PHYLOGENETIC DIVERGENCE IN A DESERT FISH: DIFFERENTIATION OF SPECKLED DACE WITHIN THE BONNEVILLE, LAHONTAN, AND UPPER SNAKE RIVER BASINS

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ABSTRACT.—Historical events have had a great impact on the biogeography of fishes of western North America. We examined the genetic variation of the speckled dace (*Rhinichthys osculus*) to determine the effects on this species of extensive hydrological changes during the last 10 million years in the Bonneville and Lahontan basins of the Great Basin and the upper Snake River Basin. Eight hundred sixty-nine base pairs of the mitochondrial gene cytochrome *b* were sequenced from 97 individuals representing 22 populations within these 3 basins, as well as from 2 individuals of long-nose dace (*Rhinichthys cataractae*) that served as outgroups. Additionally, 13 speckled dace sequences representing 3 Bonneville populations were used from GenBank. Phylogenetic relationships were reconstructed using maximum parsimony and maximum likelihood criteria. Analysis of molecular variance was used to determine population structure and to estimate the amount of gene flow across the community boundaries. Three distinct clades were reconstructed representing the Lahontan Basin, the northern Bonneville and upper Snake River basins, and the southern Bonneville Basin. Additionally, most of the population structuring was explained by variation among basins (65.33%). Speckled dace demonstrated high genetic variation. As hypothesized, the northern and southern Bonneville specimens formed separate clades; however, the southern Bonneville clade was basal to a sister clade formed by the northern Bonneville/upper Snake River and Lahontan clades. These relationships indicate that Pliocene connections between the Snake, Lahontan, and Bonneville drainages, rather than more recent Pleistocene connections, best explain population structuring in speckled dace.

Key words: Rhinichthys osculus, Great Basin, phylogeography, Snake River, genetic diversity, Lake Bonneville.

Past climatic and geologic events can impact the evolutionary trajectory of populations within a species. In the absence of gene flow, isolated populations may diverge because of genetic drift or different selection regimes and then evolve along independent trajectories (Felsenstein 1976, Endler 1977). Phylogeography attempts to understand these phenomena by using molecular data to address questions of genealogical lineages across potential geographic barriers (Avise 2000), thus providing insights into the geographic and evolutionary history of a species. Aquatic taxa that are incapable of migrating across marine or terrestrial barriers offer an opportunity to study the impacts of geologic and hydrologic history on the phylogeography of these taxa (Daniels et al. 2006).

Drainage basins of western North America have changed considerably since the Miocene, with connections between drainages shifting with volcanism, climatic cycles (glaciation followed by aridity), and tectonic processes (Johnson 2002, Smith et al. 2002, Oakey et al. 2004, Spencer et al. 2008). The Great Basin is a region of internal drainages within western North America. It is made up of 5 major subdivisions, which collectively contained over 80 lakes during pluvial times (Hubbs and Miller 1948, Shiozawa and Rader 2005). Two of these major subdivisions, the Bonneville and Lahontan basins, adjoin one another in northeastern Nevada, at the northern edge of the Great Basin. These 2 basins, along with the neighboring Snake River Basin to the north, are thought to have had periodic interconnections over the past 10 million years (Link et al. 2002).

Connections followed by long periods of isolation have had a profound effect on the biogeography of fishes in these basins. As a result, many western fish species are endemic to particular basins, limiting their usefulness

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TABLE 1. Collection sites for speckled dace (*Rhinichthys osculus*) examined in this study, including the basin, the locality name, the 2-letter locality code used in this paper, and the number of individuals sampled per locality.

Basin	Locality name	Locality code	Individuals sampled
Lahontan	East Walker River	WK	2
	East Fork Carson River	CR	5
	Truckee River	TR	7
	McDermitt	MD	2
	Humboldt River	HB	3
	Upper Mary's River	MR	3
	T Creek	TC	3
Snake River	Deep Creek	DP	2
	Cold Creek	CD	3
	Raft River	RF	3
	Snake River	SN	3
	Blackfoot River	BF	3
	Fall River	FL	3
North Bonneville	Cotton Creek	CT	5
	Rock Springs	RS	4
	Blue Creek	BL	9
	La Chappelle Creek	LC	7
	Yellow Creek	YL	5
	Bear River	BR	8
South Bonneville	Sevier River	SV	4
	Lake Creek	LK	9
	Fish Springs	FI	1
	Salina Creek	SA	13
	Tributary of Sevier River	TS	2
	Fish Creek	FC	1

in comparing their phylogeography to large-scale geologic patterns (e.g., comparison of geologic and hydrologic history among multiple drainages). The speckled dace (*Rhinichthys osculus*), however, is a good model organism for such studies. This species has a wide distribution across much of the western United States, and recent studies have shown that speckled dace have unique genetic differentiation within each of the drainage basins they occupy (Oakey et al. 2004, Smith and Dowling 2008).

