

Connectivity among populations of pygmy whitefish (*Prosopium coulterii*) in northwestern North America inferred from microsatellite DNA analyses

E.B. Taylor, J.L. Gow, J. Witt, and R. Zemlak

Abstract: We studied microsatellite DNA variation in 15 populations of northwestern North American pygmy whitefish (*Prosopium coulterii* (Eigenmann and Eigenmann, 1892)), an enigmatic freshwater fish thought to be highly fragmented by residency in deep, cold postglacial lakes. Population subdivision (θ) across 10 loci was 0.12 ($P < 0.001$) across samples, but one western Alaskan population was more divergent than all others ($\theta = 0.31\text{--}0.41$, $P < 0.001$). Within the Williston Reservoir watershed (WRW), θ averaged 0.08 ($P < 0.001$) and was positively associated with both the geographic distance between localities ($r^2 = 0.36$, $P < 0.001$) and the number of branch points interconnecting them ($r^2 = 0.33$, $P < 0.001$). Differentiation among populations was modeled as the sum of the genetic distances for the stream sections interconnecting them ($r^2 = 0.74$). Differences among subwatersheds with the WRW accounted for 5.1% of the total variation in allele frequencies ($P < 0.001$). Assignment tests suggested limited movement among lakes, with most inferred dispersal between adjacent watersheds. Coalescent analysis strongly supported a gene flow–drift equilibrium model of population structure over a drift-only model. Effective management of diversity in pygmy whitefish requires the maintenance of stream networks that interconnect lakes within a watershed.

Résumé : Nous étudions la variation de l'ADN des microsatellites dans 15 populations du nord-ouest de l'Amérique du Nord du ménomini pygmée, *Prosopium coulterii* (Eigenmann et Eigenmann, 1892), un poisson d'eau douce énigmatique dont on croit les populations fortement fragmentées à cause de leur résidence dans des lacs postglaciaires profonds et froids. La subdivision de la population (θ) dans 10 locus est de 0,12 ($P < 0,001$) dans l'ensemble des échantillons, bien qu'une population de l'ouest de l'Alaska soit plus divergente que toutes les autres ($\theta = 0,31\text{--}0,41$, $P < 0,001$). Dans le bassin hydrographique du réservoir Williston (WRW), θ a une valeur moyenne de 0,08 ($P < 0,001$) et est en corrélation positive tant avec la distance géographique entre les localités ($r^2 = 0,36$, $P < 0,001$) qu'avec le nombre de points de branchement qui les relie ($r^2 = 0,33$, $P < 0,001$). La différenciation entre les populations peut se modéliser comme la somme des distances génétiques des sections de cours d'eau qui les rattachent ($r^2 = 0,74$). La différenciation entre les sous-bassins au sein de WRW explique 5,1 % de la variation totale dans la fréquence des allèles ($P < 0,001$). Des tests d'assignation indiquent des déplacements limités entre les lacs, avec la dispersion le plus souvent retrouvée entre des bassins versants adjacents. Une analyse de coalescence appuie fortement un modèle de structure de population avec équilibre entre le flux génique et la dérive, plutôt qu'un modèle de dérive seule. Une gestion efficace de la diversité chez les ménominis pygmées nécessite le maintien des réseaux de cours d'eau qui relient les lacs dans un même bassin versant.

[Traduit par la Rédaction]

Introduction

In geographically widespread species, the extent of population subdivision, or the degree to which species are subdivided into at least partially genetically distinct units, will be a function both of historical and of contemporary processes. Historical processes such as major climatic and geomorpho-

logical events (tectonics, glaciation, climate change) can impact both the extent and the pattern of neutral genetic variation within and between populations (Hewitt 1996; McGlashan and Hughes 2000). Similarly, both natural (land or waterscape features, gene flow between populations) and anthropogenic (man-made barriers, hybridization with introduced species, exploitation) contemporary factors can also

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influence the distribution of genetic variation within and between populations (e.g., Costello et al. 2003; Riley et al. 2006).

Understanding of the extent and patterns of genetic variation within species across their geographic range is an important piece of information used to design conservation programs (e.g., Allendorf and Luikart 2007; Schwartz et al. 2007). Genetic variation exists in the form of quantitative variation that is typically associated with controlling, at least in part, expressed variation in phenotypic traits such as body size, behaviour, physiology, and morphology. Qualitative variation is also present in the array of neutral or selected variation at the biochemical (e.g., allozymes) or molecular (e.g., DNA sequence variation, microsatellite DNA allele frequency) level. Whereas an understanding of the patterns of expressed and potentially adaptive variation and the processes that influence it (e.g., gene flow, natural selection) are desirable to understand and help predict how species respond to environmental change, such information is challenging to collect especially for a broad array of populations. By contrast, neutral variation at molecular loci is more easily collected and can contribute to understanding certain processes that influence contemporary populations such as historical perturbations, changes in population size, and dispersal and gene flow between localities. In addition, studies of population structure can indicate the potential for adaptive variation between populations because high levels of population subdivision suggest low degrees of gene flow between populations that reduces any constraining influence of gene flow on adaptive differentiation (Slatkin 1987; Hendry and Taylor 2004; Morjan and Rieseberg 2004).

There have been many studies of molecular-based population subdivision in salmonid fishes (salmon, trout, char, grayling, and whitefishes), owing to their cultural, recreational, and commercial importance (e.g., Allendorf and Waples 1996). Although considerable work has been completed on whitefishes (Coregoninae) of the genus *Coregonus* L., 1758 (e.g., Patton et al. 1997; Turgeon and Bernatchez 2001; Säämä et al. 2008), comparatively little work has been focused on members of the round whitefishes, *Prosopium* Jordan, 1878. The genus *Prosopium* consists of six recognized species that are distributed across eastern Siberia and northern North America with a concentrated distribution in northwestern North America (Norden 1970). Only very recently has detailed study of this genus been undertaken (i.e., mountain whitefish, *Prosopium williamsoni* (Girard, 1856): Whiteley et al. 2004, 2006).

