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[Article]

Patterns of Molecular Diversity in Naturally Occurring and Refugial Populations of the Least Chub

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Abstract.—The least chub *Iotichthys phlegethontis* is a small, rare cyprinid fish endemic to the Bonneville basin, Utah. Although it was once widely distributed within the basin, naturally occurring populations are now known to exist only in four isolated geographical regions. We used nuclear (amplified fragment length polymorphism) and mitochondrial genetic markers to describe the patterns of genetic divergence and diversity within and among populations from these regions and to assess genetic diversity in two refugial populations. We found that a large proportion of the diversity in this species was attributable to population structuring (F_{ST} = 0.52). Given that a high proportion of the genetic variation is accounted for by population differentiation and that three of the existing populations have been discovered in the last 10 years, we suggest that surveys to discover additional populations be a high management priority. We also recommend that existing populations be studied for evidence of adaptive divergence. The refugial populations that we studied closely resembled their source populations with respect to both genetic divergence and diversity, indicating that the current policy of founding these populations with large numbers of fish is effective.

The least chub *Iotichthys phlegethontis* is a rare cyprinid fish endemic to the Bonneville basin, Utah, and the only member of its genus. Historical data and collections indicate that this species was once widely distributed in ponds, marshes, springs, and tributaries to the Great Salt Lake as well as in Utah and Sevier lakes and their tributaries and associated springs (Sigler and Miller 1963; Page and Burr 1991; Muck 1999). Declines in the range and local abundance of this species have been noted since the 1940s (Holden et al. 1974; Workman et al. 1979; Christ 1990; Perkins et al. 1998). The factors contributing to this decline are thought to include the introduction of nonnative fish and the reduction of habitat quality and quantity owing to water diversion and the impacts of livestock (Muck 1999). Naturally occurring populations of this species are currently restricted to two isolated spring systems in central Utah (the Mona Springs and Mills Valley Springs complexes) and a third spring system in the Snake Valley of western Utah (separate populations exist in the Gandy Salt Marsh, Leland Harris Spring, and Bishop Springs complexes of the Snake Valley region) (Figure 1). Very recently, least chub have also been discovered in Clear Lake south of Delta, Utah, but this population has not yet been surveyed and was not included in this study. Through a multiagency Conservation Agreement and Strategy established in 1998 (Perkins et al. 1998), a variety of conservation measures have been implemented to prevent the extinction of this fish. Actions taken under this agreement have included the initiation of monitoring programs and the establishment of refugial populations (Lucin Pond and Walter Springs; Figure 1) from the Snake Valley populations. These refugial populations were established for the purposes of replacement (in the event of loss of the original population) and supplementation.

The biotic history of the Bonneville basin has been dominated by the rise and recession of the ancient Lake Bonneville, which reached a maximum elevation approximately 16,000 years ago and has receded in an erratic fashion since that time, water levels fluctuating dramatically (Currey et al. 1984; Jarrett and Malde 1987; Currey 1990). The Great Salt Lake and Utah Lake are present-day remnants of the ancient Lake Bonneville. Interestingly, although the three locations of naturally occurring populations in our study are latitudinally proximal, they lie in distinct subbasins. Each subbasin represents a different arm of Lake Bonneville, and each has a unique prehistory of isolation as the ancient

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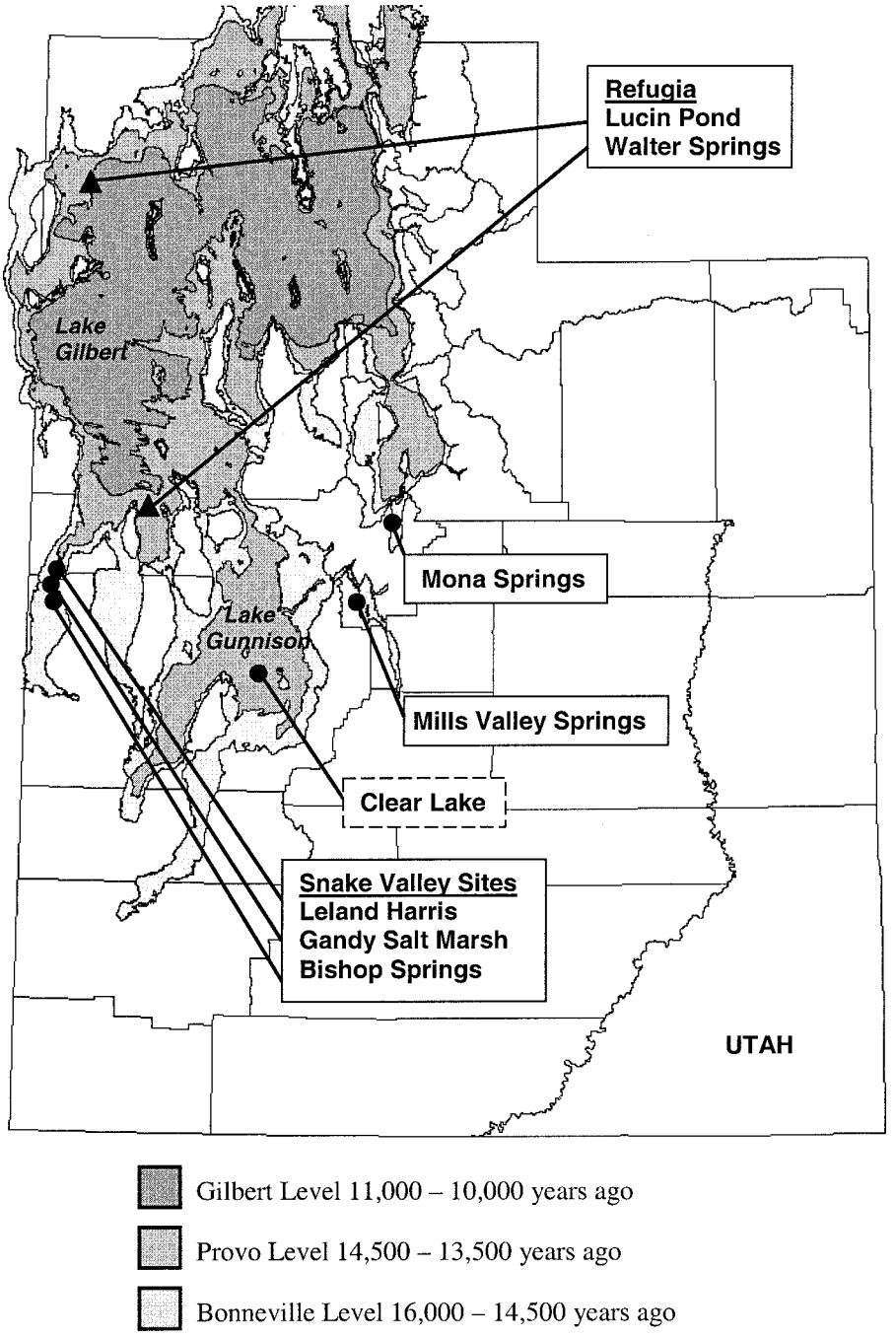