We examined genetic variation in the speckled dace in the Bonneville and Lahontan basins of the Great Basin and the Snake River Basin. Our objective was to examine phylogeographic patterns among these neighboring basins and then compare these patterns with known paleohydrological events of this region.

METHODS

Sampling

We collected speckled dace from 22 locations in the Lahontan ($n = 7$), Snake River ($n = 6$), and Bonneville ($n = 9$) basins (Table 1, Fig. 1). Locations in the Bonneville Basin were split between the northern ($n = 5$) and the southern ($n = 4$) portions of the basin. We preserved whole fish from each site in 95% ethanol. From each collection site, we sampled 1–13 individuals (mean = 4.4), for a total of 97 individuals. These were archived in the Monte L. Bean Museum fish collection at Brigham Young University. In order to improve our geographical sampling and increase sampling in underrepresented localities, we downloaded 13 additional published sequences, adding 3 more locations in the Bonneville Basin (1 northern and 2 southern populations; Genbank accession numbers DQ990252–DQ990253, DQ990278–DQ990281, and DQ990298–DQ990304) from Smith and Dowling (2008). Two individuals of longnose dace (*Rhinichthys cataractae*) collected from Goose Creek (Box Elder County, UT) were used as outgroups, based on Woodman (1992).

Data Collection

We extracted whole genomic DNA from alcohol-preserved fin clips (a few samples were obtained from alcohol-preserved muscle tissue) using standard methods. We homogenized the tissue in a DNA lysis buffer, added proteinase K, and incubated the mixture overnight at 55 °C. We completed the extraction
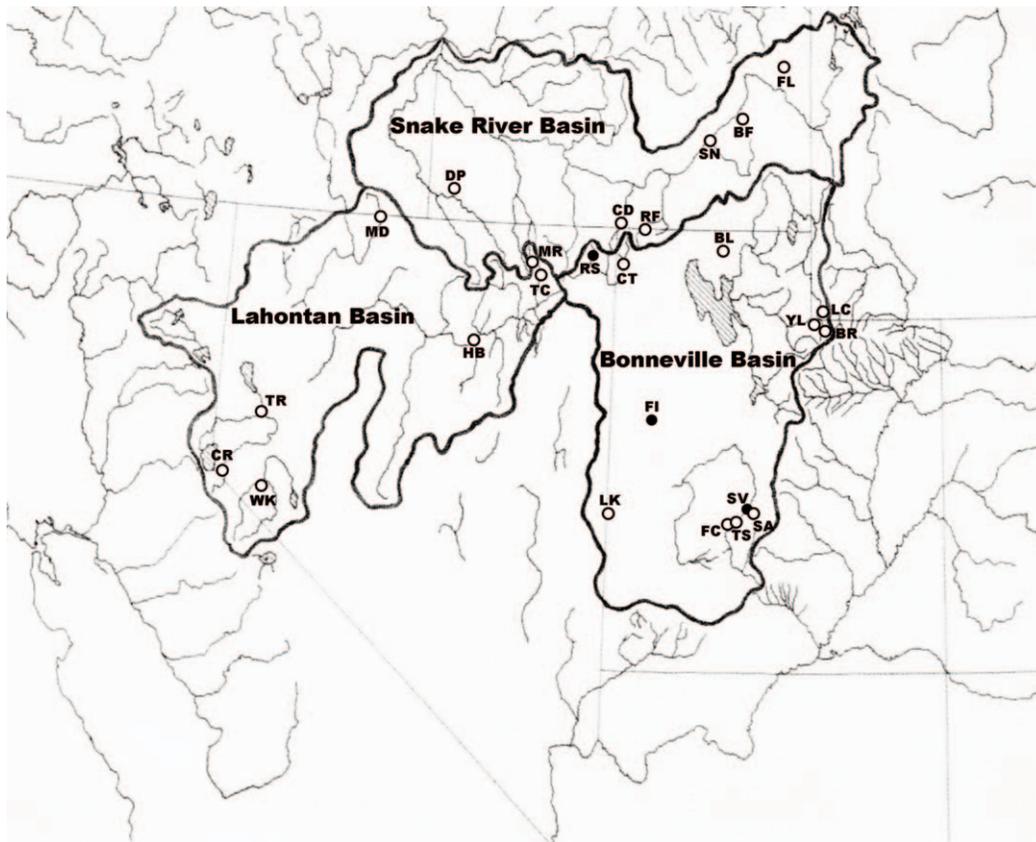
Fig. 1. The 25 collecting locations of speckled dace examined in this study from the Lahontan, Snake River, and Bonneville basins. Locality codes follow those in Table 1. Localities sampled in this study have open dots, and localities sampled from previous studies have filled dots.