The pygmy whitefish (*Prosopium coulterii* (Eigenmann and Eigenmann, 1892)) is a particularly enigmatic member of the genus and is generally considered to be a glacial relict that inhabits cold, deep lakes (Scott and Crossman 1973; Weisel et al. 1973; Becker 1983). The pygmy whitefish is essentially a western North American species with populations scattered from Washington, Idaho, and Montana north to Alaska and the Yukon Territory. It also occurs, however, in a few scattered localities east of the Continental Divide (e.g., western Lake Superior, Lake Athabasca, Great Bear Lake, and Waterton Lake), as well as on the Chukotsk Peninsula, Siberia (Chereshnev and Skopets 1992). Witt et al. (2011) studied the phylogeography of the pygmy whitefish across its North American range and resolved two major genetic lin-

eages (based both on mitochondrial and nuclear DNA sequences). The presence of these two major lineages and their geographic association with areas that served as glacial refugia support the idea that the current range disjunctions in North America probably result from historical patterns of isolation and recolonization during the glacial cycles of the Pleistocene (McPhail and Lindsey 1970; Witt et al. 2011).

The pygmy whitefish occurs in the Peace River drainage, part of the Mackenzie River system, of British Columbia and Alberta, Canada, the upper portion of which has been impacted by the construction of the W.A.C. Bennett Dam in the 1960s. The resulting reservoir, Williston Reservoir, and its tributaries are known as the Williston Watershed (about 70 000 km²) and The Peace/Williston Fish and Wildlife Compensation Program (PFWWCP) was created in 1988 to conserve and enhance fish, wildlife, and their habitats within the watershed. Although the PFWWCP initially concentrated its resources on species exploited in recreational fisheries, recently increased efforts have been placed on protecting biodiversity more generally (Olmsted and Bolin 1996). Consequently, in 1998 the PFWWCP began a study of the indigenous nongame fishes in the Williston Watershed and the pygmy whitefish was the first species selected for study. For instance, Zemplak and McPhail (2006) reported new information on the ecology of pygmy whitefish in this area and indicated that approximately 17 lakes (out of approximately 360 surveyed) in the Williston Watershed contained pygmy whitefish.

Our research on the evolutionary genetics of pygmy whitefish focuses on two geographic and temporal scales of relevance to conservation. Witt et al. (2011) studied variability across the broadest geographic and temporal scales to better understand the historical processes that have contributed to determining the origin of the contemporary range disjunctions and major phylogeographic lineage in pygmy whitefish. In this sense, we have examined the origin of populations and lineages that have arrived from the past. In this study, our goal was to assess the levels of molecular population structure among a subset of populations driven by factors that have operated largely on a postglacial and, indeed, contemporary time scale. Both perspectives on evolutionary genetics contribute to more comprehensive inputs to genetic aspects of conservation (Bowen 1999). Specifically, in the current study, we sought to assess the degree of subdivision among localities that differed in their extent of geographic separation both in terms of riverine distance and spatial complexity (migration barriers, complexity of migration). In this way, we hoped to infer the level of genetic interconnectedness among populations, i.e., whether each comprised a completely isolated population with little influence of contemporary or recent historical gene flow with other populations, or whether populations were more genetically interconnected at least within the recent past so that they might also be demographically interconnected (Bohonak 1999; Waples and Gaggiotti 2006). The pygmy whitefish often reside in deep lakes and its riverine biology is much less well understood (McPhail 2007). Consequently, it is possible that the primary determinant of population genetic structure is dominated by genetic drift in highly isolated lakes. Alternatively, pygmy whitefish are occasionally reported from rivers (McPhail 2007), and the lakes within the

Williston Reservoir watershed (WRW) are interconnected to varying degrees and pygmy whitefish may disperse among lakes using these river corridors so that population structure is determined more by a balance between genetic drift and gene flow. Our study represents a test of these alternative scenarios for this little known member of the Nearctic freshwater fish fauna.

Materials and methods

Sample localities and fish collection

Seventeen distinct collections were made from 15 different localities. Two localities had collections made in successive years: Dina Lake (No. 1; Zemplak and McPhail 2006) and lower Tacheeda Lake. Sixteen of these samples came from localities within the Williston Reservoir drainage area (Fig. 1) and one came from Black Lake (56°27'N, 158°59'W) on the Alaska Peninsula near the community of Chignik Lake, southwestern Alaska. The localities from the Williston Reservoir drainage were chosen to represent sub-regional “pairs” of localities that varied in levels of interconnectedness between each member of the pair and the other pairs (Table 1). For instance, Quentin and Weissener lakes are at the far northwestern portion of the watershed and are separated from each other by approximately 150 km of interconnecting river habitat. This pair of lakes is separated from upper and lower Tacheeda lakes (in the southeastern portion of the watershed) by approximately 500 km of intervening riverine–reservoir habitat, but the Tacheeda lakes are separated from each other by a small 90 m long creek. In total, and not including the Black Lake sample, the Williston Reservoir samples had a mean pairwise distance between any two localities of about 279 km (SD = 129). Some systems also were characterized by different habitats between paired localities (Table 1). For instance, the distance between the Omineca Arm and Peace Reach localities of the Williston Reservoir itself was only 77 km and consisted of essentially open water. By contrast, whereas Aikten and Tutizzi lakes are separated by only 56 km, the intervening habitat is riverine and fish potentially moving between these lakes must first move downstream into the intervening Mesilinka River (in the case of Aiken Lake) or into the Tutizzi River (in the case of Tutizzi Lake) and then upstream into the other lake.

Most pygmy whitefish were collected during August of 2004 and 2005 using multiple-panel sinking gill nets (10–25 mm stretched mesh sizes) set along the bottom usually for 4 h time periods as described in Zemplak and McPhail (2006). One sample (Black Lake, Alaska) was obtained by beach seining along the lake shoreline. Tissue samples consisted of caudal or adipose fin clips stored in 95% ethanol.

Microsatellite analyses

We assayed individuals using 11 microsatellite loci that had been isolated from lake whitefish (*Coregonus clupeaformis* (Mitchill, 1818)) (*Cocl-Lav10*, *Cocl-Lav45*, *Cocl-Lav49b*, *Cocl-Lav72*, *Cocl-Lav74*, *Cocl-Lav224*; Rogers et al. 2004), Arctic cisco (*Coregonus autumnalis* (Pallas, 1776)) (*Aut139*; Ramey et al. 2008), broad whitefish (*Coregonus nasus* (Pallas, 1776)) (*LGL-BWF1*; Patton et al. 1997), Atlantic salmon (*Salmo salar* L., 1758) (*Ssa456*, Slettan et al. 1995), brown

trout (*Salmo trutta* L., 1758) (*BT73*, Estoup et al. 1993), and bull trout (*Salvelinus confluentus* (Suckley, 1859)) (*Sco107*; S. Young, Washington Department of Fish and Wildlife, Olympia, Washington, USA, unpublished data). Samples were assayed using the polymerase chain reaction (PCR) and using fluorescently labelled primers; PCR products were assayed on a Beckman–Coulter CEQ 8000 automated genotyper using methods described in Taylor et al. (2007) and Northrup et al. (2010).