FIGURE 1.—Naturally occurring (circles) and refugial populations (triangles) of least chub employed in this study. Clear Lake contains a recently discovered population that was not included in the study. Water levels at different periods in the history of Lake Bonneville are indicated by shading. (Map adapted from Currey et al. 1984.)

lake receded (Figure 1). Hydrogeologic isolation can lead to neutral genetic divergence among separated populations owing to genetic drift and the accumulation of new population-specific mutations. Isolation can also allow adaptive divergence in populations, particularly if they have distinct environmental conditions or histories. Alternatively, populations of small, cryptic fish are frequently moved from one body of water to another via bait bucket transfers, and the presence of least chub in isolated locations may not mean that these fish evolved in situ. Thus, an understanding of these landscape-scale patterns and anthropogenic processes is important in prioritizing management actions for the conservation of species diversity.

The first objective of this study was to use nuclear and mitochondrial molecular markers to describe patterns of neutral genetic divergence and diversity among five remnant, putatively naturally occurring least chub populations representing three geographically distinct regions (Snake Valley, Mona Springs, and Mills Valley Springs; Figure 1). This type of information is useful in determining whether disjunct populations represent groups with distinct evolutionary histories or are the result of recent anthropogenic transfers. Comparative measures of neutral diversity among populations are useful in the detection of severe bottlenecks, which reduce both neutral and adaptively important genetic diversity. The three Snake Valley populations are spatially isolated but may share occasional hydrologic connections during periods of high flow. As a part of the first objective, we also sought to determine whether these three geographically proximate populations are genetically distinct.

The second objective of this study was to determine whether refugial populations of least chub reflect the neutral diversity found in their source populations. The loss of adaptive genetic diversity via population bottlenecks is a potential concern for all least chub populations since they are presumably small and isolated relative to historical populations. This is a particularly important issue in the refugial populations (Lucin Pond and Walter Springs) since they are known to have undergone a population bottleneck upon establishment (owing to the fact that limited numbers of individuals were translocated).

Study Area

Mona Springs complex (MO).—This naturally occurring population of least chub, discovered in 1995, is in the Utah Lake basin (Figure 1). Least chub continue to be detected at this site in annual surveys, but they exist in low and apparently declining numbers (UDWR 2002a). The Mona Springs complex is quite small by comparison with the Snake Valley complexes, and nonnative fish, particularly mosquitofish *Gambusia affinis*, are thought to be a major competitive threat to this population (UDWR 2002a).

Mills Valley Springs complex (MV).—This spring complex is near the Sevier River in southeastern Juab County (Figure 1) and contains a robust, naturally occurring population that was discovered in 1996. Least chub is the most common fish captured at this site. According to state monitoring reports (UDWR 2002a), this site is free of nonnative fish but is negatively impacted by livestock damage.

Snake Valley.—Naturally occurring populations of least chub exist in the Gandy Salt Marsh (GS), Bishop Springs (BP), and Leland Harris Springs (LH), all located in the Snake Valley of western Utah (Figure 1). The Gandy Salt Marsh and Bishop Springs are in Millard County, and the Leland Harris Springs are in Juab County. All three of these populations have been known for many years. Each of these spring complexes is extensive and much larger than the Mona Springs complex. According to state monitoring reports, the Snake Valley sites are generally free of nonnative fishes and are negatively impacted by livestock grazing (UDWR 2002b). The three least chub populations in the Snake Valley are spatially isolated from each other but are likely to share hydrologic connections during periods of high flow. Whether such connections lead to the exchange of individuals between sites is unknown.