after an ammonium acetate separation of proteins and cold ethanol precipitation. We amplified a fragment of the mitochondrial DNA cytochrome b (cyt b) gene from the isolated total genomic DNA by polymerase chain reaction (PCR). We sequenced 2 segments for the cyt b gene using 2 sets of flanking primers: LA-HD and LD-HA, after Dowling and Naylor (1997). The PCR thermal regime for each fragment was initiated at 95 °C for 4 minutes, followed by 35 cycles of 94 °C for 20 seconds, 50 °C for 30 seconds, and 72 °C for 90 seconds; the final extension was 7 minutes at 72 °C. We cycle-sequenced the amplified PCR products using Big Dye chemistry; products of these reactions were cleaned with sephadex. Sequencing was performed on either a Perkin-Elmer ABI Prism 377 automated sequencer or an ABI 3100 automated sequencer.

We edited the resulting sequences and checked for ambiguity using Sequencher 4.6 (GeneCodes Corp., Ann Arbor, MI). We created a consensus sequence for each individual and aligned the sequences in MUSCLE 3.6 using the default parameters, because Edgar (2004) demonstrated this procedure to be fast while maintaining a high accuracy. We collapsed individual sequences into haplotypes using the program TCS 1.21 (Clement et al. 2000) and used the subsequent haplotypes in phylogenetic analyses with redundant sequences removed.

Data Analysis

We reconstructed phylogenetic relationships among speckled dace under maximum parsimony and maximum likelihood criteria, rooting reconstructions using the cyt b sequences obtained from longnose dace. We

Fig. 2. Maximum likelihood phylogeny derived from mitochondrial cytochrome *b* sequences for 91 haplotypes from 25 populations of speckled dace in the Lahontan, Snake River, and Bonneville basins. Maximum parsimony bootstrap values appear above each branch, while maximum likelihood bootstrap values appear below the branch.

conducted a maximum parsimony analysis using equal weights under a heuristic search option, with the starting tree generated through 10 replications of random stepwise addition in the software package PAUP* (Swofford 1999). Phylogenetic reconstruction under maximum likelihood criteria requires an explicit model of evolution to reduce the number of unnecessary parameters (Daniels et al. 2006). We used Modeltest v3.06 (Posada and Crandall 1998) to determine the best-fitting model of nucleotide substitution from 56 different models. Using the best-fitting model, we conducted the maximum likelihood analysis in GARLI v.0.951 (Zwickl 2006; http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html). We analyzed nodal support using 1000 bootstraps under both optimality criteria.

To estimate gene flow between basins, we examined the genetic structure of populations of speckled dace from the 4 basins by dividing the sampling localities into 4 groups according to their basins (Table 1): Lahontan Basin, Snake River Basin, northern Bonneville Basin, and southern Bonneville Basin. We ran an analysis of molecular variance (AMOVA) in Arlequin v3.1 (Excoffier et al. 1992, Excoffier et al. 2005) to partition the total genetic variation into differences among the localities within basins (F_{SC}), among localities across the entire study (F_{ST}), and among the 3 historically distinct geographic regions (F_{CT}). This framework provided an *a priori* test of patterns of genetic subdivision consistent with other species in these regions that have shown genetic subdivision between the Lahontan Basin, Bonneville Basin, and the upper Snake River Basin, as well as species within the Bonneville Basin showing a distinct north and south subdivision (Johnson 2002, Johnson et al. 2004, Mock and Miller 2005, Mock et al. 2006).