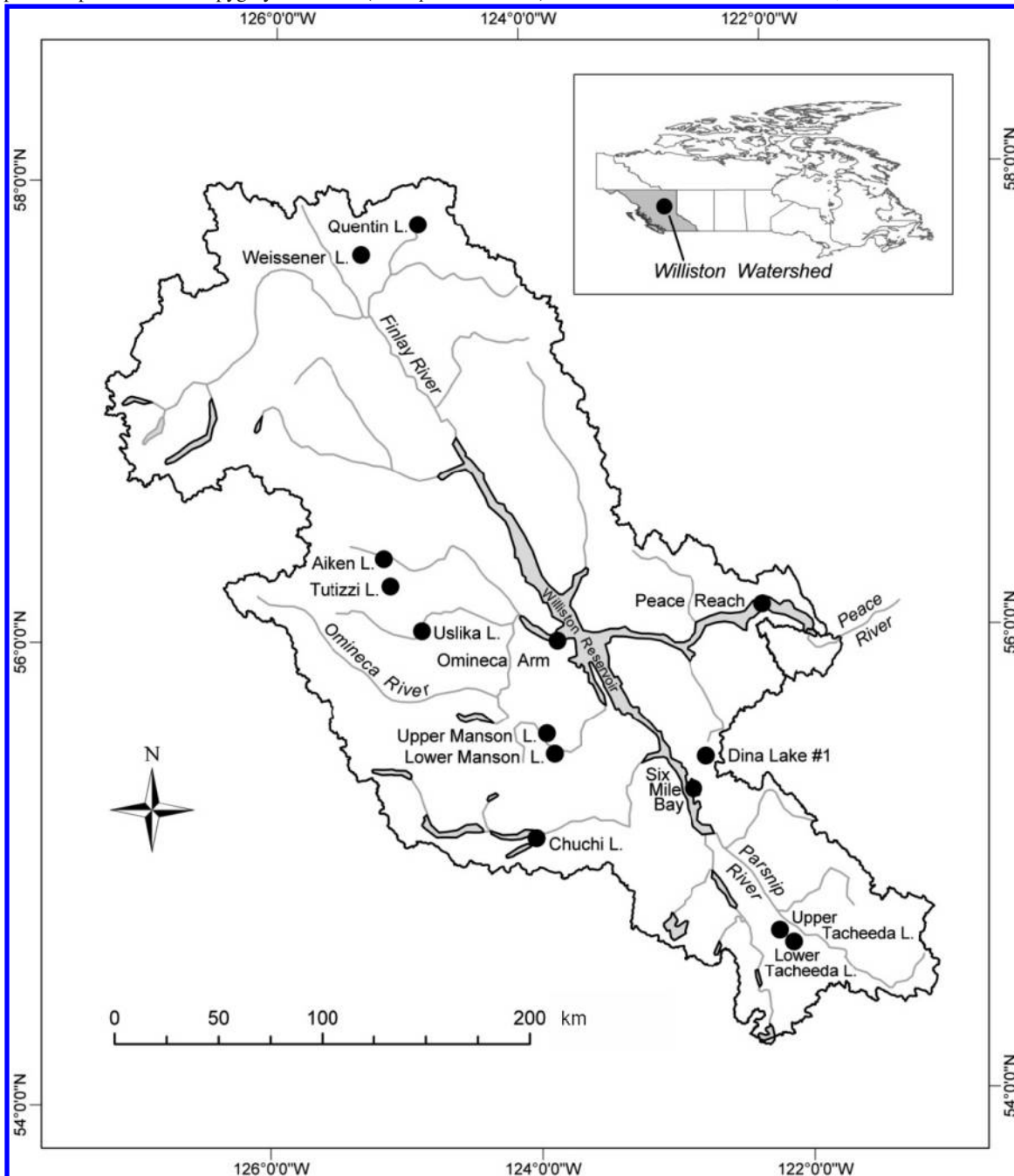
Genetic data analyses

Microsatellite data were first processed using Micro-Checker (van Oosterhout et al. 2004) to identify possible scoring errors and the presence of null alleles. Thereafter, basic descriptive statistics of sample size (N), number of alleles (N_A), and observed (H_O) and expected (H_E) heterozygosities were compiled using FSTAT version 2.9.3.1 (Goudet 1995; version 2.9.3.1 updated in 2001). The following tests were performed using GENEPOP version 3.3 (Raymond and Rousset 1995; version 3.3 updated in 2001). Tests for deviations from Hardy–Weinberg equilibrium were performed for each locus–population combination using an exact test in which probability values were estimated using a Markov chain method. Tests for genotypic linkage disequilibrium for all combinations of locus pairs within a population were also made using a Markov chain method with GENEPOP default values.

Pairwise genetic differentiation between localities was expressed as F_{ST} estimated by calculating θ (Weir and Cockerham 1984) and pairwise values were tested for significance using permutation analyses ($N = 1000$) using GENETIX version 4.05 (Belkhir et al. 2001). We also applied the analysis of molecular variance (AMOVA) approach of Excoffier et al. (1992) to partition microsatellite variation into its variance components (among populations within drainages) using ARLEQUIN version 3.11 (Excoffier et al. 2005). We pooled samples by major watershed within the Peace–Williston area across the entire sample area and compared the level of explained variance between these groupings to samples arbitrarily divided into “North” and “South” and “East” and “West” groupings of roughly equal area. We based our inferences on allele frequency variation among populations assuming that mutation-based differentiation was minor because estimates based on the former appear to be more appropriate for recently diverged populations (e.g., Gaggiotti et al. 1999). In addition, the permutation test procedure within the SPAGeDI software (Hardy and Vekemans 2002) indicated that only 1 of the 11 loci (*Sco107*) would justify the use of mutation-based statistics.

