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Ecological and life history characteristics predict population genetic divergence of two salmonids in the same landscape

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Abstract

Ecological and life history characteristics such as population size, dispersal pattern, and mating system mediate the influence of genetic drift and gene flow on population subdivision. Bull trout (*Salvelinus confluentus*) and mountain whitefish (*Prosopium williamsoni*) differ markedly in spawning location, population size and mating system. Based on these differences, we predicted that bull trout would have reduced genetic variation within and greater differentiation among populations compared with mountain whitefish. To test this hypothesis, we used microsatellite markers to determine patterns of genetic divergence for each species in the Clark Fork River, Montana, USA. As predicted, bull trout had a much greater proportion of genetic variation partitioned among populations than mountain whitefish. Among all sites, F_{ST} was seven times greater for bull trout (F_{ST} = 0.304 for bull trout, 0.042 for mountain whitefish. After removing genetically differentiated high mountain lake sites for each species F_{ST}, was 10 times greater for bull trout (F_{ST} = 0.176 for bull trout; F_{ST} = 0.018 for mountain whitefish). The same characteristics that affect dispersal patterns in these species also lead to predictions about the amount and scale of adaptive divergence among populations. We provide a theoretical framework that incorporates variation in ecological and life history factors, neutral divergence, and adaptive divergence to interpret how neutral and adaptive divergence might be correlates of ecological and life history factors.

Keywords: adaptive divergence, dispersal, neutral divergence, population genetic structure, population subdivision, *Prosopium williamsoni*, *Salvelinus confluentus*

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Introduction

Analysis of population genetic structure reveals groups of populations that share a common evolutionary history and the geographical scale at which evolutionary processes occur for a species (Waples 1995). Genetic divergence at a series of putatively neutral markers is often used to define management units, identify populations with unusual genetic characteristics and identify populations with reduced genetic variation that might have reduced probability of persistence (Avise 2004). In addition, genetic differentiation observed at neutral markers can be used as an indicator of adaptive divergence among populations (Fraser & Bernatchez 2001; Morgan *et al*. 2001). Finally, by comparing the genetic structure of closely related species we can determine if differences in their biology lead to differences in how genetic variation is distribution within and among populations.

Historical factors (e.g. vicariant fragmentation, extinction and recolonization, and range expansion) influence patterns of the distribution of genetic variation of a species and can produce patterns similar to the effects of ongoing gene flow (Felsenstein 1982; Templeton *et al*. 1995; Hewitt 2000; Turgeon & Bernatchez 2001). In particular, landscape features that disrupt gene flow are often responsible for among-population genetic differentiation, or neutral divergence (Angers *et al*. 1999; Keyghobadi *et al*. 1999; Castric *et al*. 2001; Cassel & Tammaru 2003; Costello *et al*. 2003). By comparing multiple species in the same environment, the effect of common landscape-level environmental factors on genetic structure can be determined (Bermingham & Moritz 1998). In addition, comparisons of multiple species that inhabit the same landscape allow us to test hypotheses regarding factors other than physical barriers,

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such as ecological and life history characteristics, that might also influence neutral divergence.

A number of studies have hypothesized that ecological and life history factors such as population size, dispersal pattern, and mating system are related to population genetic divergence through their effects on genetic drift and gene flow (Turner & Trexler 1998; McDonald *et al*. 1999; King & Lawson 2001; Dawson *et al*. 2002). There is strong support for an association between dispersal ability and neutral divergence across a wide array of taxa (Peterson & Denno 1998; Bohonak 1999). McDonald *et al*. (1999) demonstrated an association between neutral divergence and habitat-related dispersal patterns along with social system in two jays in the genus *Aphelocoma*. Use of aquatic habitat explained dispersal patterns and neutral divergence among three natricine snakes (King & Lawson 2001). Dawson *et al*. (2002) noted a relationship between larval duration, habitat-mediated dispersal patterns, and population size with patterns of neutral divergence in two marine gobies (Gobiidae) and many studies of marine organisms have tested for a relationship between larval dispersal ability and neutral divergence (reviewed in Bohonak 1999). In fishes residing in linear stream habitats, Turner (2001) and Turner & Trexler (1998) tested for an association between neutral divergence and life history traits in species of darters (Percidae) and Castric & Bernatchez (2004) found differences in patterns of genetic structure for two salmonids that were expected to differ in dispersal potential in the same landscape. However, the association between genetic subdivision and dispersal patterns, population size and mating system has not been considered simultaneously in stream-dwelling fishes.

