

EVOLUTION AFTER THE FLOOD: PHYLOGEOGRAPHY OF THE DESERT FISH UTAH CHUB

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Abstract.—The Bonneville Basin and upper Snake River drainage of western North America underwent extensive hydrological changes during the late Pleistocene, potentially influencing the geographic distribution and evolutionary trajectories of aquatic species that occupied this region. To test this hypothesis, I reconstructed the phylogeographic history of the desert fish Utah chub (*Gila atraria*) by examining 16 populations that span the natural distribution of this species across the Bonneville Basin and upper Snake River. I compared mitochondrial control region sequences (934 bp) among 77 individuals revealing 24 unique haplotypes. Geographic and phylogenetic relationships among haplotypes were explored using parsimony, maximum likelihood, nested clade analysis, and analysis of molecular variance. I found that *G. atraria* is composed of two distinct clades that represent an early Pleistocene split between the upper Snake River and Bonneville Basin. Within each of these clades, geographic structuring was highly concordant with the hydrological history of late Pleistocene Lake Bonneville and the upper Snake River, suggesting that glacial-induced shifts in climate and unpredictable geological events have played a major role in shaping genetic subdivision among populations. To examine the effects of vicariant events on phenotypic divergence among Utah chub populations, I mapped chub life histories to the control region haplotype network. I found a nonrandom association between haplotypes and life-history phenotypes. These results suggest that historical events responsible for population fragmentation may have also contributed to phenotypic shifts in life histories, both indirectly by limiting gene flow among populations and directly by altering the selective environments where populations persisted.

Key words.—Climate change, intraspecific evolutionary contrasts, Great Basin, historical contingency, Lake Bonneville, Pleistocene glaciation, predation, Snake River.

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Past climatic and geological events can have a profound impact on evolutionary change within species. Historical contingencies can directly affect evolution by altering levels of gene flow in species, thereby modifying the degree of evolutionary independence among populations in response to drift or natural selection (Felsenstein 1976; Endler 1977). Historical events can also indirectly shape evolution by altering the selective environments in which populations exist (Schluter 1998; Bernatchez et al. 1999). Phylogeographic distributions of neutral molecular markers have been used to link historical events to population genetic structuring (Bosart and Prowell 1998; Templeton 1998a). Such studies have become increasingly common (reviewed in Avise 2000) and are beginning to provide rich insight into the geographical history of species throughout much of the world (e.g., Hewitt 2000; Ritchie and Butlin 2001). Yet, despite considerable advances in understanding neutral population subdivision through time (Hudson et al. 1992; Templeton 1998a), relatively little is known about how deterministic factors interact with stochastic historical events to ultimately shape evolutionary divergence in natural systems (Travisano et al. 1995; Taylor and McPhail 2000).

The Bonneville Basin and upper Snake River drainage of western North America (Fig. 1) provide a model system to examine the effects of past geological and climatic events on evolutionary change. Shifts in geography and climate in this region over the past 35,000 years are well documented and potentially important in the evolutionary diversification of aquatic species. In brief, during the late Pleistocene, ice sheets covered the uppermost reaches of the Snake River, limiting organisms to downstream habitats (Currey and James 1982). Coincident with this glacial period was an increase in precipitation to the Bonneville Basin (Currey and James

1982; Oviatt 1997). Tectonic faulting around 35,000 years ago diverted Bear River from the Snake River to the Bonneville Basin, creating an opportunity for faunal exchange from the Snake River to the Bonneville system (Bright 1963). The Bonneville Basin began to fill, ultimately forming a deep pluvial lake (Lake Bonneville) that by 17,000 years ago covered most of the northeastern Great Basin of North America (51,700 km²; Oviatt et al. 1992). Lake Bonneville remained a closed system until 14,500 years ago, when it breached its northern border, causing rapid erosion and catastrophic flooding back into the Snake River (Jarrett and Malde 1987). This provided a second opportunity for faunal exchange between drainages. The consequential reduction in water level (100-m drop), coupled with subsequent climatic shifts to drier conditions, again isolated the Bonneville Basin from the Snake River. Persistent dry conditions further reduced Lake Bonneville (9700 years ago) to two smaller lakes (Lake Gunnison in the south and Lake Gilbert in the north) that ultimately fragmented into present-day springs, rivers, and lakes (see Fig. 1). This shift in climate to warmer, drier conditions also exposed areas of the uppermost Snake River previously covered by glacial ice.

The impact of historical events in western North America on aquatic organisms has been the focus of considerable speculation, particularly with respect to fish distributions (Smith 1978; Minkley et al. 1986). The prevailing hypothesis to account for a distinct faunal affinity between the Bonneville Basin and upper Snake River and to account for the absence of most Columbia River fishes above the Shoshone Falls barrier (Fig. 1) is that Pleistocene volcanic activity prior to 35,000 years ago exterminated fishes in the upper Snake River (Hubbs and Miller 1948). Such events would have limited recolonization of the upper Snake River to late Pleistocene

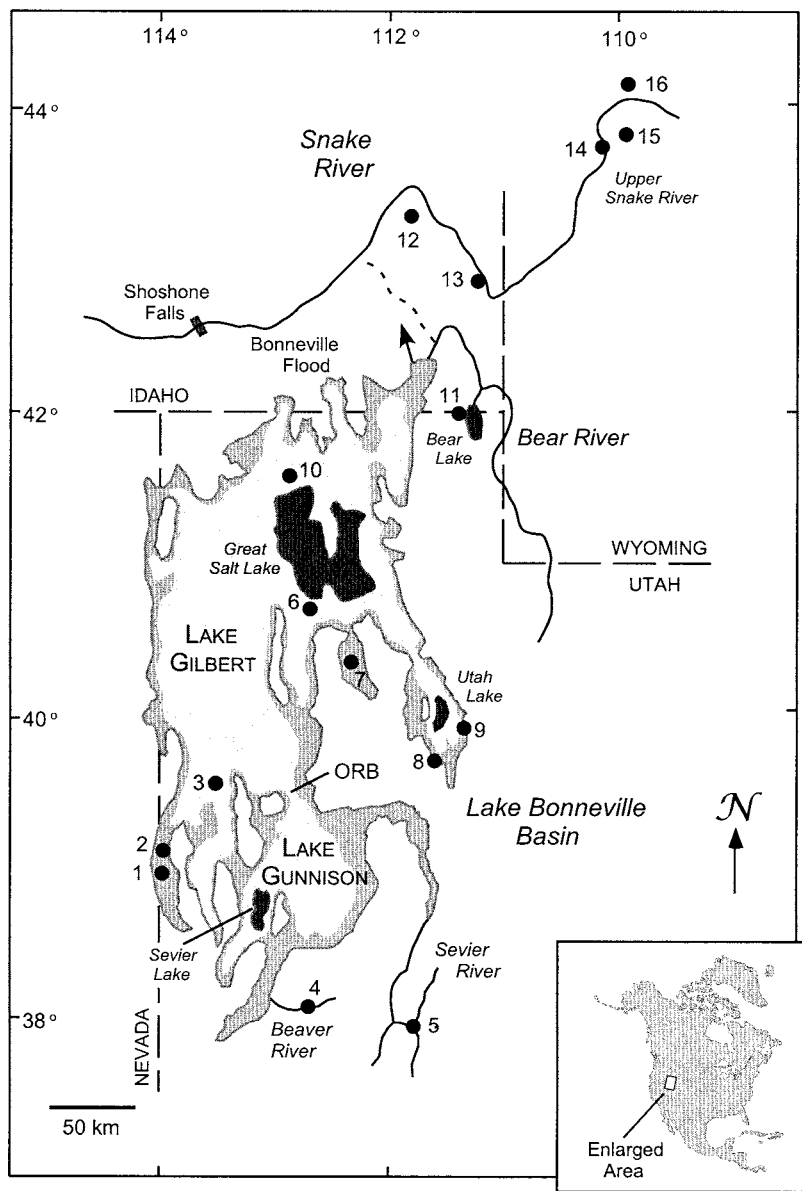


FIG. 1. Geographic distribution map of 16 collecting locations of Utah chub examined in this study (solid, numbered circles). The dashed line shows the course of Bear River into the Snake River prior to its late Pleistocene capture by the Bonneville Basin. Intermediate gray shading represents the high water mark of Pleistocene Lake Bonneville (14,500 years ago) and the arrow identifies the location and direction of the Bonneville Flood; light gray shading shows the subsequent reduction in water levels (9700 years ago) resulting in the isolation of Lake Gunnison from Lake Gilbert through the Old River Bed formation (ORB). Darkest shading and solid lines identify present-day lakes and rivers. See text for a detailed description of the geological history of this region.

exchanges with the Bonneville Basin. If this hypothesis is correct, phylogenetic divergence within species that span these regions should be recent and shallow, and hydrological events linking basins should be reflected in neutral genetic structuring and evolutionary change within such species. Similarly, geographic structuring in aquatic species within the Bonneville and Snake River Basins should reflect the late Pleistocene hydrological history described above. An alternative hypothesis is that biotic exchange between the two basins occurred prior to Pleistocene volcanic activity in the Snake River, and that cataclysmic destruction of aquatic organisms in the upper Snake River was incomplete, with some

species persisting despite volcanic events. Under this scenario, phylogenetic divergence within species across regions could be relatively deep. Advances in acquiring and analyzing DNA sequence data now provide a means to test these hypotheses. In addition, phylogeographic data can be coupled with comparative studies of evolutionary change to examine the effects of historical events on phenotypic diversification within species (Templeton 1998b).