Geographic distances between tributaries within the study area was determined using the geographic information system (GIS) program ArcView version 3.1 (Environmental Science Research Institute (ESRI), Redlands, California, USA). In addition, using the approach of Costello et al. (2003), we constructed a drainage pattern matrix between all pairs of samples. The matrix represents the spatial arrangement of all populations with respect to their interconnectivity in the hydrographic “network”. The matrix represented, therefore, pairwise values of whole numbers that represent the number of “drainage nodes” or branch points that an individual fish would need to transverse in moving from one locality to an-

Fig. 1. Map of sample localities of pygmy whitefish (*Prosopium coulterii*) in north-central British Columbia, Canada.



other through the main body of the Williston Reservoir, which served as the “root” of the network, i.e., that area that all fish would need to travel moving from one locality to another (cf. Costello et al. 2003). These nodes were intended as a representation of the number of discrete “choices” that a fish would need to make in moving from one locality to another with, presumably, the number of choices being inversely related to the likelihood of successful movement between localities. We then tested for isolation by distance using the Mantel test option in FSTAT to assess the significance of correlations between geographic (fluvial) distance and genetic distance estimated by θ . Partial Mantel tests

were conducted using both geographic distance and number of drainage nodes separating localities. None of the localities are separated from each other by any known complete migration barriers (e.g., insurmountable waterfalls; R. Zemlak, personal observations) with the exception of Dina Lake No. 1, which is an isolated lake basin (Fig. 1). To account for its lack of current connectivity with other systems, we added 2 to the drainage matrix values involving Dina Lake No. 1. To better visualize the spatial distribution of genetic differentiation, we constructed a “stream tree” of genetic distances among all localities within the Peace–Williston drainage system using the program StreamTree (Kalinowski et al. 2008)

Table 1. Pairwise $F_{ST}(\theta)$; Weir and Cockerham 1984) between populations of pygmy whitefish (*Prosopium coulterii*) assayed at 10 microsatellite loci.

	QL	WL	BL	PR	SMB	OA	AL	TU	CH	UL	UM	LM	DL*	UT	LT*
QL	—	0.18341	0.43540	0.09938	0.07794	0.12641	0.20100	0.17061	0.17520	0.16429	0.13595	0.13392	0.14715	0.15540	0.15576
WL		0.30157	0.09311	0.09311	0.11216	0.10183	0.06615	0.08734	0.14009	0.08470	0.07347	0.09755	0.05650	0.07996	0.11548
BL			0.35973	0.37799	0.36273	0.34295	0.37283	0.3283	0.41896	0.35211	0.32493	0.33375	0.34555	0.33023	0.38471
PR				0.00153	0.00968	0.06483	0.05215	0.04569	0.10469	0.03388	0.04569	0.05259	0.08199	0.06294	0.05716
SMB					0.01356	0.08726	0.07486	0.04978	0.09943	0.04978	0.03921	0.04259	0.07513	0.06976	0.05831
OA						0.06468	0.06722	0.09853	0.09853	0.02326	0.03312	0.03641	0.08881	0.08340	0.08173
AL							0.01910	0.10318	0.10318	0.01007	0.07179	0.09358	0.10007	0.04723	0.06574
TU								0.07917	0.07917	0.01590	0.08938	0.10616	0.12090	0.06084	0.06408
CH									0.05940	0.05940	0.12192	0.14055	0.09916	0.06033	0.07876
UL											0.04924	0.06385	0.08804	0.04583	0.04688
UM												-0.00589	0.05128	0.07159	0.08609
LM													0.07570	0.09713	0.10907
DL*														0.05950	0.11287
UT*															0.02108

Note: Underlined values are not significantly different from zero. Population codes and sample sizes are given in Table 2 and Fig. 3.

*Consists of pooled 2004 and 2005 samples that were not significantly different from one another.

to map genetic distances (θ) to the various stream sections that connect localities. The model of Kalinowski et al. (2008) assumes that genetic distances between populations are a function of the sum of genetic distances for the stream sections that connect them and thus helps to better visualize how the total genetic differentiation within a watershed is spatially distributed among its components. The fit of the stream tree model to the empirical data is summarized by calculating the coefficient of determination (r^2) as described by Kalinowski et al. (2008).

We used the program 2MOD (Ciofi et al. 1999) to assess the likelihood of two demographic models of population structure in pygmy whitefish: the first (the gene flow model) being that allele frequencies among populations are largely determined by a balance between gene flow and drift and the other (drift model) that allele frequencies have been determined largely by drift among populations separated since founding. Both models operate under the assumption that mutation since founding is a negligible factor organizing population structure (Ciofi et al. 1999). The method employs the coalescent framework and Markov chain Monte Carlo simulations to calculate the relative likelihoods of the two models over a series of iterations (250 000) given the allele frequency data. The probability of either model is then determined by the proportion over all iterations for which either model was the most likely. The 2MOD analysis was conducted 10 times with distinct random number seeds to assess consistency of the final results.

We also used GENECLASS version 2.0 (Piry et al. 2004) to test for migration between populations by calculating the likelihoods of individuals being first-generation migrants. Using variation across loci, the likelihood score was calculated as L_h , where L_h is the likelihood of drawing a particular individual's genotype from the population from which it was sampled given the observed set of allele frequencies (Paetkau et al. 2004). We chose to calculate L_h because it is probable that we did not sample all potential source populations in the watershed (Paetkau et al. 2004). We calculated the probability that an individual was born in the population from which it was sampled by using the genotype resampling procedure recommended by Paetkau et al. (2004). Here, repeated sets of simulated genotypes are generated from the empirical allele frequency distribution, where each set is the size of the total sample of individuals (a mean of 30 per population in our case). The number of sets is repeated until 10 000 simulated diploid genotypes are generated. An individual fish was rejected as a member of the population from which it was sampled if the observed likelihood of population membership fell with the lowest 5% of the simulated, randomly generated values for the population in question. Because we probably did not sample all potential habitats in the watershed, fish identified as potential immigrants to the locality from which they were sampled were inferred to have originated from a specific potential donor locality only if the probability of membership to that locality was at least 90%.