Lucin Pond (LP).—Least chub (42 individuals) were introduced into Lucin Pond in January 1989 from the Snake Valley, but the specific source population (Gandy Salt Marsh, Bishop Springs, and/or Leland Harris Springs) was not well documented. Subsequent monitoring failed to detect least chub in Lucin Pond, and a second introduction into Lucin Pond was made from Leland Harris Springs (89 fish) in October 1989.

Walter Springs complex (WS).—Least chub were introduced into the Walter Springs complex on the Fish Springs National Wildlife Refuge in 1996 from the Leland Harris Springs complex (230 individuals). Least chub was the most common fish species detected at this site until 2001, when mosquitofish became dominant. Least chub continue to be detected in monitoring efforts, but recruitment does not seem to be occurring (Mark Belk,

TABLE 1.—Number of samples included in amplified fragment length polymorphism (AFLP) and mitochondrial (cytochrome *b* [Cyt *b*]) analyses and their respective population diversity indices: percent polymorphic loci (%P), estimated heterozygosity (H), and number of mitotypes present in the samples analyzed.

Population	AFLP			Cyt *b* (*n*)	No. of mitotypes
	n	%P	H		
Mona Springs	31	42.86	0.178	17	5
Mills Valley Springs	41	41.43	0.149	5	3
Leland Harris Springs	6	42.86	0.192	5	2
Gandy Salt Marsh	12	52.86	0.222	5	2
Bishop Springs	24	55.71	0.214	5	4
Lucin Pond	25	51.43	0.181	5	1
Walter Springs	28	50.00	0.164	4	3

Brigham Young University, personal communication).

Methods

Samples from the five naturally occurring populations and two refugial populations were collected by the Utah Division of Wildlife Resources (UDWR) during monitoring efforts in 1997. Additional samples were collected from Mona Springs by UDWR in 2002. Samples of DNA were extracted from tissues by following a salt–chloroform protocol (Mullenbach et al. 1989), and DNA quality and quantity were assessed using 0.7% agarose gels with appropriate size (100-base-pair [bp] ladder) and concentration (λ *Hin*d III digest) standards. The numbers of these samples used for subsequent analyses are presented in Table 1.

An approximately 1,200-bp amplicon containing the mitochondrial cytochrome *b* gene was amplified using polymerase chain reaction (PCR) primers La-A and Ha-A (Dowling and Naylor 1997). Each 50-μL PCR reaction contained 50–100 ng of extracted DNA template, 1× PCR buffer, 0.2 mM deoxynucleotide triphosphates (dNTPs), 2.5 mM $MgCl_2$, 0.5 μM of each primer, and 1 unit (U) of *Taq* polymerase. The reaction was denatured at 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, with a final 5-min extension at 72°C. Amplicons were purified using Microcon-PCR spin columns (Millipore). Using the primers described above, sequencing reactions were performed from both ends of the amplicons with an ABI BigDye 2.0 kit and an ABI 3100 automated sequencer. A third internal sequencing primer (5′GCCTGTACTACGGGTCATA) was designed and used to provide coverage of the intermediate section of the amplicon. For each individual, these three sequences were used to assemble a contiguous sequence with DNA Star SeqMan software (Lasargene). These contiguous sequences were aligned with DNA Star Megalign software (Lasargene), and the aligned sequences were trimmed to a total length of 1,101 bp (nucleotides 10–1,110 of the cytochrome *b* gene; GenBank accession numbers AY641413-AY641427). A haplotype network was constructed via statistical parsimony using TCS software (Clement et al. 2000).

Amplified fragment length polymorphism (AFLP) analysis was used to characterize the nuclear divergence and diversity among naturally occurring and introduced populations of least chub. Marker profiles were generated for a total of 167 individuals from the seven populations studied (6–41 individuals per population; Table 1) by following the basic procedures described by Vos et al. (1995) with some modifications. The restriction step was carried out in a 50-μL reaction volume containing 5 μL of 10× restriction–ligation buffer (100 mM Tris–HAc, 100 mM MgAc, 500 mM KAc, 50 mM dithiothreitol pH 7.5), 5 U *Eco*RI, 5 U *Mse*I, and 50–100 ng isolated DNA. This digest was incubated for 1 h at 37°C. After digestion, 10 μL of a ligation mixture (1 μL 10× restriction–ligation buffer, 5 pmol of the forward *Eco*RI adaptor 5′-CTCGTAGACTGCGACC, 5 pmol of the reverse *Eco*RI adaptor 5′-AATTGGTACGCAGTCTAC, 50 pmol of the forward *Mse*I adaptor 5′-GACGATGAGTCCTGAG, 50 pmol of the reverse *Mse*I adaptor 5′-TACTCAGGACTCAT, 1.2 μL of 10-mM ATP, and 1 U T4 DNA ligase) was added to the restriction reaction and incubated for an additional 3 h at 37°C. The restriction–ligation mixture was diluted 1:10 in Te buffer (10 mM tris, 0.1 mM EDTA; pH 8.0). The preamplification was performed using adenine as the selective nucleotide in a 50-μL reaction volume (1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 μM dNTPs, 0.1 μM *Eco* +A primer 5′-GACTGCGTACCAATTCA, 0.1 μM *Mse* +A primer 5′-GATGAGTCCTGAGTAAA, 1 U *Taq* DNA polymerase, and 5 μL of the diluted restriction–ligation

reaction product). Preamplification reactions were held at 72°C for 2 min and subjected to 30 PCR cycles (94°C for 30 s, 58°C for 30 s, and 72°C for 1 min). The PCR products from the preamplification were viewed on a 1.4% agarose gel containing ethidium bromide to assure that they produced a smear in the 100–600-bp size range. The preamplification reactions were then diluted 1:10 in Te buffer.