In this study, I examine geographic patterns of molecular variation in the desert fish Utah chub (*Gila atraria*). Fossil evidence shows that the Utah chub historically lived in Lake Bonneville and was geographically widespread throughout

the Bonneville Basin (Smith et al. 1968; Broughton 2000). Native populations of Utah chub currently occupy only the Bonneville Basin and upper Snake River, a pattern repeated across a suite of aquatic species (Hubbs and Miller 1948; Minkley et al. 1986). Furthermore, *G. atraria* is ideal for investigating the effects of historical events on evolutionary changes across populations. This species shows remarkable variation among geographic isolates both in color and in morphology (Hubbs and Miller 1948; Smith 1978), reflected by at least 15 taxonomic synonyms (Tanner 1936). Utah chub also live in a variety of selective environments ranging from harsh desert springs to high alpine lakes, exposure to which has been contingent upon past hydrological connections (Sigler and Sigler 1996). Differences in predation covary with these environments, and previous work suggests that predator-mediated mortality has played a major role in shaping life-history phenotypes among Utah chub populations (Johnson and Belk 1999). Thus, I used Utah chub in this study as a model to examine the effects of late Pleistocene historical events on population subdivision, and to test for an association between phenotypic life-history divergence and phylogeographic structuring.

MATERIALS AND METHODS

Sampling and Molecular Methods

Utah chub were sampled from 16 locations throughout the Bonneville Basin and upper Snake River drainage of western North America; these samples span the natural distribution of extant populations (Fig. 1) and include eight sites where Utah chub life histories have previously been described (Table 1; Johnson and Belk 1999). From each collecting site, I examined five individuals (except Spring Creek [*n* = 3] and Big Spring [*n* = 4]) for a total of 77 individuals. Genomic DNA was extracted from alcohol-preserved muscle tissue using a proteinase K digestion, ammonium acetate separation of proteins, and cold ethanol precipitation. I amplified the mitochondrial control region (d-loop) by polymerase chain reaction using flanking primers L-Pro (Meyer et al. 1994) and MRT-2 (Ptacek and Breden 1998). The thermal profile (2 min at 94°C; 1 min at 55°C; 1.5 min at 72°C) was repeated 35 times, followed by a 10-min extension at 72°C. Purified double-stranded DNA was then used as template (~ 80 ng) in 15 µl cycle sequencing reactions using Big Dye chemistry (Applied Biosystems, Inc., Palo Alto, CA). For each individual, I sequenced 934–936 bp (variation due to insertions/deletions) in both forward and reverse directions using external primers L-Pro and MRT-2, and an internal primer 12R (reverse of H16498 in Shields and Kocher 1991). Products of the cycle sequencing reactions were cleaned with sephadex packed in CentriSep spin columns (Princeton Separations, Inc., Adelphia, NJ) and visualized by electrophoresis using an ABI 310 automated sequencer (Applied Biosystems). Sequences are archived in the GenBank database (accession numbers AF481739–AF481762).

Data Analysis

Utah chub control region sequences were edited and unambiguously aligned using Sequencher 3.0 (GeneCodes

TABLE 1. Collection sites for Utah chub (*Gila atraria*) including location identification numbers, population names, and geographic drainage basins. Lakes Gilbert and Gunnison are remnants of Lake Bonneville. ID numbers correspond to those in Figure 1 and haplotypes correspond to those in Figures 2–4. Counts in columns under each haplotype category identify the number of individuals from each population with a particular haplotype; empty cells indicate zeros.

ID	Population	Drainage	Haplotypes																								
			A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	
1	Bishop Spring	Gilbert							1																		
2	Leland Harris Spring	Gilbert						2				3															
3	Fish Springs ^{PF}	Gilbert	1	1	1	1	1																				
4	Beaver River	Gunnison																									
5	East Fork Sevier River	Gunnison																									
6	Big Spring ^{PF}	Gilbert			3					1																	
7	Rush Lake ^{PF}	Gilbert																									
8	Spring Creek	Gilbert																									
9	Mona Spring	Gilbert			1																						
10	Locomotive Spring ^{PF}	Gilbert							1																		
11	Bear Lake ^P	Bear ¹																									
12	Ririe Reservoir	Snake River																									
13	Pallisades Reservoir	Snake River																									
14	Jackson Lake ^P	Snake River																									
15	Two Ocean Lake ^P	Snake River																									
16	Heart Lake ^P	Snake River																									

^{PF} Populations for which life-history data are also known from Johnson and Belk (1999). P, predator life history; PF, predator-free life history.
¹ The Bear River drainage (including Bear Lake) was historically connected to the Snake River; volcanic faulting in the late Pleistocene (35,000 years ago) diverted Bear River into the Bonneville Basin (where it currently drains; see Fig. 1) contributing to the subsequent flooding (14,500 years ago) of Lake Bonneville into the Snake River, thereby introducing Bonneville Basin fishes into the Snake River drainage (see Introduction).

Corp., Ann Arbor, MI). However, ambiguities in alignment between Utah chub haplotypes and the outgroup required cropping 28 bp from the upstream end of sequences evaluated in maximum-parsimony and maximum-likelihood phylogenetic analyses (described below). Single base-pair insertions and deletions within the contiguous sequences were retained and coded as fifth base characters. Individual sequences were collapsed to common haplotypes using the software program TCS 1.13 (Clement et al. 2000) and these haplotypes, including frequencies, were used in subsequent phylogenetic and population genetic analyses. Basic sequence attributes, such as raw haplotype divergence and base composition, were calculated using PAUP* 4.0b8 (Swofford 1999).

I employed two sets of techniques to resolve phylogeographic associations among Utah chub haplotypes: (1) maximum-parsimony and maximum-likelihood analyses (Swofford et al. 1996); and (2) statistical parsimony coupled with nested clade analysis (Templeton et al. 1992; Templeton 1998a). These techniques are complementary in resolving intraspecific phylogenies (Crandall 1994). Traditional parsimony and likelihood approaches are well suited for the analysis of deep haplotype divergence, characterized by bifurcating phylogenetic groups supported by many synapomorphic traits. Statistical (or TCS) parsimony is designed to resolve shallow phylogenetic divergence where few characters distinguish haplotypes, and when coupled with nested clade analysis, can be used to infer historical processes responsible for genetic structuring among populations (Templeton 1998a). I also tested explicitly for congruence between genetic differentiation among chub populations and known geological events using analysis of molecular variance (AMOVA; Excoffier et al. 1992). Finally, I used the nested cladistic framework to test for associations between historical haplotype structuring and phenotypic life-history divergence among Utah chub populations. Details of each of these analyses are outlined below.

Phylogenetic analyses

I reconstructed phylogenetic relationships among Utah chub haplotypes under maximum-parsimony and maximum-likelihood criteria. Both analyses were performed using PAUP* 4.0b8 (Swofford 1999). I conducted an equally weighted maximum-parsimony analysis using the heuristic search option with the starting tree generated through 10 replications of random stepwise addition and TBR branch swapping. A strict consensus was used to reconcile equally parsimonious topologies. Support for each node was evaluated by 1000 bootstrap replicates (Felsenstein 1985).

Phylogenetic analysis under the maximum-likelihood criteria requires an explicit model of molecular evolution. To identify the best-fitting model for the Utah chub control region, I followed the procedure outlined in Posada and Crandall (1998). In brief, I arbitrarily generated a neighbor-joining tree calculated under a Jukes-Cantor model of evolution (Jukes and Cantor 1969) and held this tree constant to evaluate the fit of 56 different models of DNA sequence evolution (models described in Swofford et al. 1996). Negative log-likelihood scores (generated in PAUP* 4.0b8) described the fit of each model to the observed sequence data; I selected

the best-fitting, simplest model from this set based on hierarchical likelihood-ratio tests executed in MODELTEST 3.06 (Posada and Crandall 1998). This technique identified the HKY + I + Γ model (Hasegawa et al. 1985) as the simplest model (-ln likelihood score = 1599.44) statistically indistinguishable from more complex models with slightly better scores; hence, I used this model (including parameter estimates) to conduct a heuristic maximum-likelihood search (again with 10 replications of stepwise addition and TBR branch swapping). Nodal support for the resulting tree was evaluated by 100 bootstrap replicates.