Within streams, ecological and life history characteristics should have a large impact on neutral divergence. Spatial separation of reproduction sites will affect dispersal patterns because more closely situated downstream sites are more likely to be encountered by a dispersing individual. In addition, the probability of individual dispersal will be reduced if individuals must navigate through a complex environment to reach spatially separated sites in order to reproduce. Aspects of the mating system might act as a prezygotic isolating mechanism reducing gene flow because a dispersing individual might have a lower probability of successfully mating in systems with more complex behaviours (i.e. paired matings that involve mate choice vs. group spawning without mate choice). Other aspects of life history that might be important for dispersal are philopatry and specificity of reproductive timing (Avise 2004). Finally, populations of different sizes experiencing the same migration rate (m, defined as the proportion of individuals in each population that are from outside that population) have very different patterns of neutral divergence; larger populations will be much less divergent than smaller populations because the absolute number of migrants per generation (N_em) will be larger and drift will not cause as much population divergence.

Bull trout (*Salvelinus confluentus*) and mountain whitefish (*Prosopium williamsoni*) are two species in the family Salmonidae that co-occur throughout much of western North America (Scott & Crossman 1979). Within the same river systems, these species differ markedly in spawning location, mating system and population size and thus lie at the extreme ends of a continuum of factors that might influence patterns of dispersal and gene flow. Bull trout spawn in upstream portions of tributary streams that are generally characterized by environmental heterogeneity among locations (Rieman & McIntyre 1995; Swanberg 1997). Mountain whitefish spawn in downstream locations that are less environmentally heterogeneous (Davies & Thompson 1976; Northcote & Ennis 1994). Because of their spawning locations, dispersing bull trout must move further to spawn in adjacent tributary streams than mountain whitefish spawning in river mainstems or near the mouths of tributaries. Bull trout home to natal spawning sites with high precision (McPhail & Baxter 1996; Spruell *et al*. 1999; Neraas & Spruell 2001). There is some evidence that mountain whitefish return to experimental release sites within the same season (Liebelt 1970) and that they home to spawning locations (Pettit & Wallace 1975). Bull trout spawning migrations must be closely matched to environmental conditions such as seasonally reduced stream flow (Pratt 1992), while there is little evidence of such habitat specificity for mountain whitefish. Bull trout females choose dominant males and the pair spawn in a nest, or redd, often with one to several satellite males involved (Stearley 1992). Mountain whitefish spawn in groups without digging redds (Northcote & Ennis 1994) and appear to have less complex mating behaviour (Brown 1952; A. R. Whiteley personal observations). Finally, bull trout have small population sizes (Swanberg 1997) while mountain whitefish populations are often very large (Northcote & Ennis 1994). These combined factors should lead to less gene flow among populations of bull trout than mountain whitefish.

In this paper, we compared neutral molecular divergence among populations of bull trout and mountain whitefish from the Clark Fork River, Montana, USA. We predicted a priori that mountain whitefish would have greater within-population genetic variation and reduced neutral divergence among populations. We tested this hypothesis by describing the genetic structure of each species using microsatellite markers. We also tested for common landscape factors that influence the distribution of genetic variation in each species. The same ecological and life history factors that allowed us to predict relative amounts of neutral divergence are also consistent with differences in likelihood of local adaptation. We use our results to suggest a general framework for the interactions among ecological and life history factors, neutral divergence

among populations, and divergence among populations in traits likely to be important for local adaptation (adaptive divergence).

Materials and methods

Study location

The Clark Fork River forms a portion of the headwaters of the Columbia River and has three major tributaries: the Blackfoot, Bitterroot and Flathead Rivers (Fig. 1). Bull trout and mountain whitefish occur throughout the Clark Fork River system, including some high mountain lakes. Several dams occur in this system and three are most relevant to fish dispersal in this study. Milltown Dam is located at the confluence of the Blackfoot and Clark Fork Rivers and has blocked upstream movement of both species since 1907 (Schmetterling 2001). Turbines and predatory fish in the upstream reservoir impede downstream movement of juveniles and adults of both species, although downstream movement of adult bull trout has been observed (Swanberg 1997). Kerr Dam is located at the outlet of Flathead Lake and has blocked upstream fish movement since 1938 and Hungry Horse Dam is located where the South Fork of the Flathead River joins the Flathead River and has blocked upstream movement of fish since 1951.