The selective amplifications were performed in 10-μL reaction volumes (1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 μM dNTPs, 0.05 μM *Eco*-AXX primer with 5′ 6-FAM labels, 0.2 μM *Mse*-AXX primer, and 2.5 μL of the diluted preamplification reactions) using two additional selective nucleotides (X) per primer. The following five selective primer combinations were used: *Eco*-AGG and *Mse*-ACT, *Eco*-ACG and *Mse*-ACT, *Eco*-AGG and *Mse*-AGA, *Eco*-ACG and *Mse*-AGA, and *Eco*-ACG and *Mse*-ACA. These reactions were denatured at 94°C for 2 min and then subjected to a ''touchdown'' PCR procedure involving a 30-s denaturation step (94°C), a 30-s annealing step (65°C), and a 1-min extension step (72°C). This cycle was repeated nine times with incremental 1°C reductions in the annealing temperature from cycle to cycle. These cycles were followed by 30 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s. Multilocus AFLP marker profiles were resolved on an ABI 3100 automated DNA sequencer following the manufacturer's protocol, using an internal Rox 400 (ABI) size standard. Genographer 1.6 software (http://hordeum.oscs.montana.edu/genographer) was used to visualize and score the gel image. Markers were scored if they were polymorphic across the data set (95% criterion) and could be scored unambiguously. Scoring was performed without reference to sample or population identity. Thirty-two of the 167 samples (19%) were replicated prior to the restriction–ligation stage to assess the methodological and scoring error rate. After final gel scoring, the overall error rate based on these replicates was 1.3%.

The genetic variance in the AFLP data set due to differentiation among the five naturally occurring populations was assessed by means of an analysis of molecular variance (AMOVA) with Arlequin software (Schneider et al. 2000). This analysis, based on simple matching distances among individual AFLP profiles, provided estimates of the population-level structure index F_{ST} for all naturally occurring populations collectively and for all pairs of these populations. Statistical significance was determined using 1,000 permutations of the AFLP profiles among populations to estimate a null distribution. Differentiation between the refugial populations and the naturally occurring Snake Valley populations was assessed in the same fashion. The approach of Weir and Cockerham (1984) was also used to estimate θ_{ST} (an F_{ST} estimator) among naturally occurring populations using Tools for Population Genetic Analysis (TFPGA) software (Miller 1997). For this analysis, population-specific allele frequencies were estimated by means of the Taylor expansion method (Lynch and Milligan 1994) assuming Hardy–Weinberg equilibrium. The standard deviation for θ_{ST} was assessed by jackknifing over loci, and 95% confidence intervals were estimated by bootstrapping 1,000 times over loci. Locus-specific exact testing for population differentiation based on AFLP marker phenotype frequencies (Raymond and Rousset 1995) was also performed for all pairwise populations, including refugia, using the Markov-chain Monte Carlo approach provided in TFPGA (Miller 1997). For both AMOVA and exact testing among naturally occurring populations, a Bonferroni-corrected significance level of $\alpha < 0.005$ was used for interpretation of results. The TFPGA program was also used to construct a dendrogram of all populations (including refugia) based on the unweighted pair group method with arithmetic averages (UPGMA) using Nei's (1972) distance and to assess population-level diversity using percent polymorphic loci (95% criterion) and Nei's (1978) unbiased heterozygosity. For the heterozygosity and distance calculations, the allele frequencies of the recessive genotype were estimated as described above. The relative strengths of the nodes in the population-level UPGMA dendrogram were assessed by bootstrapping 1,000 times over all loci. A simple matching distance matrix of individuals was also constructed and used to perform principal coordinates analyses (PCoA) in NTSYS (Rohlf 2002) in order to visualize the strength and clustering of individuals among naturally occurring populations and source or refugial populations. For the PCoA analyses, separate distance matrices were constructed for (1) naturally occurring populations, (2) the LH and WS populations only, and (3) the LP, BP, LH, and GS populations. Each of these contrasts yielded a separate PCoA diagram using data from the first and second axes (those capturing the most variation).

