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# Genetic diversity and divergence among freshwater mussel (*Anodonta*) populations in the Bonneville Basin of Utah

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## Abstract

**Populations of the freshwater mussel genus *Anodonta* appear to be in a state of rapid decline in western North America, following a trend that unfortunately seems to be prevalent among these animals (Mollusca: Unionoida). Here we describe the patterns of molecular divergence and diversity among *Anodonta* populations in the Bonneville Basin, a large sub-basin of the Great Basin in western North America. Using amplified fragment length polymorphism (AFLP) analysis, we found a striking lack of nuclear diversity within some of these populations, along with a high degree of structuring among populations ($F_{ST}$ = 0.61), suggesting post-Pleistocene isolation, due either to a long-term loss of hydrologic connectivity among populations or to more recent fish introductions. We also found evidence of recent hybridization in one of these populations, possibly mediated by fish-stocking practices. Using mitochondrial sequence data, we compared the Bonneville Basin populations to *Anodonta* in several other drainages in western North America. We found a general lack of resolution in these phylogenetic reconstructions, although there was a tendency for the Bonneville Basin *Anodonta* (tentatively *A. californiensis*) to cluster with *A. oregonensis* from the adjacent Lahontan Basin in Nevada. We recommend further investigation of anthropogenic factors that may be contributing to the decline of western *Anodonta* and a broad-scale analysis and synthesis of genetic and morphological variation among *Anodonta* in western North America.**

*Keywords*: AFLP, *Anodonta*, Bonneville Basin, mussels, phylogeography, Unionoida

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## Introduction

A major geomorphic feature of western North America is the endorheic Great Basin (Fig. 1a). Considerable endemism of aquatic taxa has developed within the Great Basin (e.g. Johnson & Jordan 2000; Hershler & Sada 2002; Johnson 2002; Polhemus & Polhemus 2002; Smith *et al*. 2002). The Bonneville Basin, a large sub-basin of the Great Basin, is geologically dominated by the footprint of the ancient Lake Bonneville (Fig. 1b). During recession of the Wisconsin glaciers, the combined effects of precipitation increase and the diversion of the Bear River from the Snake River drainage into the Bonneville Basin resulted in inundation of the eastern third of the Great Basin, resulting in the formation of Lake Bonneville (Jarrett & Malde 1987). Lake Bonneville was a pluvial freshwater lake covering 51 700 km$^2$ at its maximum, ~17 000 years ago (Oviatt *et al*. 1992). Lake Bonneville receded rapidly following a dramatic breach at its northern boundary 14 500 years ago to form the Provo shoreline, and continued to recede following the end of the last ice age (Fig. 1b) (Currey *et al*. 1984; Jarrett & Malde 1987). The Gilbert Shoreline, formed between 10 000 and 11 000 years ago, resulted from a partial refilling of Lake Bonneville, and was followed by a series of fluctuations and a general decline in lake levels. The Great Salt Lake in Utah is the current remnant of Lake Bonneville. As a result of these processes, the current distribution and population genetic structure of aquatic fauna in the Bonneville Basin may have been influenced by pre-Wisconsonian glaciation vicariance, post-Pleistocene desiccation and/or recent anthropogenically mediated gene flow among relict populations. An understanding of the contribution of each of these processes in particular taxa is an important prerequisite to their effective monitoring and management.

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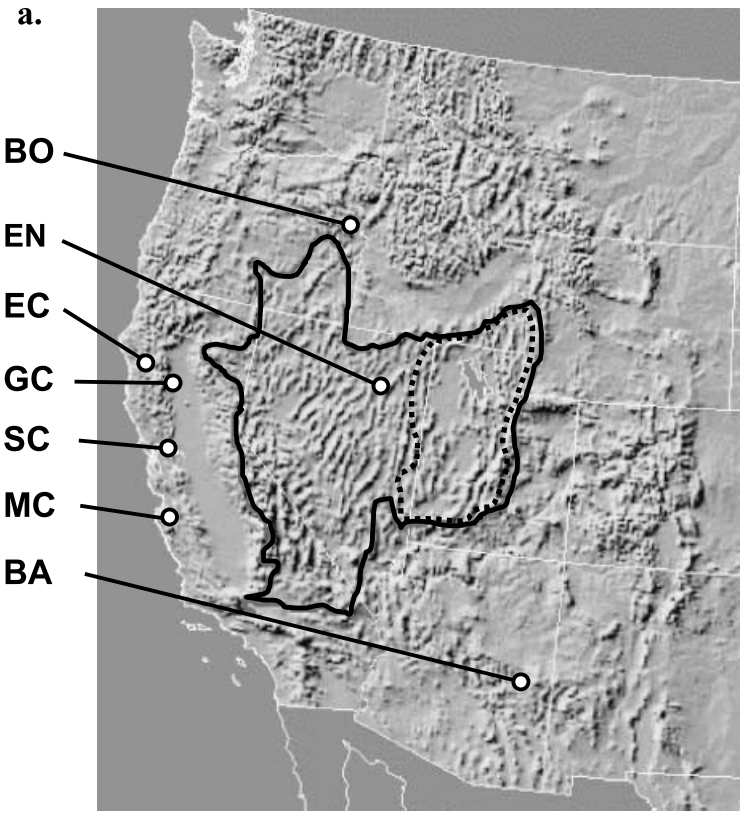


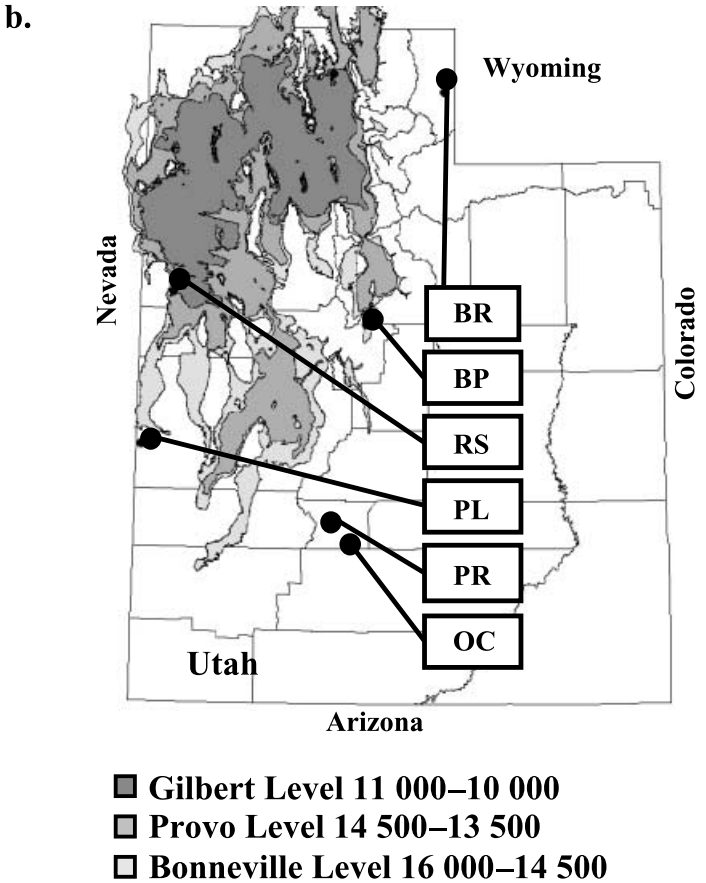


**Fig. 1** (a) Location of the Great Basin (solid line) and Bonneville Basin (broken line) in western North America. *Anodonta* sample locations outside the Bonneville Basin are as follows: Baker County, OR (BO); Elko, NV (EN); Eel River, CA (EC); Glenn County, CA (GC); Monterrey County, CA (MC); Solano County, CA (SC); Black River, AZ (BA). (b) Bonneville Basin, UT, *Anodonta* sample locations relative to previous Lake Bonneville levels (years bp) (adapted from Currey *et al*. 1984). Sample locations included the Bear River (BR), Redden Spring (RS), Pruess Lake (PL), Piute Reservoir (PR), Otter Creek Reservoir (OC) and Burriston Ponds (BP).