Maximum-parsimony and maximum-likelihood reconstructions were rooted using the control region sequences of *Gila bicolor*, a representative outgroup selected based on the phylogenetic hypothesis of Simons and Mayden (1998). I sequenced two *G. bicolor* individuals taken from the Pit River (Ash Creek National Wildlife Refuge, Modoc County CA) following protocols described above. In addition to outgroup rooting, I evaluated whether a molecular clock could be enforced in a maximum-likelihood search, potentially revealing ancestral relationships among Utah chub haplotypes. This was accomplished using a likelihood-ratio test to compare the unconstrained maximum-likelihood tree based on the best-fitting model (described above) to a tree topology constrained under a molecular clock. Thus, both outgroup comparisons and a molecular clock were used to reveal polarity among chub haplotypes.

Nested clade analysis

I used nested clade analysis to identify genetic relationships among Utah chub populations and to infer historical processes responsible for geographic structuring among haplotypes (reviewed in Templeton 1998a). In brief, I used the statistical parsimony criterion (Templeton et al. 1992) to arrange Utah chub haplotypes into a minimum spanning phylogenetic network (TCS 1.13; Clement et al. 2000). Under this Bayesian algorithm, I found that haplotypes differing by as many as 13 substitutions could be connected with a 95% probability that no multiple substitutions had occurred (see eq. 2 in Templeton et al. 1992). Ambiguities (loops) in the resulting network were resolved as outlined in Crandall and Templeton (1993). I then organized the network into a set of nested clades (following rules in Templeton et al. 1987; Templeton and Sing 1993) that were used to test for significant associations between haplotypes (or nested groups of haplotypes) and geographic sampling locations (statistical tests described below). Pairwise geographic distances were measured among sampling locations based on late Pleistocene water connections (Jarrett and Malde 1987; Currey 1990) and are available upon request. I used the software program GeoDis 2.0 (Posada et al. 2000) to calculate the geographic spread of each clade (D_c , clade distance), the geographical distribution of each clade relative to other clades in the same higher-level nesting category (D_n , nested clade distance), and the average distance between tip clades and interior clades in the network ($I-T$, interior-tip distance) at both clade and nested clade levels. Combined, these distances describe the geographic spread of older clades (interior) relative to younger clades (tip) and can be used to infer patterns of geographic

structuring through space and time (Castelloe and Templeton 1994). Interior and tip designations were unambiguous across the nested network, except at the final nesting level, where I scored clade 3-2 as interior and clade 3-1 as the tip (based on degree of similarity of each clade to the outgroup, assuming a molecular clock).

For each nesting level, a contingency table was constructed with nested clades (including haplotypes) forming the rows and geographic locations forming the columns. Because of relatively small sample sizes within some nesting groups, traditional chi-square tests may not be valid (Templeton et al. 1988). Thus, contingency tables were randomly permuted 1000 times (preserving marginal values of clade frequencies and sample sizes) under the null hypothesis of no association between clades and geographic locations (Templeton and Sing 1993). Clade distances, nested clade distances, and interior-tip distances were calculated during each iteration and provided the null distribution against which observed distance values were tested to be significantly large or small ($P = 0.05$ criterion). These statistical tests were executed in GeoDis 2.0 (Posada et al. 2000). I explored the results of these analyses using the inference key of Templeton (1998a), which summarizes patterns of haplotype distributions expected when genetic structuring is the result of restricted gene flow, vicariant fragmentation events, or past range expansions.

Analysis of molecular variance

To explicitly test the effects of geological isolating events on genetic subdivision among Utah chub populations, I used AMOVA (Excoffier et al. 1992) to examine genetic structuring among three geographical regions: (1) Snake River/Bear River (populations 11–16); (2) Lake Gilbert (populations 1–3 and 6–10); and (3) Lake Gunnison (populations 4 and 5). I partitioned total genetic variation into differences among localities within geographical regions (F_{SR}), among localities across the entire study area (F_{ST}), and among the three historically distinct geographical regions (F_{RT}). This framework provided a test of the a priori hypothesis that genetic divergence among populations has been shaped by late Pleistocene hydrological events that separated the Bonneville Basin from the upper Snake River, and that fragmented Lake Bonneville into Lake Gilbert and Lake Gunnison (Fig. 1). Analyses were run in ARLEQUIN 2.0 (Schneider et al. 2000).

Mapping life histories to phylogeography

I also used the nested cladistic framework described above to examine phylogeographic patterns of phenotypic life-history divergence among a subset of eight Utah chub populations (Table 1). In previous work (Johnson and Belk 1999), I found that Utah chub from localities where cutthroat trout predators exist (populations 11, 14, 15, and 16) showed delayed maturity, larger size at maturity, decreased reproductive effort, and increased growth rate compared to chub from predator-free environments (populations 3, 6, 7, and 10). Size-selective mortality directed at smaller, younger chub by gape-limited trout provided the best overall explanation for observed life-history shifts. However, differences among

populations in density, habitat type, and temperature were partially confounded with predation: Chub that lived with trout tended to live in lower densities, in larger water bodies, and at colder temperatures (Johnson and Belk 1999). Thus, “predation environment,” as defined in this study, is potentially composed of a suite of interacting ecological factors that could combine to create the selective pressure responsible for the observed life-history shifts—such composite selective environments appear to be typical of fish systems where predator-mediated life-history evolution has been demonstrated (Reznick et al. 2001; Johnson 2002).

To use the nested cladistic framework to evaluate life histories (analysis described below), I first used a discriminant function analysis (Sokal and Rohlf 1995) to collapse the four observed life-history traits to a single composite variable that I found to differ between populations classified by the presence or absence of predators (Wilke’s $\lambda = 0.005$, $F_{4,2} = 91.8$, $P = 0.01$). Based on this finding, I scored each of the eight chub populations categorically as having either predator or predator-free life-history phenotypes. I then tested the null hypothesis that life-history phenotypes were not associated with phylogeographic structuring as defined by the hierarchy of nested clades. This involved scoring individuals with either predator or predator-free phenotypes and then testing for an association between phenotypes and haplotypes (or clades) at each applicable level of the nested hierarchy. These tests were conducted as chi-square permutation tests (1000 replicates maintaining marginal values) within nested clades 2-2, 3-1, and the total cladogram. Rows consisted of categorical life-history phenotypes (predator vs. predator-free) and columns consisted of nested clades. Permutation tests were run in CHIPERM 1.2 (Posada 2000). Nonrandom associations between haplotypes (or clades) and life-history phenotypes were used to identify cases in which vicariant processes responsible for genetic structuring (determined by the phylogeographic analyses) could also have contributed to divergent shifts in chub life histories.

RESULTS

I found 24 unique mitochondrial haplotypes (lettered A–X; Table 1) from Utah chub populations sampled across the natural geographic distribution of this species (Fig. 1). Maximum corrected sequence divergence among all haplotypes was 3.79% (Appendix). Of the 22 variable nucleotide positions observed (of 936 bp), 17 were parsimony informative. Average nucleotide base frequencies among haplotypes were A = 0.32, T = 0.32, C = 0.22, and G = 0.14.