Results

Microsatellite variation

Initial analysis of microsatellite variation suggested the presence of one or more null alleles in 7 of the 15 popula-

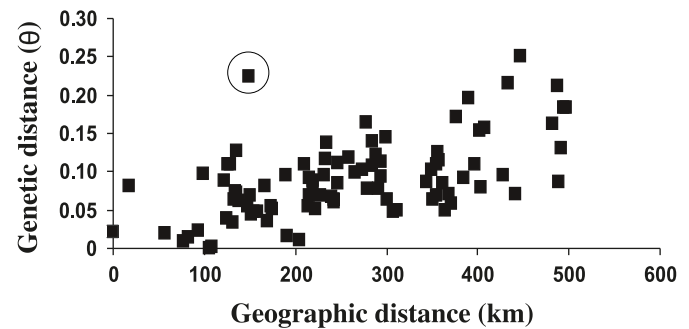
tions at BT73 with estimated frequencies within populations ranging between 0.16 and 0.24. Indeed, this locus had a consistent deficiency of heterozygotes in most populations (Supplementary Table S1).¹ Consequently, this locus was eliminated from subsequent analyses. Across all populations, *Sco107* was the most variable locus with a total of 47 alleles resolved and a mean expected heterozygosity of 0.88 (SD = 0.17), while both *Cocl-Lav74* and *Cocl-Lav224* had only 5 alleles each and mean expected heterozygosities of 0.40 (SD = 0.13) and 0.05 (SD = 0.05), respectively (Supplementary Table S1).¹ Across all populations, levels of allelic variation were remarkably consistent. The mean number of alleles across loci ranged from 4.3 to 4.5 in Quentin and Black lakes and 8.0 in lower Tacheeda Lake with most populations having between 5 and 7 alleles per locus (Supplementary Table S1).¹ Mean expected heterozygosity ranged from a low of 0.44 in Black Lake to a high of 0.59 in Weissener Lake (overall mean across populations and loci of 0.51 (SD = 0.05)).

Tests for deviations from Hardy–Weinberg equilibrium expectations resulted in 10 significant deviations across 170 possible tests. Most of these involved *Cocl-Lav10* and were associated with an excess of heterozygotes (Supplementary Table S1).¹ Tests for linkage disequilibrium were all non-significant within populations (all $P > 0.05$ for 45 simultaneous tests within populations) or between any pair of loci pooled across populations (all $P > 0.1$).

Population structure

Population subdivision (F_{ST}) as measured by θ was 0.12 and significantly greater than 0 (95% confidence intervals of 0.08–0.15 from bootstrapping over loci), and between all possible pairs of samples θ ranged from a low of 0.0 to 0.41 (Table 1). The lowest values of 0.0 were observed between upper and lower Manson lakes and θ was 0.001 between Williston Reservoir's Peace Reach and Six Mile Bay samples (all $P > 0.05$). The only other nonsignificant pairwise comparisons, out of 136 total, were between Dina Lake samples collected in 2004 and 2006 and between lower Tacheeda Lake's samples collected in 2004 and 2005 ($\theta = 0.0$ and 0.007, respectively; both $P > 0.1$), suggesting that genetic structure is generally temporally stable at least over the short term. The highest values of θ were between all WRW samples and those from Black Lake on the Alaska Peninsula (0.31–0.41, all $P < 0.001$). When the Alaskan sample was removed, θ across all WRW samples dropped to 0.080 but was still significantly greater than zero (95% confidence intervals of 0.047–0.126). The Quentin Lake sample was consistently the most distinct from all other samples (θ range of 0.09–0.20; Table 1). When the populations were grouped into five sub-drainages (Aiken–Tutuzzi–Uslika lakes; Manson lakes; Tacheeda lakes; Weissener–Quentin lakes; Williston Reservoir proper), 5.1% of the variation was attributable to these sub-drainage groupings ($P < 0.001$), 3.2% to variation among localities within these groups, and 92.6% to variation within localities (all $P < 0.001$). By contrast, when grouping the populations into northern and southern or eastern and western portions of the Williston watershed, the between group

Fig. 2. Association between pairwise measures of genetic distance (F_{ST} estimated by θ) and geographic distance (river kilometres) between all localities within the Williston Reservoir watershed (WRW). The circled value represents the comparison between Quentin and Weissener lakes (see text).

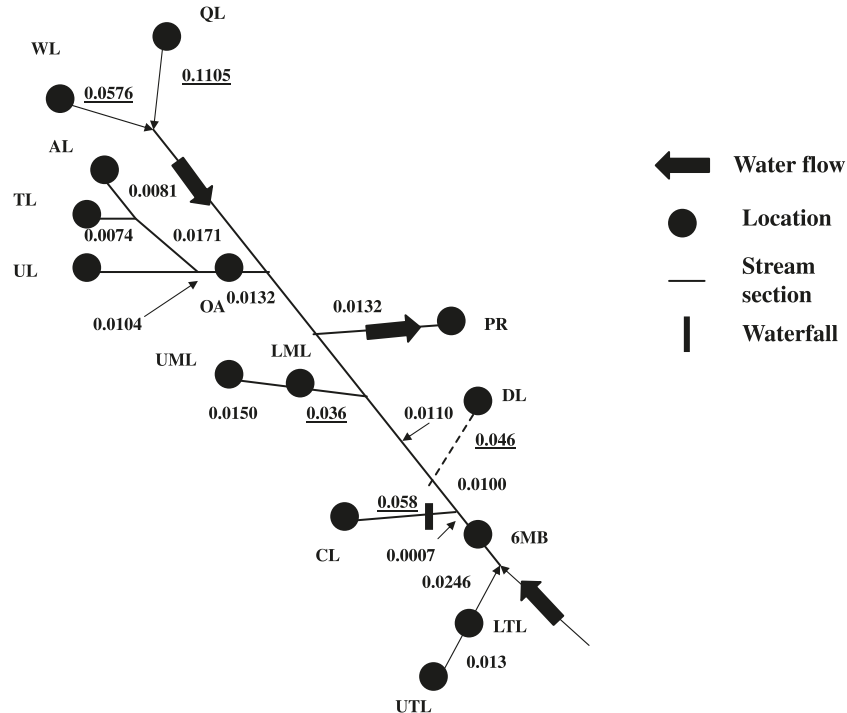


variance components explained 0.34% and 1.8% of the variance ($P = 0.2$ and 0.02 , respectively), the among populations within these groups explained 10.1% and 8.1% of the variance (both $P < 0.001$, respectively), and the within populations components explained 88.9% and 91.1% of the variance (both $P < 0.001$, respectively).