Freshwater mussels (Mollusca: Unionoida) are a taxonomically diverse group of bivalves which have an obligatory parasitic larval stage (glochidia) involving a suitable host fish (McMahon 1993). North America is a global centre of endemism for freshwater mussels, with over 300 species and subspecies recognized in the USA and Canada, many possessing very unique morphological adaptations (Williams *et al*. 1993; Turgeon *et al*. 1998). Unionid mussels were historically an integral component of many aquatic ecosystems (Ortmann 1925). In part because of their sensitivity to a myriad of pollutants and ecosystem alterations (Neves *et al*. 1997; Brim Box & Mossa 1999) freshwater mussels are now one of the most endangered faunal groups in North America (Bogan 1993, 1997; Stolzenburg 1995; Williams & Neves 1995). Startling declines in the abundance of these animals have been noted recently, particularly in the southeastern USA (Lydeard & Mayden 1995), where there is a concentration of species diversity.

In comparison to other portions of North America, species richness of the Unionoida (freshwater mussels) west of the continental divide is quite low (e.g. Burch 1973). Eight species from this region, representing three genera, were listed in a recent checklist of unionoid taxa north of Mexico (Turgeon *et al*. 1998). Six of these species are in the genus *Anodonta* (Bivalvia: Unionidae). *Anodonta* are widely distributed in western North America, ranging from Mexico to Alaska (Burch 1973; Clarke 1981; Taylor 1981, 1985). The taxonomy of western *Anodonta* has been problematic, however, with no general agreement about the number or distribution of species (Chamberlin & Jones 1929; Henderson 1936; Burch 1973; Taylor 1981). Unfortunately, very little is currently known about the population sizes, life histories, habitat requirements or important predators of *Anodonta* in western North America.

*Anodonta* have an extensive fossil record in the Great Basin, extending into the Tertiary period (Henderson & Rodeck 1934; Eardley & Gvosdetsky 1960; Currey *et al*. 1983; Oviatt *et al*. 1999). Historically, four species and one subspecies have been reported for *Anodonta* from the Bonneville drainage of the Great Basin (Chamberlin & Jones 1929; Henderson 1936), although the number of species represented in the fossil record or in historical surveys has not been firmly established. *A. californiensis* Lea 1852 is considered the only extant species in the Bonneville Basin (Hovingh 2004). However, these species identifications are tentative and based only on conchological features (Oliver & Bosworth 1999; Hovingh, submitted), which are sometimes misleading (Hoeh 1990; Mulvey *et al*. 1998).

Recent surveys suggest that western *Anodonta* populations are in decline (e.g. Hovingh, submitted). For instance, the conservation status of *A. californiensis* throughout its range is considered vulnerable (Nature Serve 2001, http://www.natureserve.org/), it is in decline in California (Taylor 1981; Frest & Johannes 1995) and in Utah it is a species of special concern. Although western unionoids may be locally abundant (J. C. Brim-Box, M. E. Gordon & P. Hovingh personal observations), modification of lotic habitats, water diversion and introduction of exotic fish species seem to have led to the extirpation of many populations and induced faunal shifts relative to species dominance (e.g. Taylor 1981; Vannote & Minshall 1982; Sada & Vinyard 2002; Hovingh, submitted).

Our study was designed to: (i) examine patterns of gene flow and genetic variation among extant populations of *Anodonta* in the Bonneville Basin, Utah; (ii) relate these patterns to the hydrologic history of the area; (iii) place the observed genetic variation from the Bonneville Basin in the context of genetic variation observed in *Anodonta* from other western North American locations; and (iv) recommend future study directions and management strategies directed toward the conservation of these animals.

## Materials and methods

### *Sample collection and DNA isolation*

*Bonneville Basin Populations* Based on historical records (Ingersoll 1877; Chamberlin & Jones 1929; Henderson 1931, 1936; Jones 1940) existing survey data (Hovingh, submitted), information available through the Utah Division of Wildlife Resources, and interviews with local fisheries and wildlife managers, surveys were conducted in 2000, 2001 and 2002 throughout the Bonneville Basin for extant populations of *Anodonta*. Live *Anodonta* specimens were collected in August 2001 from six locations within the Bonneville Basin, Utah, as described in Fig. 1(b) and Table 1: Bear River (BR), Redden Spring (RS), Pruess Lake (PL) (sometimes referred to as Garrison Reservoir), Piute Reservoir (PR), Otter Creek Reservoir (OC) and Burriston Ponds (BP). All these locations have been described by Hovingh (submitted) as extant populations. Extensive visual surveys of 14 additional Great Basin sites, including 9 historical locations in the Bonneville Basin, yielded no live *Anodonta*, although at a few of these sites *Anodonta* shells were found. These survey results suggest that the six locations where *Anodonta* were found represent most of the extant populations in this region. All molluscs were collected by hand using either SCUBA, snorkelling, or by direct observation in shallow areas. Specimens were preserved in 95% ethanol and subjected to several fluid changes during the first few days of preservation.

Maximum shell length, an approximate indicator of age, was recorded for all individuals collected (Table 1). In the Bear River and Otter Creek Reservoir populations, several of the specimens were brooding glochidia at various stages of development, but no other reproductive activity was noted at the time of collection. Mantle tissue was dissected from each specimen, rinsed with distilled water to remove any parasites or other organisms from the surface, and