Results

Mitochondrial Sequencing Analysis

Mitochondrial sequencing analysis revealed 15 distinct mitotypes in least chub across all popu-

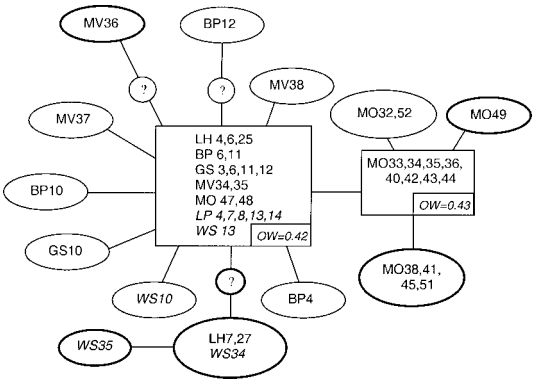

FIGURE 2.—Mitotype network showing mutational changes among least chub cytochrome *b* mitotypes. Individuals are designated according to population: Mona Springs (MO), Gandy Salt Marsh (GS), Leland Harris Springs (LH), Lucin Pond (LP), Bishop Springs (BP), Walter Springs (WS), and Mills Valley Springs (MV). Lines represent single mutational changes. Hypothesized mitotypes that were not observed in the sample are denoted by question marks. The mitotypes with the highest ancestral probabilities are bounded by rectangles, and the outgroup weights (OW) for these mitotypes are denoted in italics. Bold outlines indicate mutations resulting in an amino acid change relative to the haplotype from which it was derived.

lations, most differing by only one or two silent nucleotide changes (Figure 2). Four of the five mitotypes present in MO were not shared by samples from any of the other populations and appeared to be a monophyletic group. However, differences between the MO mitotypes and other mitotypes seen in least chub were very small (1–2 bp). With the exception of MO, there seemed to be little or no geographic structuring among mitotypes. Two of the mitotypes, differing by a single mutational change, had a high probability of being ancestral to the rest (based on TCS gene genealogy estimation; Figure 2). One of these ancestral mitotypes was found only in MO, along with the three mitotypes apparently derived from it (Figure 2). All of the populations except LP contained at least two mitotypes. This suggests that the LP population has lower overall genetic diversity (possibly because of a bottleneck created when the population was established). However, each population (except for MO) was represented by sequences from only four or five individuals, so additional mitotypes may have been undetected.

AFLP Analysis

The AFLP analysis yielded 70 polymorphic loci that were scored and used in subsequent data analyses. The AMOVA results indicated that there was significant structuring among samples from the five naturally occurring populations ($F_{ST} = 0.52$, $P < 0.0001$). The F_{ST} estimates using the approach of Weir and Cockerham (1984) yielded similar results ($\theta_{ST} = 0.48$, 95% confidence interval = 0.399–0.543, SD = 0.038). The AMOVA among all pairs of naturally occurring populations (Table 2) suggested a high degree ($P < 0.005$) of population structuring among all population pairs except GS and BP, two of the Snake Valley populations. These two populations were nonetheless significantly distinct at the $\alpha = 0.01$ level. Exact testing (Table 3) of the differentiation among naturally occurring populations yielded the same pattern, except that results for comparisons of both GS versus BP and GS versus LH were nonsignificant.

With respect to the refugial populations, pairwise exact testing and AMOVA indicated that WS was distinct ($P < 0.001$) from GS and BP but not from LH (exact testing: $P = 0.999$; AMOVA: $P = 0.561$, which is consistent with its having been derived from that population (Table 4). Exact test-

TABLE 2.—Half-matrices of AMOVA results among pairs of naturally occurring populations of least chub, based on amplified fragment length polymorphism data. Populations are denoted as follows: MO, Mona Springs; MV, Mills Valley Springs; LH, Leland Harris Springs; GS, Gandy Salt Marsh; and BP, Bishop Springs. Estimates of F_{ST} are provided above the diagonal and the *P*-values associated with each contrast are provided below the diagonal. Probabilities ≥ 0.005 are in bold italics.

Population	MO	MV	LH	GS	BP
MO		0.6006	0.6242	0.5837	0.5894
MV	0.0000		0.5372	0.4931	0.4887
LH	0.0000	0.0000		0.2372	0.3064
GS	0.0000	0.0000	0.0000		0.0415
BP	0.0000	0.0000	0.0000	***0.0088***	

TABLE 3.—Half-matrix of pairwise exact test results among naturally occurring populations of least chub, based on amplified fragment length polymorphism data. Population codes are explained in Table 2. Probabilities of being drawn from the same population are presented. Probabilities ≥ 0.005 are in bold italics.

Population	MO	MV	LH	GS	BP
MO					
MV	0.0000				
LH	0.0000	0.0000			
GS	0.0000	0.0000	***0.3139***		
BP	0.0000	0.0000	0.0000	***0.9895***	

TABLE 4.—Measures of divergence between refugial populations of least chub (Walter Springs [WS] and Lucin Pond [LP]) and naturally occurring Snake Valley populations (LH, BP, and GS; see Table 2), based on amplified fragment length polymorphism data.