Sample collection

Spawning groups of mountain whitefish (Fig. 1) were collected in 2000 and 2001 by electrofishing. In one case (Rattlesnake Creek, W2a and W2b; see Table 3) we collected spawning mountain whitefish from the same location in both 2000 and 2001. Care was taken to sample ripe adult fish that appeared to be spawning in the vicinity with the exception of the Flathead River sample (W9), where nonspawning adults were collected from the mainstem Flathead River. Bull trout juveniles were collected in tributary streams (Fig. 1) in 1998 and 1999 by electrofishing. Bull trout typically reside in their natal streams for at least 1–3 years, after which they either migrate to larger rivers or lakes or remain in their natal or closely associated stream (Dunham & Rieman 1999; Nelson *et al*. 2002). By restricting the bull trout collections to juveniles, it is highly likely that each site contained individuals from their natal stream. In addition, it is unlikely that juveniles move between sites at the scale of the comparisons made in our study. For both species, care was taken to minimize the occurrence of siblings or the representation of single cohorts in each sample. In general, the samples were distributed across at least three age classes. Both species were collected from the same tributary in two cases (B2, W2 and B3, W4; Fig. 1). Fin tissue was collected and stored in 95% ethanol until DNA extraction.

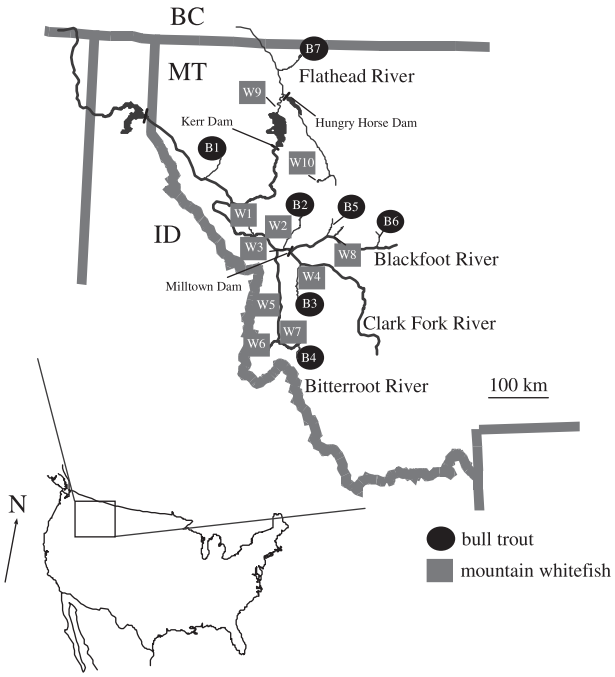

Fig. 1 Sample locations of bull trout (black circles) and mountain whitefish (grey squares) in the Clark Fork River, Montana. Sample numbers correspond to Tables 2 and 3.

Microsatellites

The general methods used for polymerase chain reaction (PCR) and visualization of subsequent PCR products followed Spruell *et al*. (1999) and Neraas & Spruell (2001). The seven variable microsatellite loci used for bull trout (*SCO19*, *FGT3*, *SSA456*, *SSA311*, *SFO18*, *BT73*, *OGO2* and *ONEμ7*) were described in Spruell *et al*. (1999) and Neraas & Spruell (2001). DNA was extracted from each fin clip by standard methods. All PCRs were performed using an MJ thermal cycler (MJ Research, Inc.). We visualized fluorescently-labelled PCR products on acrylamide gels and used individual fish of known genotypes as standards for scoring.