**Table 1** Sample sizes, metric data and genetic diversity indices for *Anodonta* populations in the Bonneville Basin, Utah, USA: Bear River (BR), Redden Spring (RS), Pruess Lake (PL), Piute Reservoir (PR), Otter Creek Reservoir (OC), and Burriston Ponds (BP). *n* = number of samples collected (first column) or analysed (subsequent columns),%*P* = percent polymorphic AFLP loci (99% criterion). No. mitotypes include combined COI and cytb data

| Location | Av. length (mm) (SE) | *n* | Sex ratio (F:M) | No. mitotypes (*n*) M | No. mitotypes (*n*) F | %*P* | Av. intrapop'n Jaccard distance | No. unique AFLP profiles (*n*) |
|---|---|---|---|---|---|---|---|---|
| BR | 50.0 (11.2) | 20 | 9:9 | 1 (5) | 1 (5) | 1.5 | 0.0041 | 2 (18) |
| RS | 53.4 (12.3) | 19 | 11:8 | 1 (3) | 1 (5) | 2.9 | 0.0131 | 3 (16) |
| PL | 99.5 (22.3) | 20 | 10:10 | 2 (5) | 1 (8) | 74.6 | 0.3857 | 20 (20) |
| PR | 79.4 (20.5) | 14 | 11:4 | 2 (3) | 1 (5) | 16.4 | 0.1189 | 11 (12) |
| OC | 73.0 (16.3) | 20 | 13:7 | 1 (5) | 1 (5) | 19.4 | 0.1111 | 15 (19) |
| BP | 75.7 (16.9) | 20 | 4:16 | 1 (3) | 1 (5) | 22.3 | 0.1210 | 15 (19) |

blotted with laboratory wipes. The tissue was then suspended in a lysis buffer (Longmire *et al*. 1988) and stored in a freezer. Genomic DNA was isolated from preserved mantle tissue using a Qiagen Dneasy Tissue kit, following the manufacturer's protocols. DNA quality and quantity was assessed by electrophoresis in 0.7% agarose gels stained with ethidium bromide. All extracted DNA was found to be high quality with minimal degradation.

Gender determination was also performed on each individual from these six populations by microscopic examination of gametogenic tissue from the visceral mass for the presence of egg or sperm (Table 1). In males, when possible, testicular tissue was sampled for the purpose of sequencing genes from the male-specific (M-type) mitochondrial lineage.

### *Mitochondrial sequencing and data analysis*

Unionid mussels are characterized by an unusual pattern of mitochondrial inheritance called doubly uniparental inheritance (DUI) (Skibinski *et al*. 1994; Zouros *et al*. 1994; Hoeh *et al*. 1996; Liu *et al*. 1996). Under DUI, females possess one F-type mitochondrial lineage and pass it on to all their offspring. Males possess two distinct mitochondrial lineages; the M-type and the F-type. Males pass on the M-type, which is found only in the testicular tissue, to their male offspring, but do not pass on the F-type. Although recombination has been documented in some *Mytilus* species (Ladoukakis & Zouros 2001; Burzynski *et al*. 2003; Rokas *et al*. 2003), it is generally considered a rare event, resulting in widely divergent male- and female-specific mitochondrial lineages (Hoeh *et al*. 2002).

*Bonneville Basin* Anodonta *analyses*. Both F- and M-type cytochrome c oxidase subunit I (COI) sequences were obtained using the primers LCO1490 and HCO2198 (Folmer *et al*. 1994; King *et al*. 1999) (Table 1, Fig. 2). Polymerase chain reactions (PCRs) were carried out in a total volume of 20 μL, with 100 ng of isolated DNA, 1 × reaction buffer, 2 mM $MgCl_2$, 0.25 mM dNTPs, 0.5 μM primers and 0.6 U *Taq* DNA polymerase. The reaction was denatured at 94 °C for 2 min, followed by 35 cycles (94 °C for 30 s, 54 °C for 1 min, 72 °C for 90 s), with a final 5-min extension step at 72 °C. Amplicons were purified using Microcon-PCR spin columns (Millipore). Sequencing reactions were performed from both ends of the amplicons using the same primers with an ABI BigDye Kit and an ABI 3100 automated sequencer, yielding sequences of ~650 bp. For the F-type COI sequences, a comparative, trimmed alignment of 573 bp was used to assess variation within and among Bonneville Basin populations (nucleotides 21–594, GenBank Accession no. AY476830). For the M-type COI sequences, a trimmed, comparative alignment of 577 bp was used (nucleotides 1–577, GenBank Accession no. AY476832).

We also obtained F- and M-type cytochrome *b* (cytb) sequences using the primers UcytB151F and UcytB270R (Merritt *et al*. 1998) (Table 1, Fig. 2) PCRs were carried out in a total volume of 50 μL, with 100 ng of isolated DNA, 1 × reaction buffer, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.5 μM primers and 1 U *Taq* DNA polymerase. The reaction was denatured at 94 °C for 2 min, followed by 40 cycles (94 °C for 10 s, 50 °C for 10 s, 72 °C for 10 s), with a final 3-min extension step at 72 °C. Sequencing reactions were performed from both ends of the amplicons as described above. M- and F-type comparative sequence sets were obtained following trimming and alignment (F-type 282 bp, M-type 328 bp). All sets of mitochondrial sequences were aligned and trimmed to uniform lengths using SEQMAN and MEGALIGN software (DNAStar). Patterns of molecular variation among populations were assessed using both the M- and F-type COI and cytb sequences using haplotype networks (Fig. 2).

*Western USA* Anodonta *sequence data*. We compared the M- and F-type COI sequences from the Bonneville Basin populations to those from a limited number of specimens from several western USA locations outside the Bonneville Basin (Downing, Gordon and Hoeh, unpublished) (GenBank Accession nos AY493462–AY493507) (Table 2, Fig. 1a). These samples, collected from October 1999 to August 2001, were