Divergence measure	LH	BP	GS
Nei's distance			
WS	0.023	0.114	0.099
LP	0.078	0.032	0.048
Pairwise exact test			
WS	$P = 0.999$	$P < 0.001$	$P < 0.001$
LP	$P = 0.098$	$P = 0.004$	$P = 0.489$
Pairwise AMOVA			
WS	$F_{ST} = -0.006$	$F_{ST} = 0.370$	$F_{ST} = 0.415$
LP	$F_{ST} = 0.234$	$F_{ST} = 0.096$	$F_{ST} = 0.092$

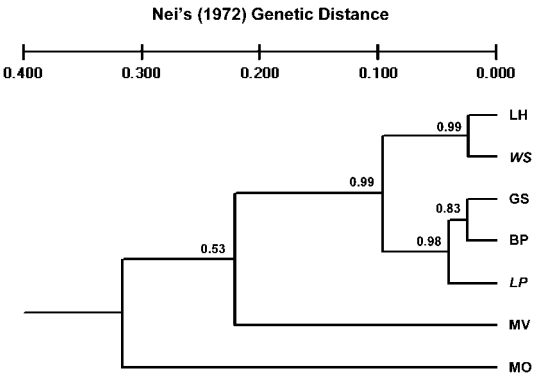

FIGURE 3.—An UPGMA dendrogram of extant least chub populations (natural and refugial) based on data from 70 polymorphic AFLP loci. Naturally occurring population designations are Leland Harris Springs (LH), Gandy Salt Marsh (GS), Bishop Springs (BP), Mona Springs (MO), and Mills Valley Springs (MV). Refugial population designations are Walter Springs (WS) and Lucin Pond (LP), which are shown in italics. Bootstrap proportions (1,000 replicates) are shown at the nodes.

ing indicated that the LP population was distinct from BP but not from GS or LH, although the latter divergence approached significance. The AMOVA indicated that LP was significantly distinct from all three Snake Valley populations ($P < 0.0001$), although it had a greater affinity for BP and GS than for LH (Table 4).

The topology of the UPGMA dendrogram (Figure 3) was generally consistent with the degree of geographic isolation among the naturally occurring populations: Snake Valley populations (LH, GS, BP) were closely associated with each other, while those from MV and MO were distinct from each other and from the Snake Valley populations. Bootstrap analysis indicated that the topology of the UPGMA dendrogram with respect to the positions of the MO and MV populations was not strongly supported. The refugial populations (WS and LP) were associated with the Snake Valley populations: WS was closely allied with its source population LH, and LP was associated with GS and BP, which was consistent with the exact testing and AMOVA results. There was strong bootstrap support for this topology. Principal coordinates analysis of individuals from the naturally occurring populations (Figure 4a) identified three markedly distinct clusters (MO, MV, and the Snake Valley populations). The Snake Valley populations were not markedly distinct from each other in this projection. Principal coordinates analysis of LP and the naturally occurring Snake Valley populations (Figure 4b) indicated that LP is more closely allied with the GS and BP populations than the LH population, consistent with the results discussed above (Table 4; Figure 3). Principal coordinate projections indicated that WS and its source population, LH, were quite similar genetically (Figure 4c). Although the small sample size from LH limited the sensitivity of this contrast, the WS population did seem to encompass the diversity in the LH sample.

The two measures of nuclear genetic diversity used in this study, percent polymorphic loci and heterozygosity, gave somewhat inconsistent patterns among populations (Table 1). However, none of the populations were particularly homogeneous, including the populations established by translocation. Overall, the BP and GS populations appeared to be the most diverse, and the MV population was the least diverse by both measures. All individual AFLP profiles were unique.

Discussion and Management Recommendations

Genetic Divergence among Naturally Occurring Populations

The MO and MV populations of least chub were distinctly divergent from each other and from the Snake Valley populations with respect to AFLP allele frequencies (Tables 2, 3; Figure 3). This divergence contributes to a pronounced partitioning of nuclear genetic variance at the population level in this species ($F_{ST} = 0.52$). The three Snake Valley populations of least chub were less distinct, although the LH and BP populations were consistently divergent from each other. These results suggest that the MO, MV, and Snake Valley popula-