Pairwise comparisons of geographic distance and θ indicated that pygmy whitefish were characterized by a moderate pattern of isolation by distance ($r^2 = 0.36$, $P < 0.005$; Fig. 2). Taking the natural logarithm of geographic distance or using Slatkin's linearized F_{ST} , $F_{ST}/(1 - F_{ST})$ (Slatkin 1995), did not strengthen the relationship. In addition, a second Mantel test indicated that the residuals were strongly positively associated with geographic distance ($r^2 = 0.60$, $P < 0.001$). One comparison, between Quentin and Weissener lakes, however, appeared to show greater genetic distinctiveness than suggested by geographic distance between them (149 km) alone (Fig. 2). The number of drainage network nodes between these two localities, eight, was greater than between other locality pairs separated by comparable geographic distances (e.g., Aiken Lake vs. Omineca Arm = 5 nodes, 150 km; upper Manson River vs. Peace Reach = 3 nodes, 148 km). Overall, the number of nodes between localities ranged from one (between Peace Reach and Six Mile Bay, upper and lower Manson lakes, and upper and lower Tacheeda lakes) to 12 (between Dina Lake No. 1 and Weissener Lake). Indeed, the number of nodes in the hydrographic network was strongly positively associated with geographic distance between localities ($r^2 = 0.57$, $P < 0.005$), and accounting for node number in a partial Mantel test with geographic distance marginally decreased the explained variation in genetic distance ($r^2 = 0.31$, $P < 0.01$). The stream tree (Fig. 3) showed a strong relationship ($r^2 = 0.74$) between the observed interlocality distances (θ) and those fitted by assuming that such distances can be modeled as the sum of genetic distances for the stream sections that connect them. Stream sections with the greatest genetic distances involved those between Quentin, Weissener, Chuchi, and Dina lakes (all $\theta > 0.04$). A model of gene flow–drift equilibrium was strongly supported by the 2MOD analysis

¹Supplementary Table S1 is available with the article through the journal Web site (<http://www.nrcresearchpress.com/cjz>).

Fig. 3. Simplified schematic diagram of stream or reservoir sections and associated genetic distances (θ) between them estimated using the stream tree algorithm of Kalinowski et al. (2008). Any river section that is unlabelled has an associated genetic distance of zero and the highest five genetic distances are underlined. Sample codes are Quentin Lake (QL), Weisener Lake (WL), Williston Reservoir Peach Reach (PR), Williston Reservoir Six Mile Bay (6MB), Williston Reservoir Omenica Arm (OA), Aiken Lake (AL), Tutizzi Lake (TL), Chuchi Lake (CL), Uslika Lake (UL), lower Manson Lake (LML), upper Manson Lake (UML), Dina Lake No. 1 (DL), upper Tacheeda Lake (UTL), and lower Tacheeda Lake (LTL). Lengths of stream sections are not drawn to scale. The broken line between DL and the main stream axis represents the fact that DL is an isolated lake. The vertical line across the connection between CL and the main river axis represents a waterfall.



($P_{\text{gene flow model}} = 0.99$) over that of drift only model across all 10 runs of the analysis.

Detection of putative immigrants

Of the 503 pygmy whitefish assayed, 52 were determined to have a probability of assignment to the locality of collection of <5% and were identified as potential immigrants (Table 2). Typically, most fish identified as potential immigrants were suggested to have emigrated from an immediately adjacent population. For instance, fish were inferred to have moved between Peace Reach and Six Mile Bay of the Williston Reservoir and between upper and lower Tacheeda lakes. By contrast, Dina Lake is completely isolated from all other localities that we sampled, yet two fish collected in Dina Lake were inferred to be migrants from another locality and four fish collected in other localities were inferred to be recent emigrants from Dina Lake (Table 2). In addition, 28 putative immigrant fish were unable to be assigned to a specific donor population with a probability of at least 0.9 (Table 2).

Discussion

Microsatellite variation in pygmy whitefish

There has been detailed study of geographic variation in microsatellite DNA in only three coregonine fishes in North America: the lake whitefish, cisco (*Coregonus artedii* Lesueur, 1818), and mountain whitefish. In cisco, mean num-

bers of alleles per locus (across seven loci) per population was 9.3 and expected heterozygosity (H_E) was 0.64 (Turgeon and Bernatchez 2001). Across 19 lakes in the St. John River system, northeastern North America (about 21 000 km²), microsatellite variation over six loci averaged about five alleles per locus and H_E was 0.55 in lake whitefish (Lu et al. 2001). Across a smaller geographic scale (Lake Superior), microsatellite variation in lake whitefish averaged about 6.5 alleles per locus and H_E averaged 0.69 (Stott et al. 2004). Microsatellite variation in mountain whitefish (primarily a riverine species) has ranged from two to nine alleles per locus (eight loci) and H_E ranged from 0.40 to 0.58 within the Clark Fork River in Montana (Whiteley et al. 2004) to mean values of 1.0–4.8 alleles per locus and H_E of 0.0–0.54 across their complete geographic range (Whiteley et al. 2006). Comparisons across studies are difficult when different loci and geographic ranges are studied, but our data (mean alleles per locus per population of 5.7, mean H_E of 0.51) are broadly consistent with these previous studies and at least do not suggest that our choice of loci resulted in overly conservative assessments of variability within and between populations.

Population structure

Our data showed most strikingly that the pygmy whitefish from Black Lake on the eastern margin of the Alaska Peninsula were the most divergent population. This is, of course, not surprising given the geographic remoteness of this population from the Williston Reservoir (approximately 2200 km

Table 2. Identification of 52 inferred immigrants using 10 locus genotypes for pygmy whitefish (*Prosopium coulterii*).