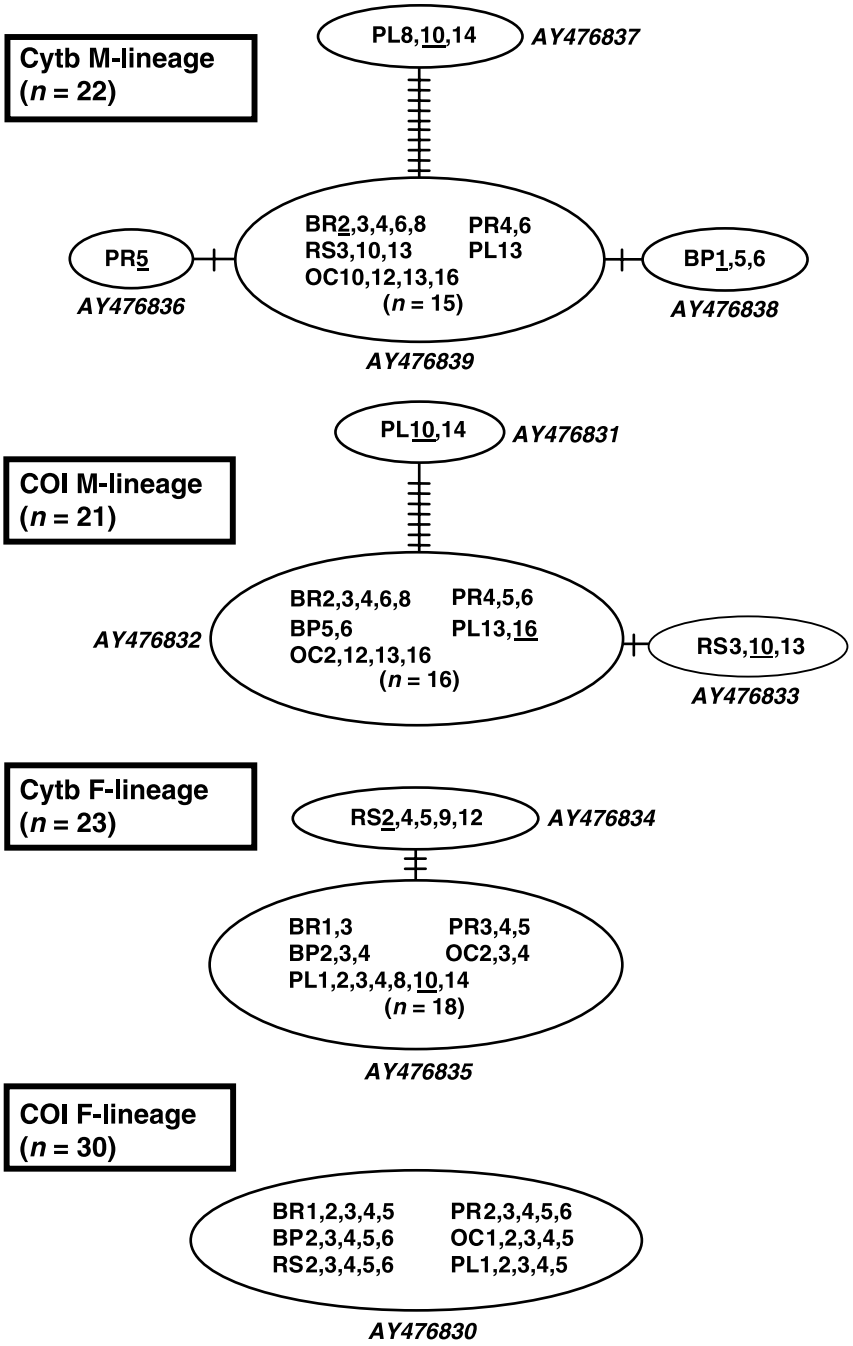


**Fig. 2** Haplotype networks constructed from F- and M-type mitochondrial cytochrome *b* and cytochrome *c* oxidase I sequences from Bonneville Basin *Anodonta*. Sample locations included the Bear River (BR), Redden Spring (RS), Pruess Lake (PL), Piute Reservoir (PR), Otter Creek Reservoir (OC) and Burriston Ponds (BP). Note that only one haplotype was detected for F-type COI sequences. Individual sample numbers are provided within ovals. GenBank Accession nos for the underlined samples are provided in italics outside the ovals.

previously preserved, sampled and sequenced in the manner described above. Tentative species identifications were made using conchological features, based on comparisons to type specimens and/or type illustrations. To assess overall sequence divergence in the combined dataset, a pairwise distance matrix including these sequences and the Bonneville Basin COI sequences was constructed using the Kimura 2-parameter model (Kimura 1980).

This combined dataset was also used to assess phylogenetic relationships among taxa. For this assessment, best-fit evolutionary models for each sex-specific lineage were identified using MODELTEST (Posada & Crandall 1998). Data from each lineage were subjected to a maximum likelihood (ML) analysis with PAUP software (Swofford 2002). For each lineage, a heuristic search was conducted with 10 random-sequence additions and tree bisection reconnection (TBR) branch swapping options. *Anodonta woodiana* F- and M-type sequences (GenBank Accession nos AB055627 and AB055626, respectively) were used as out-groups in these analyses.

**Table 2** Sample locations for *Anodonta* from western USA locations outside the Bonneville Basin

| Location (County, State) | Site | Species identification | *n* |
|---|---|---|---|
| Elko, NV (EN) | Ten Mile Creek | *A.* cf. *oregonensis* | 11 |
| Glenn, CA (GC) | Sacramento River | *A.* cf. *wahlametensis* Lea 1838 | 5 |
| Solano, CA (SC) | Union Creek | *A.* cf. *wahlametensis* | 5 |
| Monterey, CA (MC) | Pajaro River | *A.* sp. | 10 |
| Baker, OR (BO) | Burnt River | *A.* cf. *oregonensis* Lea 1838 | 5 |
| Mendocino, CA (EC) | S. Fork Eel River | *A.* sp. | 5 |
| Apache, AZ (BA) | Black River | *A. californiensis* Lea 1852 | 2 |

### *AFLP procedure and data analysis*

In order to characterize nuclear divergence and diversity among Bonneville Basin populations, amplified fragment length polymorphism (AFLP) marker profiles were generated following a modified version of basic procedures from Vos *et al*. (1995), using seven selective primer combinations: *Eco*RI-ACG and *Mse*I-ACT, *Eco*RI-AGG and *Mse*I-ATC, *Eco*RI-AGG and *Mse*I-ACA, *Eco*RI-ACG and *Mse*I-ATC, *Eco*RI-ACG and *Mse*I-ACA, *Eco*RI-ACG and *Mse*I-AGA, and *Eco*RI-AGG and *Mse*I-ACT. Resulting amplicons were run on a sequencing gel with a ROX 400 (ABI) size standard using an ABI 3100 automated sequencer. GENOGRAPHER v1.6 software (Benham 2001) was used to visualize and score the gel image. Markers were scored if they were polymorphic across the dataset (95% criterion) and if they could be scored unambiguously. Scoring was performed without reference to sample or population identity. Seventeen (16%) of the 104 samples (including representatives from all populations) were replicated following DNA extraction to assess the methodological and scoring error rate.

Genetic divergence among the six Bonneville populations was assessed using MANTEL-STRUCT software (Miller 1999). Individual AFLP profiles were used to generate a matrix of average pairwise distances among populations using the Jaccard coefficient (Jaccard 1908). The null hypothesis that average interindividual genetic distances within populations equalled average interindividual genetic distances among populations (i.e. no genetic population-level structure) was tested using a Monte Carlo randomization procedure (1000 replicates). The matrix of average pairwise Jaccard distances among populations was also used to construct a UPGMA dendrogram with NTSYS software (Rohlf 2002). UPGMA dendrograms were constructed for individual AFLP profiles as well, using both Jaccard and simple matching distances. A principal coordinates analysis was also performed in NTSYS using a matrix of interindividual Jaccard distances to illustrate the clustering of genetic variation within and among populations. We tested for structure among Bonneville Basin populations using Weir & Cockerham's (1984) theta ($\theta$), an estimator of Wright's $F_{ST}$, using TFPGA software (Miller 1997). Allele frequencies used in these analyses were estimated using Lynch & Milligan's (1994) Taylor expansion approach, under the assumption of Hardy–Weinberg genotypic proportions. 95% confidence intervals for $\theta$ were generated by bootstrapping (1000 replicates) over loci.

## Results

### *Mitochondrial sequencing analysis*

*Bonneville Basin* Anodonta *analyses*. We obtained a total of 51 COI sequences (21 M-type, 3 variants; 30 F-type, 1 variant) and 44 cytb sequences (22 M-type, 4 variants; 23 F-type, 2 variants) from individuals in our Bonneville Basin localities (Fig. 2). None of these sequences were found to include stop codons, and all exhibited a strong third position codon mutational bias, in accordance with expectations for coding sequences. Also, all sequences were subjected to Basic Local Alignment Search Tool (BLAST) (Altschul *et al*. 1990) nucleotide and protein searches in GenBank (Benson *et al*. 2000), all of which yielded the expected COI or cytb proteins. M-type sequences were only amplified from testicular tissue samples; no M-type sequences were ever amplified from DNA extracted from somatic tissue. Collectively, this evidence suggests that none of our sequences were nuclear pseudogenes.