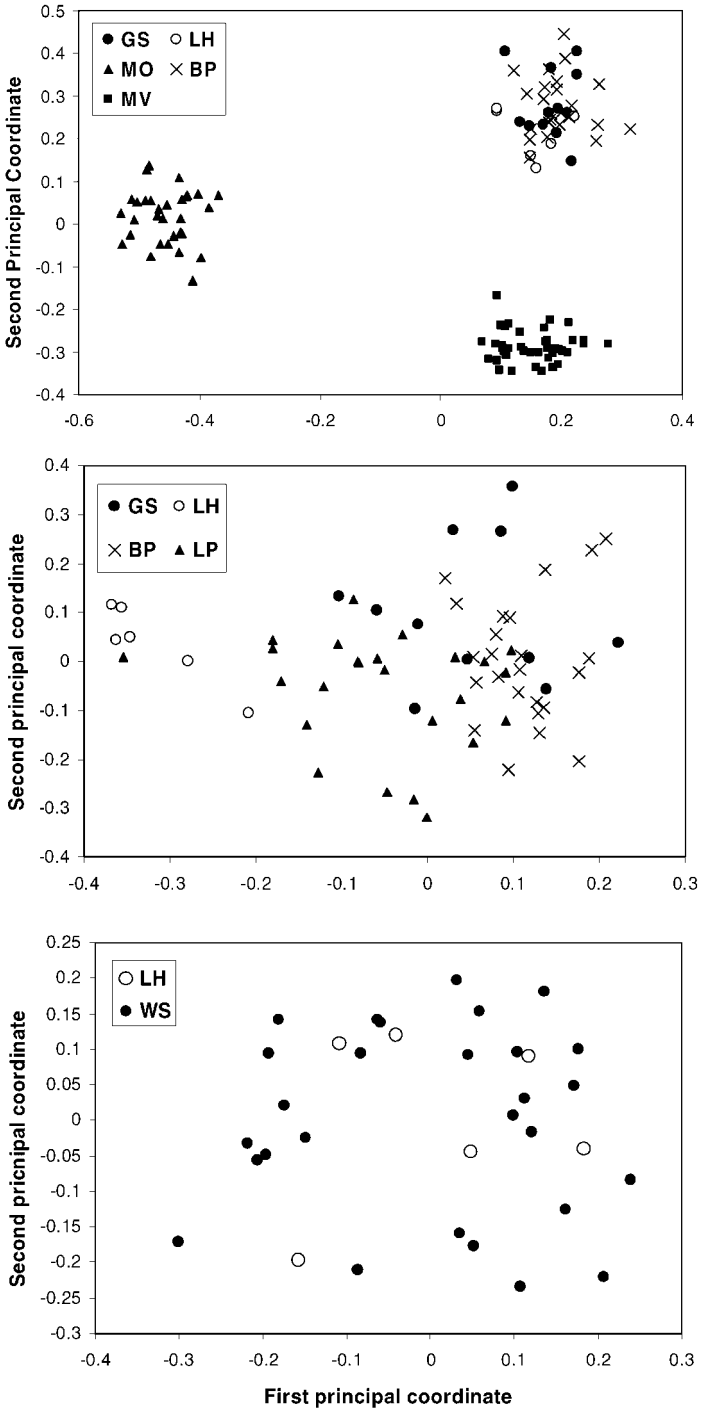

FIGURE 4.—Principal coordinate plots based on simple matching distance matrices constructed with AFLP data from least chub populations. Naturally occurring populations include Gandy Salt Marsh (GS), Leland Harris Springs (LH), Mona Springs (MO), Bishop Springs (BP), and Mills Valley Springs (MV). Refugial populations include Walter Springs (WS) and Lucin Pond (LP). Plots represent clustering of genetic variation among naturally occurring populations of least chub (top panel), Snake Valley populations and the LP refugium (middle panel), and the LH population and WS refugium (bottom panel).

tions are indeed naturally occurring and not the result of bait bucket transfers.

Mitochondrial cytochrome *b* sequences among populations were not highly divergent and produced a starlike phylogeny (Figure 2). The most common mitotype in the species was shared by all populations, and the remaining mitotypes were population-specific derivations of this mitotype differing by one or two mutational steps. None of the derived mitotypes were found to be shared among populations, although our limited sample sizes precluded a thorough assessment of interpopulation mitotype frequency differences.

In the MO population, the common mitotype was detected in 2 out of 17 individuals, and the remaining mitotypes found in this population formed a single clade (Figure 2). This pattern suggests a relatively longer period of isolation in this location. Given the relative frequencies of shared and unique mitotypes in MO, this divergence is unlikely to simply be the result of the disproportionately high numbers of MO samples sequenced. The MV population, which was also markedly divergent from the Snake Valley and MO populations with respect to AFLP profile, did not show the mitochondrial divergence detected in MO. The striking population-level AFLP differences among these three groups in the absence of deep population-specific mitochondrial divergence suggests that nuclear differences among populations are primarily the result of recent differential drift (changing allele frequencies) resulting from isolation rather than population-specific mutations accumulated in situ. We note that this pattern is consistent with the genetic evidence of pronounced, geologically recent population isolation found in other Bonneville basin cyprinids (Johnson and Jordan 2000; Johnson 2002) and mollusks (Mock et al. 2004), which is probably related to late-Pleistocene or early-Holocene vicariance or isolation by distance. The starlike pattern of the mitotype network and the existence of a shared mitotype among populations suggest recent expansion from a common stock with limited mitochondrial diversity followed by isolation and in situ single-step mutational changes and/or differential drift processes among populations. Such a scenario would be consistent with the history of dramatic water-level fluctuations in Lake Bonneville since its maximum level approximately 16,000 years ago (Figure 1).

Snake Valley and Mona Springs were in the Lake Gilbert drainage as Lake Bonneville receded, and the Mills Valley Springs were in the Lake Gunnison drainage (currently the Sevier River drainage), suggesting that these groups have been hydrologically isolated for at least 11,000 years. The presence of the MV population in the Sevier River drainage might suggest that it has been isolated for a longer period of time than the MO and Snake Valley populations, a pattern not supported by our AFLP and mitochondrial data. However, Mona Springs and Snake Valley occupy extreme ends of the ancient Lake Gilbert drainage and their hydrological isolation is likely to have occurred at approximately the same time as the hydrological separation between Lake Gilbert and Lake Gunnison (C. G. Oviatt, Kansas State University, personal communication). In addition, it is possible that the MO population was isolated by ecological factors (e.g., predators or fluctuating lake salinity) well before it was hydrologically isolated.