Results

We sequenced 869 base pairs of cyt *b* for 97 individuals of speckled dace and 2 individuals of longnose dace, and we deposited sequences on GenBank under accession numbers FJ528915–FJ528987 and GQ495958–GQ495981. For greater geographical sampling and increased sampling in underrepresented localities, we used 13 additional sequences available on Genbank for our analyses (see methods for accession numbers). Of the 869

characters in each of the 110 sequences, we found 194 positions variable, with 160 of the variable sites being parsimony informative. The 110 individuals from 25 locations represented 91 unique haplotypes. Snake River and northern Bonneville basins shared 2 haplotypes, but all other haplotypes were unique to a particular basin. Average nucleotide base frequencies were A = 0.251, C = 0.274, G = 0.183, and T = 0.292.

Maximum parsimony and maximum likelihood yielded similar topologies (Fig. 2). Maximum parsimony produced 9311 equally parsimonious trees of 390 steps, with most of the variation in topology occurring at the extreme tips of the tree and not affecting the deeper nodes. MODELTEST selected the TrN + I + G model of evolution under the Akaike information criterion (AIC). Maximum likelihood converged on a single tree, with a log-likelihood score (–lnL) of 3442.1234 (Fig. 2). We identified 3 distinct clades corresponding to major geographic regions (northern Bonneville/Snake River, Lahontan, and southern Bonneville), with high bootstrap support for each of the 3 clades.

The most basal clade consisted of 26 haplotypes unique to the southern Bonneville Basin, while the 2 remaining regions (Lahontan and northern Bonneville/Snake River) formed sister clades to one another. The Lahontan clade consisted of 27 haplotypes that made up 4 smaller subclades; however, there was no obvious geographical structure to these 4 subclades. The Lahontan clade further has 2 haplotypes that are unique to the Snake River Basin and 2 haplotypes unique to the Bonneville Basin (both from the south). The northern Bonneville and Snake River clade consisted of 38 haplotypes. Haplotypes from Cotton Creek (CT) and Rock Springs (RS) formed a more basal clade with respect to other locations in the northern Bonneville clade. Mean sequence divergence between the 3 major clades ranged from 7.8% to 9.8%, while divergence within each of the clades was low (northern Bonneville/Snake River = 1.1%, southern Bonneville = 0.7%, and Lahontan = 2.8%).

Our AMOVA results revealed significant genetic variation across the 4 regions. With the populations divided into northern Bonneville, southern Bonneville, Snake River, and Lahontan groups, most of the total variation among haplotypes (60.5%) was explained by differences

among basins ($F_{CT} = 0.60477$, $P < 0.0001$). Differences among localities overall accounted for only 20.3% of the variation ($F_{ST} = 0.79713$, $P < 0.0001$), and differences among populations within basins accounted for just 19.2% of the total variation ($F_{SC} = 0.4867$, $P < 0.0001$).

DISCUSSION

We examined the broad-scale geographic relationships between speckled dace populations in the Bonneville, Lahontan, and Snake River basins to determine if they concurred with previous studies of speckled dace and other aquatic species (Johnson 2002, Johnson et al. 2004, Oakey et al. 2004, Smith and Dowling 2008). Our results show that speckled dace contain a large amount of genetic variation, characteristic of a widespread species, as has been demonstrated in other studies (Oakey et al. 2004, Smith and Dowling 2008). The 3 distinct clades we recovered had high nodal support with 2 shared haplotypes occurring between the northern Bonneville and Snake River basins. The 3 clades coincided with geographical location, with one clade each representing the Lahontan, northern Bonneville/Snake River, and southern Bonneville basins. Our AMOVA results showed that most of the variation was explained by differences among the basins, indicating low gene flow between the 3 clades. We now examine these relationships in light of both historical geography and other fish studies.

Like other fish species in the Bonneville Basin (Martin et al. 1985, Johnson 2002, Mock and Miller 2005, Mock et al. 2006), speckled dace showed a north–south split in the Bonneville basin. The late Pleistocene capture of the Bear River from the Snake River into the Bonneville Basin (Bright 1963, Bouchard et al. 1998) has been recognized as a major event that should have transferred fishes into the Bonneville Basin (Hubbs and Miller 1948, Taylor and Bright 1987, Behnke 1992). The separation of many Bonneville Basin fishes into northern and southern lineages was recognized more recently (Loudenslager and Gull 1980, Martin et al. 1985, Johnson 2002) and argues for multiple invasions into the Bonneville Basin. In our study, the speckled dace populations in the northern and southern Bonneville drainages were not sister to each other; rather, the southern Bonneville populations

were ancestral to a northern Bonneville/upper Snake River and Lahontan clade. Further, the upper Snake River and northern Bonneville basin speckled dace fell into the same clade in both the maximum likelihood and maximum parsimony analyses. This finding is similar to other phylogeographic studies of speckled dace (Oakey et al. 2004, Smith and Dowling 2008) and suggests that speckled dace in the northern Bonneville Basin and the upper Snake River were only recently separated. We presume that this separation was due to the late Pleistocene capture of the Bear River into the Bonneville Basin.