There was little variation among Bonneville Basin populations with respect to the F-lineage mitochondrial genes (Fig. 2). There was no variation among these populations with respect to F-type COI sequences, and only two haplotypes of the F-lineage cytb gene were detected. One common allele was present in most populations and a variant differing by a pair of adjacent transitions resulting in a single amino acid change (proline to phenylalanine) was found in all five sequences from the Redden Springs population.

Among Bonneville Basin populations, the M-type genes were found to be somewhat less conserved than the corresponding F-type genes, consistent with previous findings in unionid mussels (Liu *et al*. 1996; Hoeh *et al*. 2002). M-type sequences were more difficult to obtain than F-type sequences because of a tendency for co-amplification of F-lineage sequences, likely due to the presence of small amounts of somatic tissue in the testicular tissue extractions. Four M-type cytb mitotypes were detected across Bonneville Basin populations (Fig. 2). The most common haplotype was the only one detected in the Bear River, Redden Spring and Otter Creek Reservoir populations.

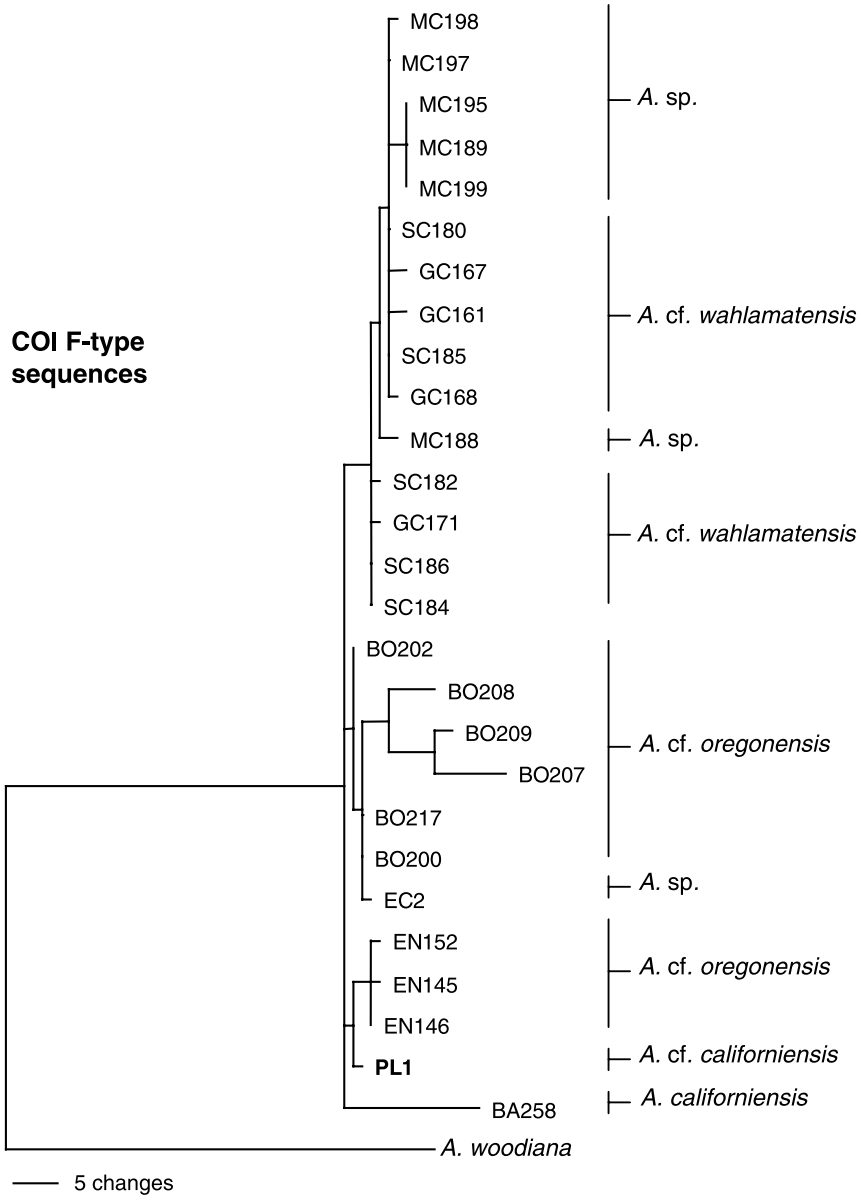


**Fig. 3** Phylogram of the single best topology identified using maximum likelihood (ML) analysis for F-type mitochondrial cytochrome *c* oxidase I sequences (604 bp) from *Anodonta* specimens in western North America. The Bonneville Basin sequence (PL1) is shown relative to sequences from Monterrey County, CA (MC); Solano County, CA (SC); Glenn County, CA (GC); Baker County, OR (BO); Eel River, CA (EC); Elko County, NV (EN); and the Black River, AZ (BA).

Another M-type cytb sequence, differing by a single missense mutation, was the only haplotype detected in the Burriston Ponds population. Two very distinct sequences were found within the Pruess Lake population; the common Bonneville Basin sequence and one differing by 10 mutational changes (9 silent). The M-type COI gene showed a similar pattern (Fig. 2), with only the common sequence found in most Bonneville Basin populations, a haplotype differing by a single silent mutation found exclusively in the Redden Spring population, and two very distinct M-type sequences in the Pruess Lake population: the common sequence and one differing by 8 nucleotide changes (7 silent).

*Western USA* Anodonta *analyses.* COI sequences from the Bonneville Basin *Anodonta* populations were compared with sequences from the western USA populations outside the Bonneville Basin (Downing, Gordon and Hoeh, unpublished). The COI F-type dataset (excluding the out-group) consisted of 604 nucleotides (56 variable, 21 parsimony informative sites). The COI-M type dataset (excluding the out-group) consisted of 607 nucleotides (56 variable, 28 parsimony informative sites). Pairwise divergences in both of these datasets ranged from 0 to 5%.

MODELTEST (Posada & Crandall 1998) indicated that the Tamura & Nei (1993) model of sequence evolution with the proportion of invariable sites estimated from the data (TrN + I), was the most appropriate model to use for maximum likelihood (ML) analysis of both F- and M-types. ML analysis of the F-type sequences resulted in a single tree (Fig. 3), whereas the M-type sequences produced nine best