Regardless of the timing of particular vicariant events in the Bonneville basin, our results clearly show that there is significant structuring with respect to nuclear markers among the naturally occurring populations of least chub in the Snake Valley, MV, and MO. This structuring suggests that these populations have experienced different evolutionary histories, potentially allowing adaptive divergence to have occurred in response to differing selective regimes. From a management perspective, the finding of significant population structure in the least chub suggests that monitoring efforts to identify additional naturally occurring populations could contribute significantly to the effective characterization and conservation of genetic diversity in this species. The genetic diversity and divergence in the newly discovered Clear Lake population should be assessed to determine whether it is an additional naturally occurring population (potentially possessing a large fraction of the species diversity) or may have been established via a bait bucket transfer. In the former case, we may expect the Clear Lake population to be most closely allied with the MV population, since it is in the Sevier drainage.

The results as to differentiation among the Snake Valley populations were inconsistent among analyses. Both the UPGMA analysis and AMOVA results indicated that the LH population was differentiated from both the GS and BP populations, but exact testing did not detect a significant difference between the LH and GS populations. None of the analyses detected a significant difference between GS and BP. The differentiation of LH from other groups may have been an artifact of small sample size ($n = 6$) in that population and warrants further investigation. Owing to their proximity, it is possible that the Snake Valley lo-

cations are hydrologically connected during periods of very high flow, and these connections are likely to have been more pronounced during the wetter periods of the late- and post-Pleistocene epochs.

Although our analyses suggested somewhat shallow, geologically recent mitochondrial divergence, it is important to note that the evolution of adaptively important (and divergent) traits in specific populations can occur over very short periods of time (Thompson 1998; Hendry et al. 2000; Reznick and Ghalambor 2001). We recommend that water quality and habitat parameters, seasonal temperature regimes, life history traits, and morphology be compared among the sites of all naturally occurring populations to assess potential adaptive differences that are not detectable with molecular markers. This information will be an important adjunct to the molecular data presented here (Crandall et al. 2000; Moritz 2002). The Mona Springs site should be a particularly high priority with respect to these studies, given its divergence from the other populations and its tenuous status. Until these determinations can be made, we recommend that the three groups of naturally occurring populations (Snake Valley, Mills Valley Springs, and Mona Springs) be maintained separately to the extent possible to maximize overall diversity in the species and that suitable refugium populations be established and maintained for all three of these unique groups.

Within-Population Genetic Diversity

Although we lack comparative historical data on species-level diversity, none of the naturally occurring populations appeared to be particularly depauperate with respect to molecular diversity. Gandy Salt Marsh and Leland Harris Springs appeared to contain the highest diversity. This suggests that, despite declines in range and local abundance, either (1) these least chub populations have remained large enough to avoid significant population bottlenecks caused by genetic drift or (2) least chub populations were large historically and their recent decline has been so rapid that the loss of population genetic diversity is not yet detectable. Under the second scenario, we would expect genetic drift to rapidly reduce population-level genetic diversity if populations stay small or are subjected to continued bottlenecks. In either case, this apparent lack of severe bottlenecks within populations is a promising result with respect to the management and potential recovery of the least chub.

We recommend that population monitoring surveys be continued to establish long-term and baseline trends, and we support current management goals to establish refugial populations and maintain large population sizes within both naturally occurring and refugial populations. Based on our data, none of the populations appear to require supplemental translocations at this time to counteract a severe population bottleneck. Over the long term, we suggest that microsatellite markers be developed in this species to provide a more sensitive and reliable tool for the detection of population bottlenecks, both in refugial and naturally occurring populations. This effort, however, should be secondary to management measures to search for additional naturally occurring populations and to increase the quantity and quality of habitat for extant populations of this species.

Genetic Diversity and Divergence in Refugial Populations

The refugial populations of least chub in Lucin Pond and Walter Springs remain allied to the Snake Valley populations. Although the power of our analyses was limited by the low sample size from LH, the WS population was indistinguishable from its known source population (LH) according to both the exact and AMOVA approaches. The LP population seemed to be most closely allied to the GS and BP populations (Figures 3, 4; Table 4), suggesting that the initial (poorly documented) introduction into LP was from one of these groups and prior to the documented introduction from LH. Both exact and AMOVA testing suggest that the initial population was established from GS. Populations of least chub established as refugia were similar to their source populations with respect to genetic diversity measures, that is, there was no evidence of reduced diversity owing to a severe bottleneck in these populations. The Walter Springs population, however, was sampled shortly after its establishment, and it is possible that pronounced population reductions have occurred subsequently. Microsatellite markers would be a more appropriate molecular tool with which to assess recent bottlenecks in these populations because such markers are codominant and have greater locus-specific allelic richness.

Our results indicate that the translocation programs, which used large numbers of individuals to establish refugia, have been successful in maintaining the neutral genetic identity of the source populations. This inference is somewhat limited by the low sample number from LH, which could

cause underrepresentation of the source diversity. It is important to recognize, however, that it is possible to lose genetic diversity and increase neutral divergence in these populations if there is a sustained bottleneck in the future. Additionally, it is important to point out that strong differential selective pressures in the source and refugial populations can lead to adaptive divergence in these populations that is not detectable with neutral molecular markers (Stockwell et al. 2003). Such adaptive divergence in refugial populations may cause a loss of fitness in naturally occurring populations if they are used in a supplemental capacity (Lynch and O'Hely 2001) and should be assessed to the extent possible prior to the initiation of supplemental programs.

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