Phylogenetic Analyses

Maximum parsimony and maximum likelihood consistently identified two evolutionarily distinct sister clades of Utah chub (Fig. 2). One clade (hereafter called the Snake/Bear clade) consisted of three haplotypes (V, W, and X) that occurred only in Bear Lake and throughout the upper Snake River drainage (Table 1). The second clade (hereafter called the Bonneville clade) was composed of 21 haplotypes found only in the Lake Bonneville drainage basin, with the following exceptions: haplotypes K, L, and N occurred in the Bonneville Basin and the uppermost Snake River and haplotype

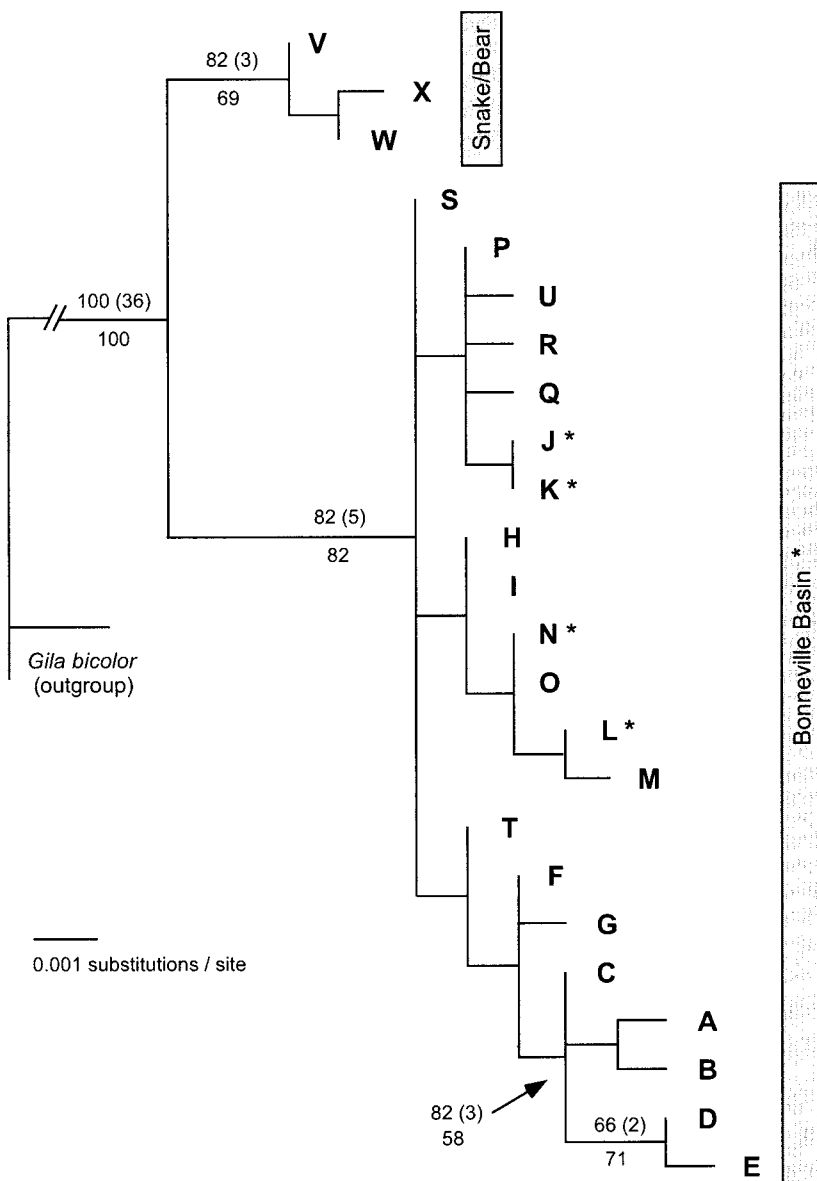


FIG. 2. Maximum-likelihood phylogeny estimated under the HKY + I + Γ model of sequence evolution ($-\ln L = 1606.61$) depicting relationships among 24 control region haplotypes from 16 Utah chub populations. Nodes supported on this tree are consistent with those from a strict consensus of 627 most parsimonious trees (71 steps, CI = 0.85, RI = 0.89) from the maximum-parsimony analysis. Numbers at each node indicate bootstrap support greater than 50% from maximum parsimony (above line) and maximum likelihood (below line) and the number of unambiguous characters supporting each node in the parsimony analysis (in parentheses above line). The bars adjacent to the haplotypes identify geographic regions where haplotypes occur (see Fig. 1) with the asterisks denoting the following exceptions: haplotypes K, L, and N occur in both the Bonneville Basin and the upper Snake River and haplotype J is found only in the upper Snake River. *Gila bicolor* is an outgroup taxon used here to root the phylogeny.

J (represented by just one individual) was found only in the Snake River drainage (Table 1).

Maximum parsimony and maximum likelihood yielded similar results. The maximum-parsimony analysis (with outgroup included) produced 627 equally parsimonious trees of 71 steps (consistency index [CI] = 0.85; retention index [RI] = 0.89) that were combined into a strict consensus tree (Fig. 2). A total of 36 unambiguous characters supported monophyly of Utah chub relative to the outgroup *G. bicolor*; three and five unambiguous characters supported reciprocal monophyly of the Snake/Bear clade and the Bonneville clade, respectively.

Maximum-likelihood analysis also produced a well-supported phylogeny that distinguished the Snake/Bear and Bonneville clades ($-\ln$ likelihood score = 1606.61). The likelihood score of this phylogeny—generated under the HKY + I + Γ model of molecular evolution—did not differ from that produced under the constraint of a molecular clock ($\chi^2 = 27.7$, $df = 24$, $P = 0.27$). However, neither maximum-likelihood nor maximum-parsimony analyses could adequately resolve relationships among individual haplotypes within either of the two Utah chub clades, despite some support for a subgroup of five haplotypes (A–E) in the Bonneville clade (Fig. 2).

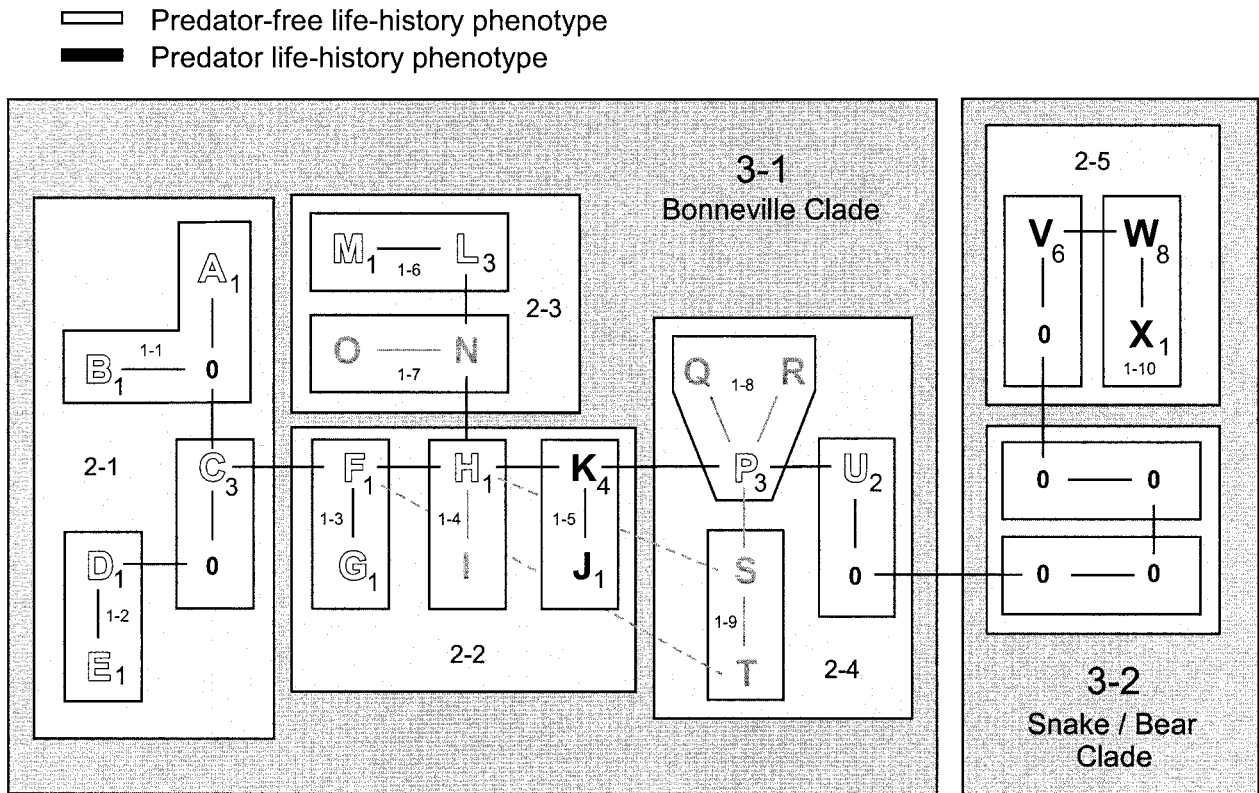


FIG. 3. Nested haplotype network (cladogram) of mitochondrial DNA sequences of Utah chub constructed under the statistical parsimony criterion. Letters represent unique haplotypes and correspond to those given in Table 1 and Figure 2. Each line in the network represents a single mutational change between haplotypes. Dashed lines represent alternative connections among haplotypes. A zero indicates a haplotype not present in the sample, but inferred to be intermediate between two nearest-neighbor haplotypes. Background shading and boxing identify the hierarchical nesting framework used in the nested clade analysis (detailed in Materials and Methods). The network was also used to test for a relationship between phylogeography and phenotypic life-history divergence among chub populations. Haplotypes are coded by their associations with life-history phenotypes scored for eight of the 16 populations (taken from Johnson and Belk [1999] and identified by superscripts in Table 1): open block letters identify haplotypes from locations characterized by predator-free life histories; solid block letters identify haplotypes from locations characterized by predator life histories (see text for details). Subscripts next to haplotypes indicate the number of individuals in the life-history sample with that particular haplotype. Light-shaded haplotypes were excluded from the life-history permutation analysis due to lack of life-history information from locations where these haplotypes occur.