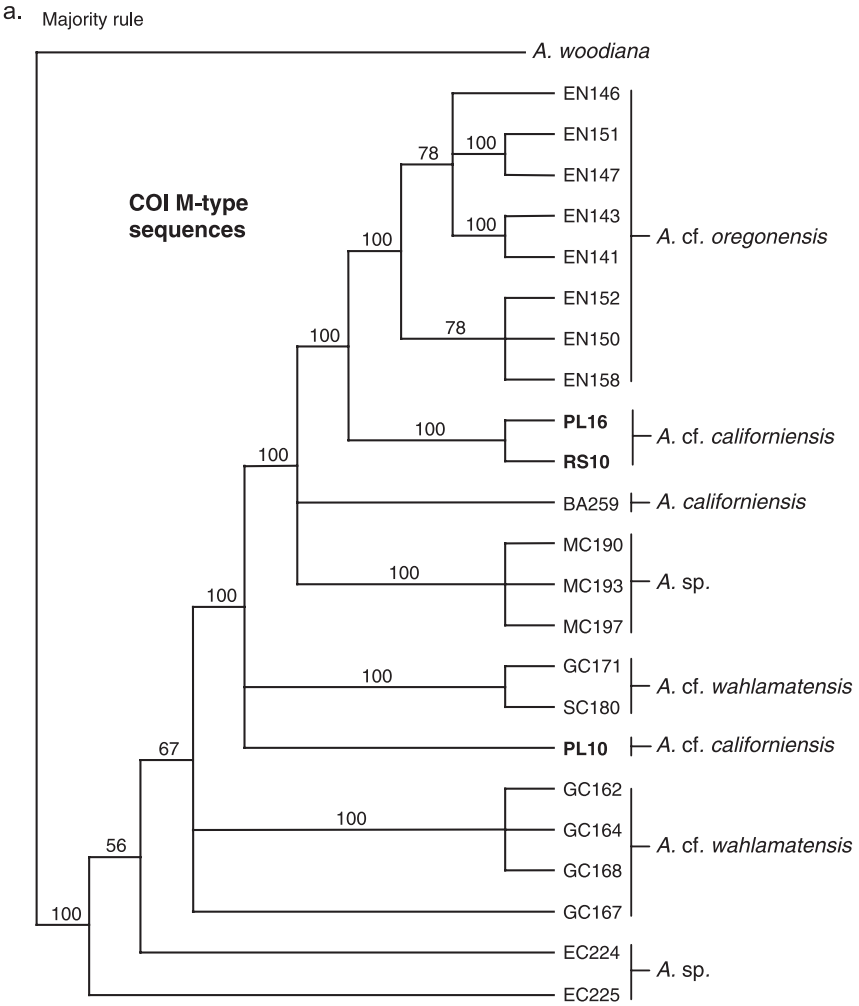


**Fig. 4** (a) Consensus tree of the nine best topologies identified using maximum likelihood (ML) analysis for M-type mitochondrial cytochrome *c* oxidase I sequences (607 bp) from western North American *Anodonta* specimens. (b) The phylogram of one of these topologies. The Bonneville Basin sequences (PL16 and RS10), and the divergent Pruess Lake sequence (PL10) are shown relative to sequences from Monterrey County, CA (MC); Solano County, CA (SC); Glenn County, CA (GC); Baker County, OR (BO); Eel River, CA (EC); Elko County, NV (EN); and the Black River, AZ (BA). Numbers on branches indicate the percent of best trees in agreement with the topology depicted on the dendrogram.

trees (Fig. 4). There was no high degree of differentiation or resolution in either of these trees, but they did suggest that the Bonneville Basin populations most closely resembled our specimens from Elko County, Nevada, the only other Great Basin population that we sampled. Interestingly, populations did not tend to group along current taxonomic boundaries.

### *AFLP analysis of Bonneville Basin populations*

AFLP analysis of the Bonneville Basin populations yielded 67 polymorphic loci. A methodological error rate of 0.6% was estimated from the replicates included in the analysis. There was a striking absence of nuclear diversity in the populations from the Bear River and Redden Spring (Table 1). In the Bear River population, 17 of the 18 individuals sampled had exactly the same AFLP profile across all 67 loci. The aberrant profile found in that population differed at a single locus. In the Redden Spring population, 13 of the 16 individuals shared a single AFLP profile, two exhibited a profile that differed from the majority type at a single locus, and one had a third profile that also diverged from the majority type at a single locus. The common profile types of the Bear River and Redden Spring populations differed at four loci. The other populations within the Bonneville Basin were more diverse with respect to AFLP profiles (Table 1). Pruess Lake was particularly diverse, with no shared AFLP profiles among individuals and all pairwise individual profiles differing by at least 11 loci.

Results from Mantel testing indicated that all populations differ significantly from each other ($P < 0.001$) with the exception of those from the Piute Reservoir and Otter Creek populations ($P = 0.29$). These two sites are physically close within the Sevier River system, and individuals from both localities appear to be a single functional population. The UPGMA dendrogram of all Bonneville Basin populations (Fig. 5a) indicated that the Bear River and Redden Spring populations are the most similar pair of populations, followed by the Burriston Ponds population,