The following microsatellites were optimized for use for mountain whitefish: *COCL4*, *SSA14*, *SSA456*, *ONE8*, *FGT25*, *BT73*, *SFO8-1* and *SFO8-2* (Table 1). We confirmed disomic Mendelian inheritance for all eight loci using three mountain whitefish families, each with 10 offspring. Parents for these families were collected in 2000 from site W2 (Fig. 1). For *SSA456*, *FGT25* and *BT73*, the following thermal cycler profile was used: 93 °C for 3 min, 92 °C for 1 min, variable annealing temperature (listed in Table 1) for 1 min, and 72 °C for 1 min, with the number of cycles listed in Table 1. For the remaining loci, we used variations of the following touchdown PCR profile (Don *et al*. 1991): 96 °C for 5 min, 94 °C for 10 s, variable initial annealing temperature for 35 s (Table 1), and 72 °C for 1 min for seven cycles during which the annealing temperature was

Table 1 Locus names, number of alleles, size range, annealing temperature, and number of cycles for mountain whitefish microsatellites

Locus	Number of alleles	Size range (bp)	Annealing temperature (°C)*	Number of cycles†	Reference
COCL4	3	146–152	57–51	7,29	L. Benatchez personal communication, 2000
SSA14	5	167–175	57–51	7,29	O'Reilly *et al*. 1996
SSA456	15	138–232	52	30	O'Reilly *et al*. 1996
ONE8	6	178–190	60	30	Scribner *et al*. 1996
FGT25	4	170–180	57–51	7,26	Sakamoto *et al*. 2000
SFO8-1	3	158–164	55–49	7,31	Angers *et al*. 1995
SFO8-2	2	195–197	55–49	7,31	Angers *et al*. 1995
BT73	51	146–280	55	32	Estoup *et al*. 1993

*A range of temperatures indicates a touchdown PCR was used, where the annealing temperature was decreased 1° per cycle for seven cycles starting at the higher temperature. The remainder of the cycles were performed at the lower annealing temperature.
†The first number represents the number of cycles where the annealing temperature was decreased 1° per cycle. The second number is the number of cycles at the lower annealing temperature. The total number of cycles is the addition of both numbers.

decreased 1 °C per cycle. At the lower annealing temperature listed in Table 1, a variable number of cycles (Table 1) were performed with the following profile: 94 °C for 10 s, variable annealing temperature for 35 s, and 72 °C for 1 min. A final extension period of 72 °C for 10 min was used for all profiles. PCR reagent concentrations are available upon request from the authors.

Data analysis

Allele frequencies, deviations from Hardy–Weinberg expectations, linkage disequilibrium, observed (H_O) and expected (H_E) heterozygosity per locus and population, mean within-population expected heterozygosity (H_S), mean allelic richness per population, pairwise exact tests for genic differentiation, *F*-statistics and pairwise F_{ST} values were calculated using GENEPOP version 3.4 (Raymond & Rousset 1995) and FSTAT version 2.9.3.2 (Goudet 2001). We used θ (Weir & Cockerham 1984) for estimates of F_{ST}. Confidence intervals (95%) for multilocus F_{ST} estimates were generated by bootstrap sampling over loci (Goudet *et al*. 1996). We used F_{ST} instead of R_{ST} because F_{ST} estimates are more conservative when relatively few microsatellite loci are used (< 20) and populations have diverged recently (Gaggiotti *et al*. 1999). We adjusted the results from tests for conformation to Hardy–Weinberg proportions and linkage disequilibrium for multiple tests using the sequential Bonferroni procedure (Rice 1989). We determined the average number of loci for which we could reject the null hypothesis that allele frequency distributions were the same between populations (determined using pairwise exact tests for genic differentiation from GENEPOP version 3.4) at the $P < 0.05$ and $P < 0.001$ levels for both species.

We used PHYLIP version 3.5 (Felsenstein 1993) to calculate Cavalli-Sforza & Edwards's (1967) genetic distance (CSE) with the GENDIST module and to construct a UPGMA (unweighted pair group method with arithmetic mean) dendrogram using the NEIGHBOUR module. CONSENSE was used to generate a consensus tree with bootstrap values from 1000 replicate data sets created in SEQBOOT. We chose to analyse genetic divergence between populations using CSE because it is drift based, does not assume any models of mutation and performs well in simulations of microsatellite data (Takezaki & Nei 1996).

Mantel tests were used with 5000 replicates to compare matrices of both CSE distance and pairwise F_{ST} estimates to a matrix of geographical distance using the program ISOLATION BY DISTANCE (IBD; Bohonak 2003). We considered the relationship between genetic and physical distance with and without high mountain lake sites for each species because the differentiation we observed for these sites appeared to result from factors other than geographical distance alone. We estimated river distances among sample locations using digital topographic maps from National Geographical TOPO! version 2.7.4.