Nested Clade Analysis and AMOVA

Statistical parsimony justified the construction of a well-resolved phylogenetic network (cladogram) of haplotypes, including haplotypes marked by extremely shallow phylogenetic divergence (Fig. 3). In addition, this network also revealed the deeper split between the Snake/Bear clade and the Bonneville clade; seven mutational steps separated the two most closely related haplotypes from each respective clade (Fig. 3). Haplotypes fully nested within the network created a four-level hierarchy that included 10 one-step clades, five two-step clades, two three-step clades, and the total cladogram (Fig. 3). Of these, clades 1-1 and 1-2 could not be tested for geographic associations among haplotypes because haplotypes in these clades showed no geographic variation within the nested clade, and clade 3-2 could not be tested because one of its two-step clades contained only unsampled haplotypes (Fig. 3).

Nested clade analysis revealed significant geographic structuring among Utah chub haplotypes at varying geographic scales across the distribution of this species (Fig. 4).

For example, regional structuring was evident in clade 1-8 and to varying degrees in clades 2-1, 2-2, and 2-4 (Table 2). Genetic structuring across the entire geographic distribution of the species was most pronounced in clades 3-1 and in the total cladogram (Fig. 4), variation that corresponds to the deeper phylogenetic divergence revealed in the maximum-parsimony and maximum-likelihood analyses. Calculated distances of geographic spread among haplotypes are reported in Figure 4 and show a mixed pattern ranging from restricted geographic distributions in some nested clades to larger than expected distributions in others. Interpretation of these distances for each clade (based on Templeton's [1998a] inference key) revealed evidence for historic range expansion, long-distance colonization, and geographic fragmentation, with these processes operating at different times in different geographic regions (Table 2).

AMOVA results were consistent with the nested clade analysis, revealing significant genetic variation across a hierarchy of three geologically subdivided regions. With populations divided into late Pleistocene drainage basins (Lake

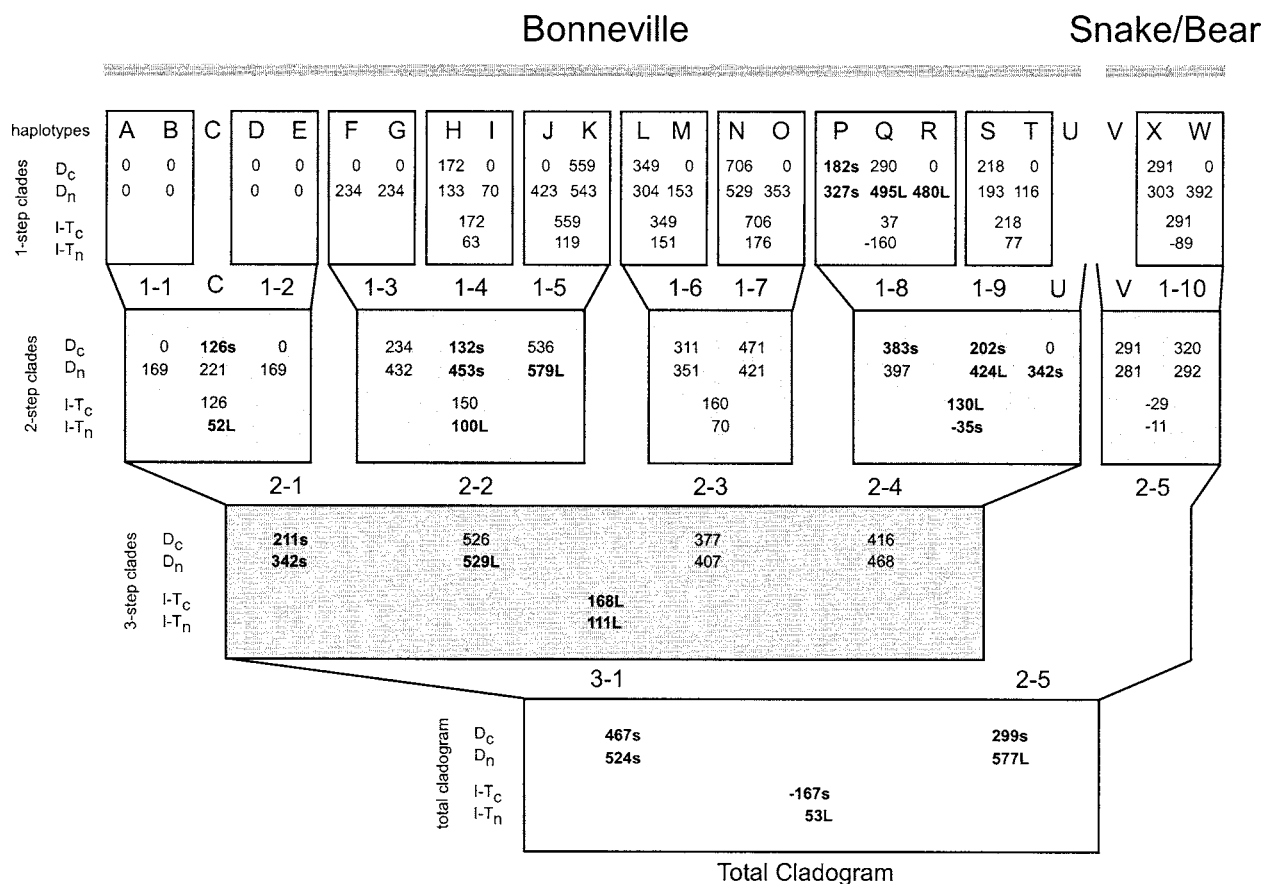


FIG. 4. Results of the nested clad analysis. Lettered haplotypes are shown across the top of the figure (and correspond to those given in Figs. 2, 3). Moving from top to bottom, haplotypes are grouped into one-step clades (white boxes), one-step clades are grouped into two-step clades (light-shaded boxes), two-step clades are grouped into three-step clades (dark-shaded boxes), and three-step clades are grouped to enclose the entire cladogram; this same nested hierarchy is depicted graphically in Figure 3. The clade (D_c) and nested clade (D_n) distances are given below each haplotype (or higher level clade), and I-T distances for D_c and D_n are given in cases where both tip and interior clades occurred in the nesting group. Significance was determined by permutation tests ($P < 0.05$) and distances found to be significantly large or small are bolded and identified by the letters L or s, respectively.

Gunnison, Lake Gilbert, and the Snake/Bear basin), most of the total variation among haplotypes (43%) was explained by differences among basins ($F_{RT} = 0.42, P < 0.01$). Differences among populations overall accounted for 40% of the total variation ($F_{ST} = 0.60, P < 0.01$) and differences among populations within regions accounted for just 17% of the total variation ($F_{SR} = 0.30, P < 0.01$).

Mapping Life Histories to Phylogeography

Phenotypic life-history variation among Utah chub populations mapped unambiguously to the haplotype network (Fig. 3). Twelve chub haplotypes were identified from predator-free sites and five haplotypes were identified from predator sites. The distribution of life-history phenotypes on the network was not random. Nested clades 1-5 and 2-5 both contained only haplotypes associated with predator phenotypes, whereas adjacent clades contained only haplotypes associated with predator-free phenotypes. Nonrandom association of haplotypes and phenotypes in clades 2-2 ($\chi^2 = 8.0, P < 0.01$) and 3-1 ($\chi^2 = 12.0, P < 0.01$) occurred independently of the nonrandom association found in clade 3-2. In other words, associations between clades and phenotypes oc-

curred separately in two distinct parts of the cladogram. Finally, a significant association also occurred across the entire cladogram ($\chi^2 = 24.0, P < 0.01$), due to the fixation of predator phenotypes in clade 3-2 (15 predator vs. none predator-free) and the relatively high frequency of predator-free phenotypes in clade 3-1 (five predator vs. 20 predator-free).