Recipient locality	No. of immigrants/ <i>N</i>	Locality of inferred immigrants
Quentin Lake (QL)	1/30	<u>DL</u>
Weissener Lake (WL)	1/30	UNK
Dina Lake Number 1 (DL)	2/60	6MB, UNK
Williston Reservoir – Peace Reach (PR)	5/30	6MB (2) , UNK (3)
Williston Reservoir – Six Mile Bay (SMB)	10/30	PR , AL, <u>DL</u> , OA , UNK (6)
Williston Reservoir – Omineca Arm (OA)	3/30	PR (2) , 6MB
Aiken Lake (AL)	3/30	UNK (3)
Tutizzi Lake (TL)	3/30	UNK (3)
Uslika Lake (UL)	2/30	OA , UM
Chuchi Lake (CL)	4/30	6MB (3), OA
Upper Manson Lake (UM)	5/30	LM , UNK (4)
Lower Manson Lake (LM)	3/30	UNK (3)
Upper Tacheeda Lake (UT)	5/30	LT (2) , WL, UNK (2)
Lower Tacheeda Lake (LT)	5/60	<u>DL</u> (2), AL, UNK (2)

Note: Immigrants were defined as fish with a probability of <0.05 of belonging to the recipient (collection) locality from exclusion tests. Given are the recipient locality, the number of fish inferred to be immigrants out of the total assayed (*N*), and the localities inferred to have contributed immigrants to the recipient locality. Names in boldface type represent localities in the same sub-watershed as the recipient locality and underlined localities indicate inferred migrants between localities that are completely isolated from one another. The number in parentheses indicates the number of inferred immigrants if greater than one and UNK indicates that the source locality of the inferred immigrant could not be determined with a probability of at least 0.9.

to the northwest). There is, however, likely a deeper historical reason for the distinctiveness of pygmy whitefish from Black Lake because sequence data from mtDNA and *ITS-1* genes indicate that the pygmy whitefish from this region of Alaska belong to a distinctive phylogeographic lineage relative to fish from the rest of the geographic range of the species (Witt et al. 2011). It is likely, therefore, that these fish represent whitefish from a distinct genetic lineage that survived the Pleistocene glaciations in a distinct western Alaskan refugium (McPhail and Lindsey 1970; McCart 1970; Witt et al. 2011). Similar distinctions between fishes inhabiting regions in Beringia (the lower Yukon River and adjacent areas in Alaska and Siberia) and farther east in North America have been noted previously (cf. Bernatchez and Dodson 1991; Stamford and Taylor 2004).

Within the WRW, pairwise F_{ST} averaged 0.08, which is very similar to a value of 0.06 reported across nine microsatellite loci in mountain whitefish in the Bitterroot River system in Montana. The geographic scale of study was slightly greater in the survey of mountain whitefish (mean distance between localities of about 400 km) than in our study (279 km not including Black Lake). The slightly greater level of differentiation, despite smaller geographic distances between localities of pygmy whitefish, might be explained by the fact that our samples came from lakes, whereas the samples of mountain whitefish were largely riverine. Although pygmy whitefish inhabit both rivers and streams, they do not appear to be particularly abundant in such habitats; lake populations may only enter adjoining river systems during spawning, which may reduce the probability of exchange among localities, especially because in lakes pygmy whitefish are often found in deep water (Scott and Crossman 1973; Nelson and Paetz 1992). Despite the potential differences between lake-dwelling and stream-dwelling *Prosopium*, our

data, in conjunction with that of Whiteley et al. (2004), suggest relatively low levels of population subdivision compared with some other co-distributed salmonids such as bull trout. For instance, Whiteley et al. (2004) reported F_{ST} values for bull trout collected across the same range in the Bitterroot River, Montana, that were almost an order of magnitude greater than in mountain whitefish. They attributed the greater differentiation in bull trout to differences in reproductive habitat use and life history that would tend to restrict dispersal in bull trout relative to mountain whitefish. Our data indicate a similar level of population subdivision in pygmy whitefish and mountain whitefish over similar geographic scales and suggest that pygmy whitefish are influenced by the same life-history features (large population sizes, lower age at maturity, prespawning movements into rivers that might promote interconnectedness) that constrain divergence at neutral loci, relative to a species like bull trout. Indeed, the two species of *Prosopium* are considered ecologically similar and rarely co-occur despite broad overlap across large portions of their geographic ranges in western North America (Scott and Crossman 1973; Mackay 2000). There are no hatchery programs for pygmy whitefish, so these results undoubtedly reflect natural patterns of population structure.

To our knowledge, the current study is one of the few to apply the “stream tree” mapping approach of Kalinowski et al. (2008) and it provided a useful summary of the spatial arrangement of genetic differentiation. The stream tree approach allows one to extend isolation-by-distance analyses to visualize more precisely the variability in genetic distances between populations and how such variability may, or may not, be explained by distance between localities. Consequently, construction of stream trees helps to better integrate variation in genetic distance with variability in the physical riverscape and can promote a more complete understanding

of what shapes patterns of genetic differentiation. For instance, the heightened genetic differentiation of Quentin Lake from all other lakes in the WRW does not appear to be explained by geographic distance alone and suggests the presence of some other, as yet unknown, barrier to movement between Quentin Lake and other localities. Although no known geographic barrier (waterfall or high-velocity cascade) exists between Quentin Lake and the other localities (R. Zemplak, unpublished data), the lake has a very distinct limnological character. It is glacially fed and is very turbid with high glacial flour content both in the lake and the outlet stream (R. Zemplak, unpublished data). It is possible, therefore, that the distinct physical character of Quentin Lake and its outlet influences movement of pygmy whitefish from and into the lake. Such anomalies stand out in stream trees and help raise the possibility that distinct environments may play an important role in influencing population structure (e.g., Hokit et al. 2010). The principal assumption of the stream tree model of Kalinowski et al. (2008) is that genetic distances between localities can be modeled as a sum of genetic distances for the stream sections interconnecting them. Our analysis, however, produced only a moderate association ($r^2 = 0.74$) between the observed genetic distances between populations (θ) and those fitted under the stream tree model. Kalinowski et al. (2008) reported much higher associations ($r^2 \geq 0.97$) in three other freshwater fishes (and lower in one other, $r^2 = 0.68$). Moderate conformance to the strict stream tree model could be explained by historical effects (e.g., watershed transfers), situations where genetic distance actually declines with distance (e.g., a large bottleneck in a geographically intermediate population that might exaggerate genetic distance estimates), life-history differences between populations that might influence gene flow (Kalinowski et al. 2008), or by an incomplete understanding of the actual level of contemporary connectivity between localities such as discussed above in term of Quentin Lake's limnological character.