Results

Bull trout

We analysed bull trout from seven locations at seven microsatellite loci. There were six river sites (B1–B6; Fig. 1, Table 2) and one high-mountain lake site (Trout Lake, B7; Fig. 1, Table 2). Average within-population expected heterozygosity (H_S) ranged from 0.073 to 0.394 and mean allelic richness ranged from 1.1 to 2.8 (Table 2). The location with the least amount of genetic variation was Trout Lake (H_S = 0.073, allelic richness = 1.1). Meadow Creek (B4) had the greatest heterozygosity (0.441) and Rock Creek (B3) had the greatest allelic richness (2.8).

We did not detect any significant departures from Hardy–Weinberg proportions ($P > 0.05$) for bull trout. Three tests for linkage disequilibrium yielded *P*-values less than 0.05. There was no pattern of significant disequilibrium within

Table 2 Allele frequencies for bull trout in the Clark Fork River. Sample size (n), average expected heterozygosities (H_S), and mean allelic richness are shown

Sample number	Sample location	Basin	*SSA456*		*SSA311*		*OGO2*					*FGT3*					
			**157*	**159*	**112*	**120*	**150*	**154*	**156*	**158*	**162*	**165*	**167*	**169*	**171*	**173*	**175*
B1	Thompson River	Clark Fork	0.238	0.762	0.107	0.893	—	0.553	0.447	—	—	0.698	0.093	0.093	—	—	0.116
B2	Rattlesnake Creek	Clark Fork	0.740	0.260	0.135	0.865	0.250	0.231	0.462	0.019	0.038	0.904	—	—	—	0.096	—
B3	Rock Creek	Clark Fork	0.250	0.750	0.318	0.682	0.118	0.206	0.662	—	0.015	0.758	0.030	0.091	—	—	0.121
B4	Meadow Creek	Bitterroot	0.538	0.462	0.481	0.519	0.200	0.380	0.400	0.020	—	0.500	0.269	—	0.038	0.192	—
B5	Monture Creek	Blackfoot	0.569	0.431	0.207	0.793	0.417	0.017	0.550	—	0.017	0.672	0.103	—	—	0.224	—
B6	Copper Creek	Blackfoot	0.857	0.143	0.714	0.286	—	—	1.000	—	—	0.946	—	—	—	0.054	—
B7	Trout Lake	Flathead	0.486	0.514	—	1.000	—	—	1.000	—	—	—	1.000	—	—	—	—

Sample number	Sample location	Basin	*ONEμ7*		*SFO18*		*SCO19*					n	H_S	Mean allelic richness
			**218*	**244*	**150*	**156*	**174*	**200*	**202*	**206*	**216*			
B1	Thompson River	Clark Fork	0.895	0.105	0.962	0.038	0.157	0.800	0.043	—	—	43	0.318	2.4
B2	Rattlesnake Creek	Clark Fork	1.000	—	0.783	0.217	0.462	0.462	—	—	0.077	29	0.344	2.4
B3	Rock Creek	Clark Fork	1.000	—	0.691	0.309	0.448	0.397	0.052	0.017	0.086	34	0.402	2.8
B4	Meadow Creek	Bitterroot	1.000	—	0.940	0.060	0.435	0.391	0.174	—	—	27	0.441	2.6
B5	Monture Creek	Blackfoot	1.000	—	0.786	0.214	0.192	0.712	0.019	—	0.077	30	0.381	2.5
B6	Copper Creek	Blackfoot	0.880	0.120	1.000	—	0.019	0.981	—	—	—	30	0.146	1.7
B7	Trout Lake	Flathead	1.000	—	1.000	—	1.000	—	—	—	—	39	0.073	1.1