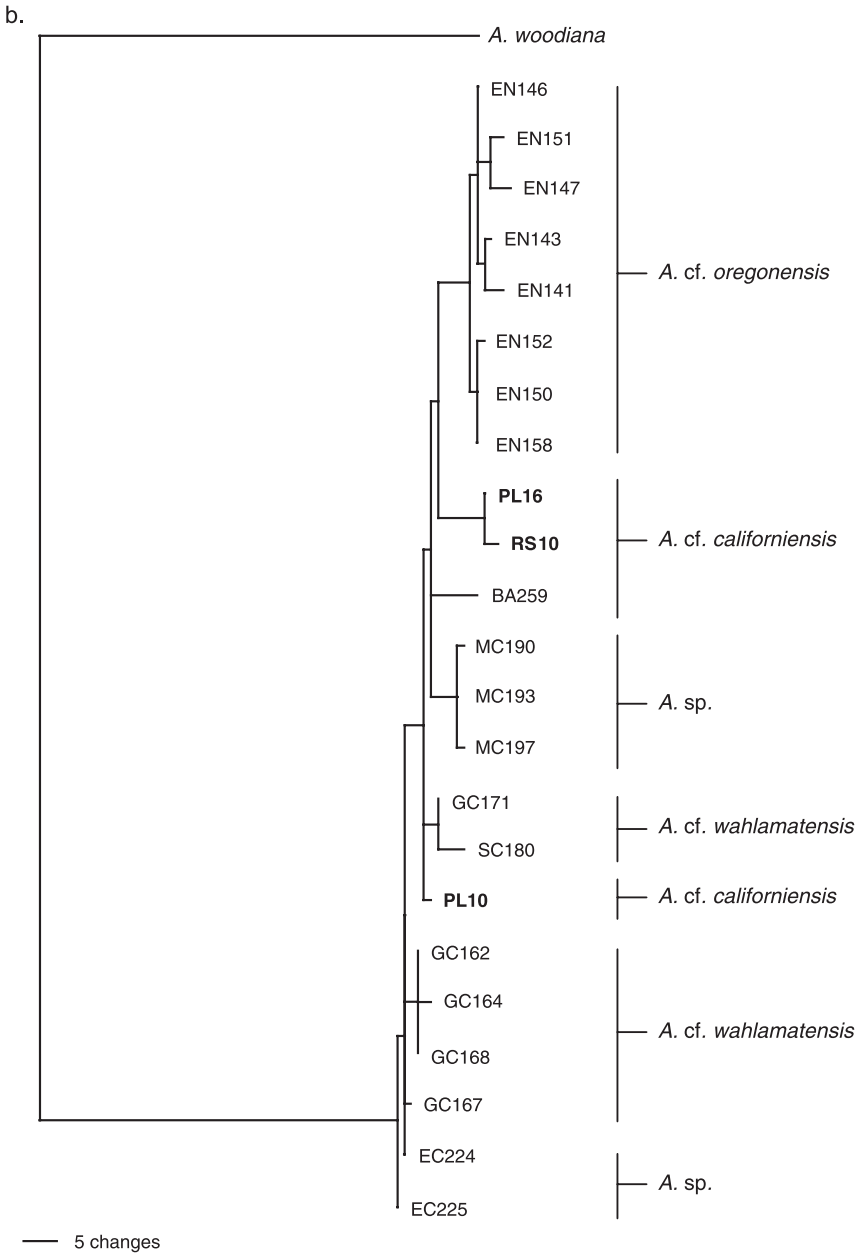


**Fig. 4** *Continued*

the Piute Reservoir/Otter Creek population and finally the Pruess Lake population, which was highly divergent from the others. A UPGMA tree of individuals, not presented due to space limitations, displayed the same topology with respect to populations, using both Jaccard and simple matching distance measures. In these trees, all individuals formed population-specific clusters with the exception of a single individual from Burriston Ponds, which clustered more closely with the Bear River population. Within the Bonneville Basin, AFLP results suggested that the populations were highly structured ($\theta = 0.61$, 95% CI 0.54–0.68). A principal coordinates analysis diagram of individuals, based on AFLP data, illustrates the extremity of this interpopulation-level structure (Fig. 5b). The first three principal coordinates in this analysis captured 32.5, 23.5 and 11.1% of the variance in the dataset, respectively.

## Discussion

### *Genetic diversity within Bonneville Basin* Anodonta *populations*

The lack of nuclear diversity within extant Bonneville Basin *Anodonta* populations (Table 1) was striking. Homogeneity

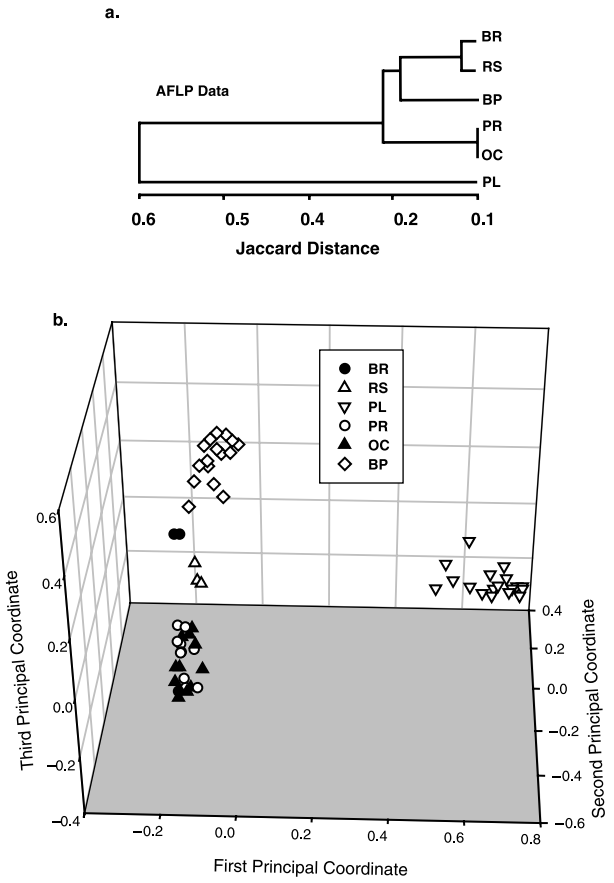


**Fig. 5** (a) UPGMA dendrogram of Utah Bonneville Basin *Anodonta* populations based on data from 67 polymorphic AFLP loci. Branch lengths reflect average interindividual Jaccard distances among populations. (b) Principal coordinates plot of individuals from these populations. Sample locations included the Bear River (BR), Redden Spring (RS), Pruess Lake (PL), Piute Reservoir (PR), Otter Creek Reservoir (OC) and Burriston Ponds (BP).

was particularly pronounced in the Bear River and Redden Springs populations. Such a pattern may be the result of severe, sustained bottlenecks or founder effects in these populations, which would be consistent with the long-term regional hydrogeological patterns: a general trend of desiccation and salinization from ~14 000 years BP, with marked short-term fluctuation (Currey *et al*. 1984; Madsen *et al*. 2001). These effects may be exacerbated by more recent anthropogenic impacts on water quality and quantity in the Great Basin, resulting in both direct and indirect (host fish) impacts on mussels (Smith *et al*. 2002). Certainly, the documented declines in western unionids seem to be occurring over a compressed time frame relative to potentially contributing geological and climatological changes. This suggests that anthropogenic impacts may be important contributors to these declines. It is possible that the factors causing such a loss of population-level diversity in presumably neutral molecular markers could also be severe enough to cause a loss of diversity in alleles of adaptive and evolutionary significance. A loss of adaptive potential in these populations, along with an increased level of inbreeding, may contribute significantly to the risk of local extinction (Lande 1993, 1996; Milligan *et al*. 1994; Frankham 1995; Dunham *et al*. 1999).

### *Gene flow and divergence among Bonneville Basin* Anodonta *populations*

North American unionid mussels are known to require attachment to a host fish for larval (glochidial) development and dispersal (McMahon 1993). Some mussel species (e.g. *Lampsilis* spp.) have developed elaborate lures to attract suitable host fish for this purpose. This parasitic life history means that unionid mussels are vulnerable to factors affecting their fish hosts, and that gene flow among populations is defined by host fish movements. In western North American *Anodonta* populations, glochidial dispersal by host fish is likely to be dramatically impacted by geologically recent desiccation of watersheds due to climate change as well as by human impacts on water quality and flows, and even by anthropogenic fish stock transfers.

In our study, *Anodonta* populations from the Bonneville Basin were strongly structured, with respect to both AFLP and mitochondrial sequence data. These results suggest that there is little or no recent gene flow among extant populations that are currently hydrologically separated. Additionally, mitochondrial sequence data suggest a lack of ancestral diversity within the Bonneville Basin. These patterns are consistent with the absence of hydrologic connections between these sites during most of the Holocene Epoch, along with a history of dramatic climatic and hydrologic fluctuations in the region (Currey *et al*. 1984; Madsen *et al*. 2001). If we presume a common ancestry for the populations within the Bonneville Basin, the pronounced distribution of diversity among, rather than within, populations suggests two possible histories: (i) post-Pleistocene vicariance among extant populations, followed by shrinking and fluctuating population sizes, resulting in a loss of ancestral diversity via population bottlenecks and drift; or (ii) post-Pleistocene founder effects resulting from the occasional redistribution of parasitized host fish into peripheral locations in the Bonneville Basin as Lake Bonneville levels fluctuated. Our data do not allow us to distinguish between these scenarios.

An alternative explanation for the pronounced differences among Bonneville Basin populations is that they were established via host fish introduced into these areas during historical times, and that these differences are simply very recent founder effects. Host fish relationships for *Anodonta* species have not been firmly established, but one potential host is *Gambusia affinis*, the Western mosquitofish

(Hoggarth 1992), a nonindigenous fish commonly introduced into isolated springs in the Bonneville Basin. Under this scenario, we would expect that recently established populations would be less diverse than source populations and that mitotypes and AFLP alleles in recently established populations would be a subset of those found in source populations. Our data were not entirely consistent with these expectations. First, our sample populations represent most of the known extant populations in the Bonneville Basin, and it is unlikely that we have missed large source populations from which founders could be drawn via host fish translocations. Utah Lake may have previously served as a large source population for anthropogenic transfers, but *Anodonta* is thought to have been extirpated from this location prior to the 1930s. Second, if these populations were recently derived from a common source population, we would expect more extensive sharing of profiles among populations. Finally, in Redden Spring, the least diverse population from the perspective of AFLP profiles, we detected exclusively M- and F-lineage mitotypes not found in any other locations. If the low AFLP diversity in Redden Spring was due to a recent founder effect, it is unlikely that a mitotype unique among the Bonneville Basin populations would be fixed or at high frequency in this population.