DISCUSSION

Phylogeography

Genetic subdivision in Utah chub is strongly associated with geography, both between the upper Snake River and Bonneville Basin and within each of these respective regions. However, the pattern of phylogenetic divergence among chub haplotypes is more complex than that predicted by only the past 35,000 years of hydrological history. In particular, I found an unexpectedly deep split between two monophyletic clades of Utah chub (Fig. 2) that coarsely distinguished populations in the Bear and Snake River drainages from those in the Bonneville Basin proper (Fig. 1). Yet, within each of these ancient clades, shallow phylogeographic structuring was remarkably concordant with the late Pleistocene history

TABLE 2. Inference chain based on geographic distance measures calculated in the nested clade analyses. Results are reported only for clades in which the observed χ^2 was found to be significantly larger than the χ^2 generated under the random permutation tests.

Clade	χ^2 probability	Chain of inference ¹	Inference
1-8	0.02	1-2-11-12-no	Contiguous range expansion between Lake Gilbert and Lake Gunnison Basins
2-1	0.04	1-2-11-12-13-14-no	Long-distance colonization between western Bonneville Basin (Fish Springs) and eastern Bonneville Basin (Big Spring and Mona Springs)
2-2	<0.01	1-2-3-5-15-no	Past fragmentation between Fish Springs/Big Spring and low-lying habitats on the south-eastern (Bishop Springs/Leland Harris Springs) and northern (Locomotive Springs) edges of Lake Gilbert
2-4	0.06	1-2-3-5-6-7-yes	Restricted gene flow dispersal with some long-distance dispersal between Lake Gunnison sites (Beaver River and East Fork Sevier River) and geographically widespread populations in the Lake Gilbert Basin (Rush Lake, Leland Harris Springs, and Mona Springs)
3-1	<0.01	1-2-3-4-no	Restricted gene flow primarily within the Lake Bonneville Basin, probably concomitant with high water levels of Lake Bonneville
Total	<0.01	1-2-3-5-15-no	Past fragmentation between the Bonneville Basin and the upper Snake River/Bear River drainages

¹ Inferences based on Templeton's (1998a) inference key.

of this region, suggesting that historical contingencies of geology and climate have had a profound effect on more recent genetic subdivision in this species.

Shallow divergence: climate change and geology

Extant Utah chub populations in the Bonneville Basin are highly fragmented, living in desert springs, streams, and lakes that are separated by vast stretches of arid land. How these localities became geographically isolated from one another can be reconstructed from the palaeohydrological chronology of Lake Bonneville and the upper Snake River (Oviatt et al. 1992). As the Bonneville Basin filled and flooded, range expansion of chub to new habitats could have occurred as high water levels provided access to an increasing distributional range. Subsequent decline in lake levels caused by the Bonneville flood would have rapidly isolated groups of populations occupying habitat at the lake periphery. Climate-induced desiccation led to fragmentation among the upper Snake River, Lake Gunnison, and Lake Gilbert, leaving the latter two lake basins connected by just a single river (Old River Bed channel; Fig. 1). Further drying of Lake Gunnison left the Sevier River and Beaver River to drain directly into terminal Sevier Dry Lake. Similarly, the transformation of Lake Gilbert to modern Great Salt Lake left Utah chub isolated to peripheral freshwater habitats. This sequence of events can be viewed as a set of null hypotheses against which observed patterns of genetic subdivision can be evaluated. Do such historical events predict specific patterns of phylogeographic divergence in Utah chub?

Two analyses employed in this study were used to answer this question. First, nested clade analysis pointed to multiple cases of genetic subdivision in Utah chub that were consistent with the outlined hydrological history (Table 2). As the lake increased to its maximum level 17,000 years ago, contiguous range expansion and colonization events were evident in clades 1-8 and 2-1, respectively. Clade 3-1 showed a pattern of isolation by distance consistent with levels of gene flow at the highest lake levels. The presence of haplotypes from the Bonneville clade in the upper Snake River drainage (Fig. 2) supports a connection between basins via the Bonneville flood. Evidence that this faunal exchange was recent is in-

ferred from the lack of extensive unique mutational change to the Bonneville haplotypes now present in the Snake River (haplotypes K, L, and N). Fragmentation in clade 2-2 is consistent with isolation expected among locales in the southwestern region of the lake basin as the lake declined. Restricted gene flow between Lake Gilbert and Lake Gunnison, through the Old River Bed formation, is consistent with the pattern in clade 2-4. In fact, only nesting at the total cladogram level revealed genetic variation that could not be explained by the late Pleistocene history of Lake Bonneville. This level of divergence most likely reflects the deeper phylogenetic split between the upper Snake River and the Bonneville Basin discussed below. Thus, the nested clade analysis revealed a remarkably good qualitative fit of the data to the hydrological history of this region. To more explicitly test for vicariant fragmentation, I also used an AMOVA (Excoffier et al. 1992). Results from this analysis were completely consistent with the nested clade findings and showed strong genetic subdivision among remnant populations from Lake Gunnison, Lake Gilbert, and the upper Snake River. Clearly, evidence for late Pleistocene vicariant events pervades the phylogeographic data.

Deep divergence: implications for regional biogeography

Phylogenetic divergence between the Bonneville clade and the Snake/Bear clade was pronounced and unexpectedly deep. Maximum-likelihood and maximum-parsimony analyses both showed strong evidence for reciprocal monophyly between these clades (Fig. 2). The statistical parsimony network confirmed this level of divergence: Seven unambiguous mutational steps distinguished even the most closely related haplotypes between clades (Fig. 3). By applying a conservative molecular clock (1% divergence per 1 million years; Bermingham et al. 1997), I found that this split dates to late Pliocene or early Pleistocene (1.6 million years ago), well before hydrological events of the past 35,000 years. Moreover, the geographic distribution of the two clades points to an early Pleistocene event that isolated chub in the upper Snake River from those in the Bonneville Basin. It also appears that isolation between regions was persistent, interrupted only by the late Pleistocene faunal exchange between

basins. Thus, these findings refute the hypothesis that the fish fauna of the upper Snake River were exterminated by volcanic activity prior to the late Pleistocene introduction of fishes via the Lake Bonneville flood.

An earlier survey of geographic variation in allozymes in Utah chub provided poor resolution of recent phylogeographic relationships among populations and called for the use of more rapidly evolving mitochondrial markers (Rosenfeld 1991). My results show that genetic structuring within Utah chub has been a temporally dynamic process, with recent patterns of gene flow overlaid on earlier patterns of divergence. Deep divergence in Utah chub between geographic regions is evident when comparing populations from the upper Snake River to the Bonneville Basin. Yet, the more recent infusion of Bonneville Basin haplotypes to the upper Snake River partially masks this deeper split. This phenomenon is important in interpreting patterns of regional biogeography, especially in the Great Basin of western North America. Here, late Pleistocene lakes spanned vast geographic basins (map in Hubbs and Miller 1948), thereby increasing opportunity for homogenizing gene flow in now fragmented aquatic species. Yet, accumulating phylogeographic evidence across the Great Basin reveals that genetic exchange through pluvial lakes provides only a partial explanation for current genetic structuring, with evidence for deep divergence frequently revealing a series of temporally varying historical effects (Hershler et al. 1999; Johnson and Jordan 2000; Smith et al., in press).

Life-History Divergence in Space and Time

The Utah chub shows distinct phenotypic divergence in life-history strategies among geographic isolates, largely coincident with environments marked by the presence or absence of trout predators (Johnson and Belk 1999). This pattern of life-history divergence is consistent with that predicted by life-history theory, suggesting that ecological determinism via natural selection is responsible. The phylogeographic data generated in this study provide a rare opportunity to examine the potential effects of historical contingencies on life-history shifts, including the possibility that historical events associated with phylogeographic subdivision have interacted with natural selection to shape chub phenotypes.

Contingency tests showed that Utah chub life histories were nonrandomly distributed among haplotypes in the nested haplotype network (Fig. 3). Predator phenotypes map to two distinct groups of haplotypes, one composed of the deeply divergent Snake/Bear clade (haplotypes V, W, and X), and the other composed of two haplotypes (K and J) nested within the Bonneville clade (Fig. 2). Haplotypes associated with the predator-free life history were found only in the Bonneville clade and formed two distinct phylogenetic groups (Fig. 3). Thus, associations between haplotypes and predator or predator-free phenotypes occurred independently at two locations in the network. The geographic distribution of life-history types was also nonrandom. Predator phenotypes occurred only in the upper Snake River and Bear Lake, but mapped on the network to haplotypes that characterize both the early Pleistocene split between regions and the late Pleistocene

hydrological connection caused by the Bonneville flood (Fig. 3). In contrast, predator-free phenotypes were associated only with haplotypes found in recently fragmented populations of the Bonneville Basin (Fig. 3). Can these nonrandom associations between haplotypes and phenotypes be used to examine the role of historical contingencies on chub life-history evolution?