Conservation implications

Stamford and Taylor (2005) examined the population subdivision of Arctic grayling (*Thymallus arcticus* (Pallas, 1776)) sampled from a comparable area of the Williston Reservoir drainage using seven microsatellite loci and reported a mean F_{ST} of 0.085 and strong isolation by distance ($r^2 = 0.41$). Taken together, the comparable levels of population subdivision among lake-caught pygmy whitefish and river-sampled Arctic grayling and mountain whitefish suggest that despite their lake-dwelling habits, pygmy whitefish from the WRW are probably linked genetically and possibly demographically through interlocality dispersal via the interconnecting rivers in the watershed across individual or a few generations. Our data also suggest that the "complexity" of interlocality movement contributes to the extent of neutral genetic divergence because one interlocality genetic comparison (Quentin vs. Weissener lakes) was much greater than suggested by the geographic distance separating them (149 km) and because these two lakes had a high number of drainage nodes separating them from each other (eight).

A general model of isolation by distance was also supported by the results from the migrant detection analysis; a number of fish were inferred to be interlocality migrants, or their recent descendants, and most interlocality dispersal was

between localities that are geographically proximate to one another. There were, however, six cases where interlocality dispersal was inferred between areas that are not presently physically connected, i.e., into or from Dina Lake, a lake that has no physical connection to the Williston Reservoir or any other area that we sampled. Dina Lake was differentiated from the other Williston Reservoir localities by a mean θ of about 0.09. The inferred immigrants among all localities could result from actual movement or a lack of assignment power owing to retention of ancestral allelic polymorphisms, large historical effective population sizes (N_E), and consequently, low divergence from drift. The latter possibility can be assessed by using the isolated nature of Dina Lake, the value of θ between it and Williston Reservoir samples and estimating N_E under a model of complete isolation. Under complete isolation, $F_{ST} = 1 - (1 - 1/2N_E)^t$, where t is the number of generations of isolation (Nei and Chakravarti 1977). Assuming that Dina Lake has been isolated from the Parnip River region of what is now the southern reach of the Williston Reservoir since deglaciation was completed 10 000 years ago (Dyke 2004), and a typical generation time for pygmy whitefish of 3 years (McPhail and Lindsey 1970), t is estimated to be 3300 generations. Substituting the observed $\theta = 0.09$ into the equation above gives an estimated N_E of 17 500 between localities or about 8 750 each. Given that single trap nets in Dina Lake have captured up to 2000 individuals (Zemplak and McPhail 2006), our estimates of long-term N_E do not seem unreasonable, especially given evidence that contemporary N_E may be much smaller than historical N_E in temperate freshwater fishes (Stamford and Taylor 2005; Swatdipong et al. 2010). It appears, therefore, that in certain habitats, population sizes of pygmy whitefish have been substantial enough historically to constrain postglacial divergence at the neutral loci that we examined even under complete isolation such as between Dina Lake and all other localities. Still, if the inferred dispersal to and from the isolated Dina Lake is interpreted as an estimate of "false" migrant detection owing to insufficient assignment power, the other 46 inferred migrants suggest some degree of contemporary movement among interconnected localities even if any individual inferred instance of migration needs to be interpreted cautiously. For instance, the several instances of dispersal among Peace Reach, Omineca Arm, and Six Mile Bay areas of Williston Reservoir, or between upper and lower Mansen or Tacheeda lakes, are quite realistic given the broad interconnectedness among these areas (Fig. 1). By contrast, inferred recent migration between the Tacheeda lakes system and Weissener Lake, located over 400 km apart at opposite ends of the watershed, is less likely.

Our data, however, also suggest that across the physically interconnected localities, restricted gene flow (in addition to population size) is also an important factor influencing genetic divergence at neutral loci. For instance, when there is a signal of recent gene flow (e.g., soon after a range expansion), F_{ST} values should show little association with distance and be consistently low over all distances. In contrast, when drift predominates, there may be little correlation between F_{ST} and geographic distance, but that relationship should be highly variable and might signal a recent range fragmentation (Hutchison and Templeton 1999). Regional equilibrium between gene flow and drift is suggested by our data because

of the strong pattern of isolation by distance resolved, the positive association between the residuals of this relationship and geographic distance (Hutchison and Templeton 1999), and the high probability of the gene flow–drift demographic model given the allele frequency data (Ciofi et al. 1999).

Before our study and that of Zemplak and McPhail (2006) and Witt et al. (2011), very little was known about the biology or evolutionary history of pygmy whitefish across its range, but it was considered to be a glacial relict that typically inhabited large, deep (>50 m), cold lakes. We now know that pygmy whitefish consist of at least two major evolutionary lineages across its geographic range (Witt et al. 2011; this study). In addition, in British Columbia, broad faunal surveys across the Williston Reservoir drainage and detailed study in one small sub-boreal lake have indicated that the species may often occur across a broader range of lake sizes and habitats (including large and small rivers) than originally perceived (Zemplak and McPhail 2006; McPhail 2007). Further, our data suggest that across large, interconnected drainages, lake populations of pygmy whitefish are genetically and possibly demographically interconnected at least over the medium to long term. This implies that the intervening stream and river habitats are critical in maintaining such interconnectedness. The demographic linkage among populations may take place during spawning periods when some lake-dwelling populations enter streams and migrate to spawning areas (Hallock and Mongillo 1998). Our data, in particular, suggest that pygmy whitefish are ecologically much more diverse than simply deepwater inhabitants of large lakes. In the Williston Reservoir drainage, they occur in several small lakes and populations in these lakes appear to be genetically linked through a pattern of isolation by distance. Whiteley et al. (2006) reached essentially a similar conclusion for the congeneric mountain whitefish in watersheds across its range and our data support these authors' contention that demographic exchange, as inferred through genetic estimates of gene flow, tends to occur over broader geographic scales in *Prosopium* than in other co-distributed salmonids, such as bull trout (cf. Costello et al. 2003; Whiteley et al. 2006). More generally, therefore, conservation programs in the Williston Reservoir drainage (and others like it) will need to maintain habitat interconnectedness to promote persistence of native species among a diversity of aquatic habitats across potentially a variety of spatial scales appropriate for each species. Our genetic analysis of pygmy whitefish is just a first step in building an appreciation of the conservation needs of this and similar species. A more precise understanding of demographic connectivity across ecological time scales in pygmy whitefish will require a greater investment in the estimation of how interlocality dispersal influences population growth and vital rates (see review by Lowe and Allendorf 2010).

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