We suggest that the strong genetic structuring found among populations is more likely the result of isolation and drift, possibly including ancient founder effects, following the recession of the ancient Lake Bonneville (Currey *et al*. 1984; Madsen *et al*. 2001). A similar phylogeographical pattern has been found in other Bonneville Basin aquatic species, e.g. the Utah chub (*Gila atraria*) (Johnson 2002), the spotted frog (Bos & Sites 2001) and the Least chub (*Iotichthys phlegethontis*) (Mock & Miller 2003). It is possible that Pruess Lake was the first site to lose its hydrological connection to Lake Bonneville, given its location at the south end of a shallow bay of Lake Bonneville (currently the Snake Valley) and its distance from the Provo and Gilbert shorelines (Fig. 1). We would predict from this history that Pruess Lake would contain the most genetically divergent population, which is clearly the case. The Pruess Lake population is so disproportionately divergent, in fact, that other processes may be involved (see below). Based on the recession pattern of Lake Bonneville, the Piute and Otter Creek populations might be expected to be the next most divergent population. The pattern of dissimilarity that we found in our AFLP data (Fig. 5) does seem to be consistent with this prediction, but these relationships could have been influenced and confounded by the operation of different demographic processes (e.g. drift, bottlenecks) within populations.

Mitochondrial sequence data did not contain enough variation to test detailed hypotheses about population divergence within the Bonneville Basin. The pattern of existing mitochondrial variation does support the hypothesis of isolation, as there were unique, fixed (or high frequency) mitotypes in the Redden Spring (M-type and F-type) and Burriston Ponds (M-type) populations. It is unclear whether these population-specific mitotypes originated in these populations or whether they represent stochastic sorting of limited ancestral diversity into current populations followed by genetic drift.

### *Potential hybridization in Pruess Lake* Anodonta

In contrast to our other sample sites, the Pruess Lake population was quite diverse with respect to AFLP profiles, with no duplicate genotypes and an average within-population interindividual genetic distance over three times greater than any other population (Table 1). In addition, AFLP data indicated that the Pruess Lake population was the most divergent of the Bonneville Basin *Anodonta* populations (Fig. 5). The principal coordinates analysis plot of individuals (Fig. 5b) indicated that there was rather continuous variation among the remaining Bonneville Basin populations, but the Pruess Lake population cluster was quite disjunct. These results are likely the result of the presence of multiple unique alleles segregating in the Pruess Lake population. The Pruess Lake population also contained two highly divergent M-type mitochondrial lineages (Fig. 2). There are three potential explanations for these patterns: (i) Pruess Lake has retained ancestral diversity that has been lost in the other populations; (ii) Pruess Lake has a different hydrogeological history than the other Bonneville Basin populations, and has been diverging from them since well before the high point of Lake Bonneville; or (iii) the *Anodonta* currently in Pruess Lake are the result of recent mixing between a population derived from the Bonneville Basin and a divergent population from elsewhere. The first explanation is not supported by the biogeological history and geography of the region: Pruess Lake is an unlikely source for the other Bonneville Basin populations, and it is not a particularly large or undisturbed habitat that would have been less susceptible to the stochastic effects of isolation and drift. The second explanation is plausible given the location of Pruess Lake at the edge of the Bonneville Basin, but does not explain the retention of high AFLP diversity relative to the other populations. Furthermore, neither of these scenarios is supported by the mitochondrial data: although this population does harbour a divergent M-type lineage, the common mitotypes found in the other populations are also represented, and there were no other variants detected. The data seem most consistent with the third explanation. This history would explain the remarkable AFLP diversity and divergence as well as the presence of a single divergent M-type mitochondrial lineage. Pruess Lake has historically received several nonnative fish introductions, including *Archoplites interruptus* (Girard) (Sacramento perch) in the

early 1900s (LaRivers 1962; Sigler & Miller 1963), and *Anodonta* glochidia encysted on these fishes could be responsible for the introgression. The divergent M-lineage mitotype (represented by PL10) found in Pruess Lake did not group most closely with the more common M-lineage mitotype in the Bonneville Basin (Fig. 4), further suggesting that this mitotype may have originated outside the Bonneville Basin. Unfortunately, the COI M-type tree lacked the resolution to identify a potential source population for this mitotype.

### *Relationships of Bonneville Basin* Anodonta *to other western* Anodonta

Overall the amount of COI sequence divergence and resolution among *Anodonta* populations in the western USA was low, although observed levels of divergence (4–5%) did exceed levels of intraspecific divergence in other unionid studies (0–2.82%) (Roe & Lydeard 1998; Roe *et al*. 2001; Machordom *et al*. 2003). Two interesting patterns did emerge that were common to both the M- and F-lineage trees. First, the Bonneville Basin and Elko County, Nevada (Humboldt River) populations formed a monophyletic group (excluding the presumably introgressed M-lineage mitotype in Pruess Lake) (Figs. 3 and 4). This is consistent with their geographical proximity and location within the Great Basin. Second, species identifications were not congruent with the observed phylogenetic structure among these populations (Figs. 3 and 4). Specimens identified tentatively as *Anodonta californiensis*, *A. oregonensis* and *A. wahlamatensis* did not consistently form monophyletic groups in our analyses. This lack of congruence could be due to the generally low level of phylogentic signal and resolution in the dataset, phenotypic plasticity in conchological features, inappropriate local taxonomic designations, or a combination of these factors.

### *Recommendations for further study*

*Assessment of factors contributing to low diversity.* In order to assess the causes underlying low diversity in some of the Bonneville Basin populations, more thorough surveys should be conducted to estimate population sizes and trends, to evaluate habitat quality and quantity, and to locate and characterize additional *Anodonta* populations.

*Assessment of host fish relationships.* Although western *Anodonta* are thought to be somewhat generalist with respect to host fish requirements, based on their widespread distribution, these relationships have not been determined for *Anodonta*. Nonnative species are frequently the target of eradication efforts in the western USA, but they may be serving as host fish for *Anodonta* in the absence of a native host fish. Host fish relationships are rarely considered in stocking programmes. Fish stocking may also result in unwanted gene flow between geographically disjunct populations of *Anodonta*. In order to protect freshwater mussel habitats and populations from nonnative unionid mussel introductions, fish health inspectors should be particularly vigilant for the presence of glochidial cysts in fish stocks.

*Taxonomy of western North American* Anodonta. In order to more fully assess the species identity of Bonneville Basin *Anodonta*, and to establish the relationship of this species to other western *Anodonta* species, a broad-scale analysis of genetic and morphological variation among *Anodonta* in western North America is necessary. We recommend that such a survey should include sequence data from additional, more variable mitochondrial genes, as well as population-level analysis of nuclear markers.

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