Mapping phenotypic traits to an intraspecific phylogenetic network and testing for an association between phylogeography and evolution hinge on the assumption that life-history shifts are temporally coupled with neutral phylogenetic divergence. In other words, the rate of mitochondrial DNA evolution (reflected by mutations in the network) or the rate of lineage sorting must approximate the rate at which life histories evolve. Such untested assumptions have been invoked in similar studies that have mapped species-specific phenotypes to haplotype networks (Templeton 1994; Shaw 1999). In this study, dated fossil evidence helps draw the link between chub life-history shifts and phylogeography. Skeletal remains from multiple sites show that Utah chub lived with trout predators in Lake Bonneville as recently as 10,000 years ago (Smith et al. 1968; Broughton 2000). This suggests that the predator-free life-history phenotype is derived and evolved as populations became isolated during the late Pleistocene, coincident with the recession of Lake Bonneville. Given this order of character evolution, it appears that the predator-free phenotype has arisen independently at least two times: once in the eastern Bonneville Basin, where haplotypes P and U (Fig. 3) occur in populations 7, 8, and 9 (Fig. 1), and once in the western Bonneville Basin and at the periphery of modern Great Salt Lake, where haplotypes A–H, L, and M (Fig. 3) occur in populations 3, 6, and 10 (Fig. 1).

Conclusions

Three general conclusions emerge from this study. First, *G. atraria* is composed of two phylogenetically distinct clades, representing a comparatively deep biogeographical split between the upper Snake River and Bear River relative to the Bonneville Basin. These findings contradict the conventional biogeographical view that aquatic organisms in the upper Snake River colonized from the Bonneville Basin only 14,500 years ago. The magnitude of divergence between clades points to a long period of evolutionary isolation, dating to the early Pleistocene, interrupted by a short period of faunal exchange during the late Pleistocene. Second, phylogenetic structuring within each Utah chub clade is largely consistent with documented hydrological events in the Bonneville Basin and upper Snake River over the past 35,000 years. Major shifts in historical geography, including flooding and fragmentation events, were evident in control region sequences compared across Utah chub populations. Third, the life-history data mapped to the haplotype network revealed a strong association between geographic haplotype divergence and phenotypic shifts in chub life histories. This finding suggests that historical contingencies responsible for late Pleistocene fragmentation in Utah chub were coincident with changes in the selective environments where populations persisted. Combined, the results of this study reveal an important

role for historical contingencies in shaping evolutionary divergence in Utah chub. Further examination of similarly distributed aquatic taxa will reveal how pervasive such historical events have been in shaping regional biogeography and evolutionary change.

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LITERATURE CITED

- Avice, J. C. 2000. *Phylogeography: the history and formation of species*. Harvard Univ. Press, Cambridge, MA.
- Bermingham, E., S. S. McCafferty, and A. P. Martin. 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. Pp. 113–128 in T. D. Kocher and C. A. Stepien, eds. *Molecular systematics of fishes*. Academic Press, New York.
- Bernatchez, L., A. Chouinard, and G. Lu. 1999. Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, *Coregonis*, as a case study. *Biol. J. Linnean Soc.* 68: 173–194.
- Bossart, J. L., and D. P. Prowell. 1998. Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Trends Ecol. Evol.* 13:202–206.
- Bright, R. C. 1963. Pleistocene Lakes Thatcher and Bonneville, southeastern Idaho. Ph.D. diss., University of Minnesota, Minneapolis.
- Broughton, J. M. 2000. Terminal Pleistocene fish remains from Homestead Cave, Utah, and implications for fish biogeography in the Bonneville Basin. *Copeia* 2000:645–656.
- Castelloe, J., and A. R. Templeton. 1994. Root probabilities for intraspecific gene trees under neutral coalescent theory. *Mol. Phylogenet. Evol.* 3:102–113.
- Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9:1657–1659.
- Crandall, K. A. 1994. Intraspecific cladogram estimation: accuracy at higher levels of divergence. *Systematic Biol.* 43:222–235.
- Crandall, K. A., and A. R. Templeton. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* 134:959–969.
- Currey, D. R. 1990. Quaternary palaeolakes in the evolution of semidesert basins, with special emphasis on Lake Bonneville and the Great Basin, U.S.A. *Palaeogeography, Palaeoclimatology, Palaeoecology* 76:189–214.
- Currey, D. R., and S. R. James. 1982. Palaeoenvironments of the northeastern Great Basin and northeastern basin rim region: a review of geological and biological evidence. Pp. 27–52 in *Man and environment in the Great Basin*. SAA papers, no. 2.
- Endler, J. A. 1977. *Geographic variation, speciation, and clines*. Princeton Univ. Press, Princeton, NJ.
- Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–491.
- Felsenstein, J. 1976. The theoretical population genetics of variable selection and migration. *Ann. Rev. Genetics* 10:253–280.
- . 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791.
- Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by molecular clock of mitochondrial DNA. *J. Mol. Evol.* 21:160–174.
- Hershler, R., H. P. Liu, and M. Mulvey. 1999. Phylogenetic relationships within the aquatic snail genus *Tryonia*: implications for biogeography of the North American Southwest. *Mol. Phyl. Evol.* 13:377–391.
- Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405:907–913.
- Hubbs, C. L., and R. R. Miller. 1948. The zoological evidence: correlation between fish distribution and hydrographic history in the desert basins of Western United States. Pp. 17–166 in *The Great Basin, with emphasis on glacial and postglacial times*. Bulletin of the University of Utah, Biological Series 10, no. 38.
- Hudson, R. R., M. Slatkin, and W. P. Maddison. 1992. Estimations of levels of gene flow from DNA sequence data. *Genetics* 132: 583–589.
- Jarrett, R. D., and H. E. Malde. 1987. Palaeodischarge of the late Pleistocene Bonneville flood, Snake River, Idaho, computed from new evidence. *Geol. Soc. Am. Bull.* 99:126–134.
- Johnson, J. B. 2002. Divergent life histories among populations of the fish *Brachyrhaphis rhabdophora*: detecting putative agents of selection by candidate model analysis. *Oikos* 96:83–92.
- Johnson, J. B., and M. C. Belk. 1999. Effects of predation on life-history evolution in Utah chub (*Gila atraria*). *Copeia* 1999:948–957.
- Johnson, J. B., and S. Jordan. 2000. Phylogenetic divergence in leatherside chub (*Gila copei*) inferred from mitochondrial cytochrome *b* sequences. *Mol. Ecol.* 9:1029–1035.
- Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules. Pp. 21–132 in H. N. Numro, ed. *Mammalian protein metabolism*. Academic Press, New York.
- Meyer, A., J. M. Morrissey, and M. Shartl. 1994. Recurrent origin of a sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. *Nature* 368:539–542.
- Minkley, W. L., D. A. Hendrickson, and C. E. Bond. 1986. Geography of Western North American freshwater fishes: description and relationships to intracontinental tectonism. Pp. 519–614 in C. H. Hocutt and E. O. Wiley, eds. *The zoogeography of North American freshwater fishes*. John Wiley and Sons, New York.
- Oviatt, C. G. 1997. Lake Bonneville fluctuations and global climate change. *Geology* 25:155–158.
- Oviatt, C. G., D. R. Currey, and D. Sack. 1992. Radiocarbon chronology of Lake Bonneville, Eastern Great Basin, USA. *Palaeogeography, Palaeoclimatology, and Palaeoecology* 99:225–241.
- Posada, D. 2000. CHIPERM 2.0. Free software available via <http://bioag.byu.edu/zoology/crandalllab/programs.htm>.
- Posada, D., and K. A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- Posada, D., K. A. Crandall, and A. R. Templeton. 2000. GeoDis: a program for the nested cladistic nested analysis of the geographical distribution of genetic haplotypes. *Mol. Ecol.* 9: 487–488.
- Ptacek, M. B., and F. Breden. 1998. Phylogenetic relationships among the mollies (Poeciliidae: Poecilia: *Mollienesia* group) based on mitochondrial DNA sequences. *J. Fish Biol.* 53A: 64–81.
- Reznick, D., M. J. Butler IV, and H. Rodd. 2001. Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *Am. Nat.* 157:126–140.
- Ritchie, M., and R. Butlin. 2001. Phylogeography, hybridization, and speciation. *Mol. Ecol.* 10:536.
- Rosenfeld, M. J. 1991. Systematic studies of members of the genus *Gila* (Pisces: Cyprinidae) from the Great Basin and Colorado