Northern and southern Bonneville populations have also had recent within-basin connections via Lake Bonneville. However, the apparently deep temporal separation of populations from the 2 drainages indicates that pluvial Lake Bonneville was not conducive to speckled dace movement and dispersal. Thus, northern and southern Bonneville populations maintained their genetic distinctness during this apparent connection.

The northern Bonneville, upper Snake River, and Lahontan basin relationships suggest that the speckled dace in these basins were strongly influenced by the premodern Snake River drainage. The headwaters of the early Snake River connected drainages in southeastern Idaho (upper Snake River) and, at least periodically, northern Utah (northern Bonneville). The Snake River flowed west and was connected to Pliocene Lake Idaho. A number of investigators have suggested that the Snake River flowed into Lake Idaho and then exited to the west in Oregon, eventually entering the Pit–Sacramento or Klamath River basins (Miller 1965, Taylor 1985, Minckley et al. 1986, Smith et al. 2000). The Lahontan Basin would have had periodic connections with the Snake–Sacramento system, as hypothesized by Taylor (1985). The sister relationship of the northern Bonneville/upper Snake River and Lahontan clades supplies evidence for such a drainage connection.

However, recent fossil mammal studies (Repenning et al. 1995) and phylogenetic investigations of mollusks (Hershler and Liu 2004) have suggested that a river draining Lake Idaho through western Oregon did not exist. Instead, Repenning et al. (1995) suggested an alternate route for the premodern Snake River. Prior to about 3 million years ago (Ma), the Snake

River did not enter Pliocene Lake Idaho but instead flowed to the southwest through the Lahontan Basin to the Pit River system. Investigations utilizing sedimentary zirconium (Link et al. 2002, Beranek et al. 2006) have supported this hypothesis. If Repenning's hypothesis is correct, then the Snake River–Lahontan Basin connection would be near the Cotton Creek and Rock Springs dace populations collected in the Bonneville Basin. The Lahontan–Snake connection would have been broken by 3 Ma, when the Snake River was captured into the Columbia River Basin by the draining of Lake Idaho (Beranek et al. 2006). The separation of Cotton Creek and Rock Springs populations from the rest of the northern Bonneville/upper Snake River clade suggests that those speckled dace populations were isolated from other northern Bonneville/upper Snake River populations by migration barriers (e.g., aridity) or they arose from early stream-capture events between the Lahontan and northern Bonneville drainages.

Connections between the southern Bonneville Basin drainages and the premodern Snake River drainage would have allowed the passage of ichthyofauna from the north into the Colorado River drainage. Taylor (1985) suggested a Late Miocene connection between the 2 drainages in the western Bonneville Basin. This connection is supported by living and fossil mollusks (Taylor 1985) and is also reflected in the distribution and relationships of *Lepidomeda* species (Johnson and Jordan 2000, Johnson et al. 2004). However, other evidence suggests a connection between the upper Colorado River, the Bonneville Basin, and the Snake River (Houston et al. 2010). The north–south connection between the Colorado River and the premodern Snake River would have been broken prior to the separation of the upper Snake and Lahontan drainages by the upper Snake River capture into the Columbia River Basin. Southern Bonneville drainages may have remained tributary to or at least intermittently connected to the Upper Colorado River until about 0.546 Ma (McKell 2003, Oakey et al. 2004, Smith and Dowling 2008). A single Lake Creek haplotype and the Fish Springs haplotypes fell outside of the southern Bonneville clade and were instead basal haplotypes in the Lahontan clade. These haplotypes may represent ancestral haplotypes from a north–south connection

between the premodern Snake and southern Bonneville basin that have persisted through time or may represent ancient stream-capture events from the Lahontan Basin into the Bonneville Basin.

The phylogeography of speckled dace has demonstrated the importance of ancient events on structuring both the drainages and ichthyofauna of the western United States. Although we found evidence supporting some of the ancient connections between drainage basins, more sampling is necessary to confirm hypotheses presented herein and by others. Similarly, phylogeographic studies of other species or genera that are common in western North America (e.g., catostomids, cottids, etc.) could also provide evidence to support or refute these hypotheses.

ACKNOWLEDGMENTS

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