>
<td>Blackfoot River</td>
<td>Upper Snake</td>
<td>12 470083E 4737750N</td>
<td>10</td>
<td>5.8-9.1</td>
<td>7.8</td>
<td>9/95</td>
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<tr>
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<td>Monture Creek</td>
<td>Clark Fork</td>
<td>12 332781E 5211938N</td>
<td>10</td>
<td>7.9-9.1</td>
<td>8.4</td>
<td>7/96</td>
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Table 2. Allele frequencies, % polymorphic loci, expected and observed heterozygosities at all loci.

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<th>Locus</th>
<th>Allele</th>
<th>Population</th>
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<td>CC</td>
<td>WER</td>
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<tr>
<td>FUM</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>MDH</td>
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<tr>
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<td>0.472</td>
</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>6PGDH</td>
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<tr>
<td>SOD</td>
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</tr>
<tr>
<td>Number of samples</td>
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<td>% Polymorphic loci</td>
<td>33.3</td>
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<td>Mean H_e</td>
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<tr>
<td>Mean H_o</td>
<td>0.074</td>
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</table>
Table 3. Values of $F_{is}$. $F_{is}$ represents the inbreeding coefficient of individuals within subpopulations.

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<tbody>
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<tr>
<td>FUM</td>
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<td>1.000***</td>
<td>0.792***</td>
<td>--</td>
<td>0.124</td>
<td>0.693**</td>
<td>0.467</td>
<td>0.780***</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>HK</td>
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<td>0.042</td>
<td>0.430</td>
<td>0.253</td>
<td>0.570*</td>
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<tr>
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<td>0.200</td>
<td>0.373</td>
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<td>0.375</td>
<td>0.733**</td>
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<td>-0.143</td>
<td>1.00**</td>
<td>-0.027</td>
<td>-0.026</td>
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<td>0.825**</td>
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<td>0.570*</td>
<td>1.00*</td>
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<tr>
<td>Weighted Mean $F_{is}$</td>
<td>0.371</td>
<td>0.369</td>
<td>0.294</td>
<td>0.449</td>
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<td>0.399</td>
<td>0.587</td>
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<td>-0.053</td>
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* denotes significant heterozygote deficiency based on Fisher's Exact Test at p=0.05  
** p=0.01  
***p=0.001
Table 4. Observed and expected heterozygosities and self-fertilization rates (1-t) for all polymorphic loci.

<table>
<thead>
<tr>
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<th>FUM</th>
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<th>LGG</th>
<th>MDH</th>
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<tr>
<td></td>
<td>H₀</td>
<td>Hₑ</td>
<td>1-t</td>
<td>H₀</td>
</tr>
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<td>0.460</td>
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<td>0.255</td>
<td>1.000</td>
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</tr>
<tr>
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<td>0.480</td>
<td>0.884</td>
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</table>
Table 5. Nei’s unbiased genetic distance.

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<td>0.051</td>
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<td>0.064</td>
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</table>
Figure 1. Distributional range of *Margaritifera falcata*. (based on Clarke 1981)
Figure 2. Sampling sites.
Figure 3. Mean observed and expected heterozygosity for all populations from West to East.
Figure 4. UPGMA based upon Nei’s Unbiased Genetic Distance

Distance

0.100 0.075 0.050 0.025 0.000

12 13 10 11 9 2 3 1 5 7 8 6 4

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