- River: protein electrophoretic and cytogenetic variation. Ph.D. diss., University of Utah, Salt Lake City.
- Schluter, D. 1998. Ecological causes of speciation. Pp. 114–129 in D. J. Howard and S. H. Berlocher, eds. *Endless forms: species and speciation*. Oxford Univ. Press, New York.
- Schneider, S., D. Roessli, and L. Excoffier. 2000. ARLEQUIN ver. 2.0: a software for population genetic data analysis. Genetics and Biometry Laboratory, Univ. of Geneva, Geneva, Switzerland.
- Shaw, K. L. 1999. A nested analysis of song groups and species boundaries in the Hawaiian cricket genus *Laupala*. *Mol. Phyl. Evol.* 11:332–341.
- Shields, G. S., and T. D. Kocher. 1991. Phylogenetic relationships of North American ursids based on analysis of mitochondrial DNA. *Evolution* 45:218–221.
- Sigler, W. F., and J. W. Sigler. 1996. *Fishes of Utah: a natural history*. Univ. of Utah Press, Salt Lake City.
- Simons, A. M., and R. L. Mayden. 1998. Phylogenetic relationships of the Western North American phoxinins (Actinopterygii: Cyprinidae) as inferred from mitochondrial 12s and 16s ribosomal RNA sequences. *Mol. Phyl. Evol.* 9:308–329.
- Smith, G. R. 1978. Biogeography of intermountain fishes. *Great Basin Nat. Mem.* 2:17–42.
- Smith, G. R., W. L. Stokes, and K. F. Horn. 1968. Some late Pleistocene fishes of Lake Bonneville. *Copeia* 1968:807–816.
- Smith, G. R., T. E. Dowling, K. Gobalet, T. Lugaski, D. Shiozawa, and P. Evans. *In press*. Biogeography and rates of evolution of Great Basin fishes. In R. Hershler and D. Currey, eds. *The Great Basin: Cenozoic geology and biogeography*. Smithsonian Institution Press, Washington, D.C.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry*. W. H. Freeman, New York.
- Swofford, D. L. 1999. PAUP*: phylogenetic analysis using parsimony (* and other methods). Ver. 4.0b8. Sinauer Associates, Sunderland, MA.
- Swofford, D. L., G. J. Olsen, P. J. Waddell, and D. M. Hillis. 1996. Phylogenetic inference. Pp. 407–514 in D. M. Hillis, C. Moritz, and B. K. Mable, eds. *Molecular systematics*, 2d ed. Sinauer Associates, Sunderland, MA.
- Tanner, V. M. 1936. A study of the fishes of Utah. *Proceedings of the Utah Academy of Sciences, Arts, and Letters* 13:155–184.
- Taylor, E. B., and J. D. McPhail. 2000. Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. *Proc. Roy. Soc. Lond. B* 267:2375–2384.
- Templeton, A. R. 1994. The role of molecular genetics in speciation studies. Pp. 455–477 in B. Schierwater, B. Streit, G. P. Wagner, and R. DeSalle, eds. *Molecular ecology and evolution: approaches and applications*. Birkhäuser Verlag, Basel, Switzerland.
- . 1998a. Nested clade analyses of phylogeographic data: testing methods about gene flow and population history. *Mol. Ecol.* 7:381–397.
- . 1998b. Species and speciation: geography, population structure, ecology, and gene trees. Pp. 32–43 in D. J. Howard and S. H. Berlocher, eds. *Endless forms: species and speciation*. Oxford Univ. Press, Oxford, U.K.
- Templeton, A. R., and C. F. Sing. 1993. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. *Genetics* 134:659–669.
- Templeton, A. R., E. Boerwinkle, and C. F. Sing. 1987. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in *Drosophila*. *Genetics* 117:343–351.
- Templeton, A. R., C. F. Sing, A. Kessling, and S. Humphries. 1988. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. II. The analysis of natural populations. *Genetics* 120:1145–1154.
- Templeton, A. R., K. A. Crandall, and C. F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132:619–633.
- Travisano, M., J. A. Mongold, A. F. Bennett, and R. E. Lenski. 1995. Experimental tests of the roles of adaptation, chance, and history in evolution. *Science* 267:87–90.

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APPENDIX

Pairwise sequence divergence among the 24 Utah chub haplotypes included in this study. Above diagonal: absolute number of changes (bp, total length 934). Below diagonal: model-corrected percent sequence divergence (HKY + I + Γ model of molecular evolution).

Haplo-type	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
A	—	2	2	4	5	3	4	4	5	6	5	6	7	5	6	6	7	7	5	4	7	12	13	14
B	0.28	—	2	4	5	3	4	4	5	6	5	6	7	5	6	6	7	7	5	4	7	12	13	14
C	0.26	0.26	—	2	3	1	2	2	3	3	3	4	5	3	4	4	5	5	3	2	5	10	11	12
D	0.57	0.57	0.25	—	1	3	4	4	5	6	5	6	7	5	6	6	7	7	5	4	7	12	13	14
E	0.41	0.41	0.38	0.16	—	4	5	5	6	7	6	7	8	6	7	7	8	6	6	5	8	13	14	15
F	0.41	0.41	0.12	0.38	0.53	—	1	1	2	3	2	3	4	2	3	3	4	4	2	1	4	9	10	11
G	0.58	0.58	0.25	0.53	0.70	0.12	—	2	3	4	3	4	5	3	4	4	5	5	3	2	5	10	11	12
H	0.63	0.63	0.26	0.58	0.75	0.12	0.26	—	1	2	1	2	3	1	2	2	3	3	1	2	3	8	9	10
I	0.63	0.63	0.26	0.58	0.75	0.13	0.26	0.00	—	3	2	3	4	2	3	3	4	4	2	3	4	9	10	11
J	0.82	0.82	0.42	0.76	0.95	0.26	0.41	0.12	0.12	—	1	4	5	3	4	2	3	3	3	4	3	10	11	12
K	0.82	0.82	0.42	0.76	0.95	0.26	0.41	0.12	0.12	0.00	—	3	4	2	3	1	2	2	2	3	2	9	10	11
L	1.03	1.03	0.58	0.94	1.14	0.41	0.58	0.25	0.25	0.37	0.37	—	1	1	2	4	5	5	3	4	5	10	11	12
M	1.24	1.24	0.76	1.14	1.36	0.58	0.75	0.38	0.39	0.54	0.54	0.12	—	2	3	5	6	6	4	5	4	9	10	11
N	0.82	0.82	0.42	0.75	0.94	0.26	0.41	0.12	0.12	0.25	0.25	0.12	0.24	—	1	3	4	4	2	3	4	9	10	11
O	0.82	0.82	0.42	0.75	0.94	0.26	0.41	0.12	0.12	0.25	0.25	0.12	0.24	0.24	0.24	4	5	5	3	4	5	10	11	12
P	1.03	1.03	0.58	0.94	1.14	0.41	0.58	0.25	0.25	0.37	0.37	0.12	0.24	0.24	0.39	—	1	1	1	2	1	8	9	10
Q	1.24	1.24	0.76	1.14	1.36	0.58	0.75	0.38	0.39	0.54	0.54	0.12	0.24	0.24	0.54	0.12	—	2	2	3	2	9	10	11
R	1.24	1.24	0.76	1.14	1.36	0.58	0.75	0.38	0.39	0.54	0.54	0.12	0.24	0.24	0.54	0.12	0.25	—	2	3	2	9	10	11
S	0.82	0.82	0.41	0.75	0.94	0.26	0.41	0.12	0.12	0.25	0.25	0.12	0.24	0.24	0.24	0.12	0.25	0.25	—	1	2	7	8	9
T	0.58	0.58	0.25	0.53	0.70	0.12	0.25	0.26	0.26	0.24	0.24	0.24	0.38	0.53	0.24	0.12	0.25	0.25	0.12	—	3	8	9	10
U	1.24	1.24	0.76	1.14	1.36	0.58	0.75	0.38	0.39	0.54	0.54	0.12	0.24	0.24	0.54	0.12	0.25	0.25	0.41	0.41	—	7	8	9
V	3.02	3.02	1.98	2.77	3.09	1.88	2.17	1.48	1.48	1.73	1.73	1.98	1.72	1.72	1.72	1.72	1.73	1.73	1.73	1.24	1.25	—	1	2
W	3.37	3.37	2.46	3.09	3.41	2.16	2.45	1.72	1.73	1.98	1.98	2.26	1.98	1.98	1.98	1.73	1.98	1.98	1.98	1.48	1.88	1.48	0.12	—
X	3.75	3.75	2.78	3.44	3.79	2.46	2.77	1.98	1.99	2.26	2.26	2.55	2.26	2.26	2.26	1.99	2.26	2.26	2.26	2.12	1.73	0.25	0.12	—