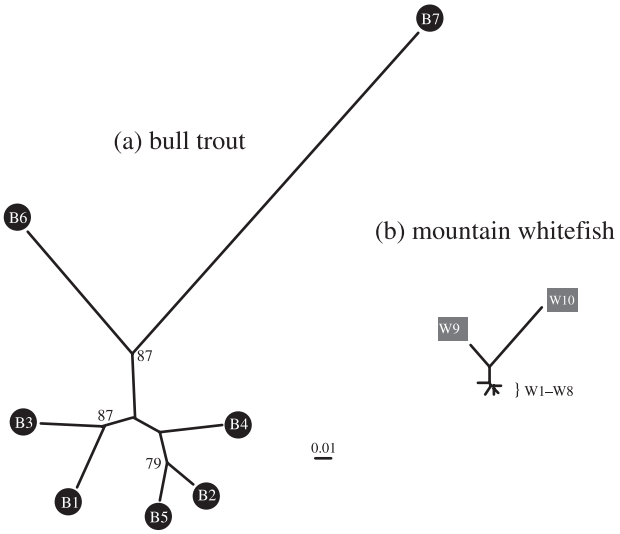

Fig. 2 UPGMA dendrogram based on Cavalli-Sforza & Edwards (1967) chord distances for (a) bull trout and (b) mountain whitefish in the Clark Fork River. There were no statistically significant differences in allele frequencies among samples W2a, W2b and W3, or among sites W5, W6 and W7. These sites were pooled into two groups for (b). Bootstrap values > 50% are shown for bull trout in (a). All bootstrap values were greater than 50% for the mountain whitefish dendrogram (b) but, for presentation purposes, were not shown.

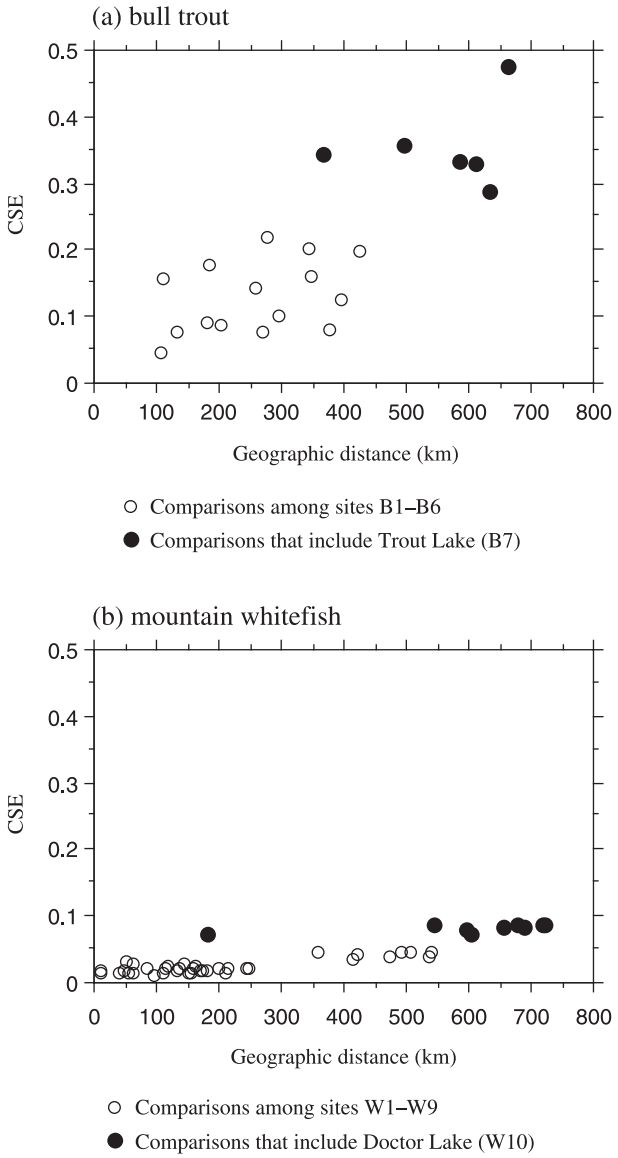

Fig. 3 Isolation by distance analysis of (a) bull trout and (b) mountain whitefish in the Clark Fork River. Pairwise Cavalli-Sforza & Edwards (1967) chord distances (CSE) are plotted against pairwise geographical distances for all sample sites for each species. Open circles, comparisons among sites B1–B6 (a) and W1–W9 (b); closed circles, comparisons that include high mountain lake sites Trout Lake, B7 (a), and Doctor Lake, W10 (b).

any of the population samples or for any of the locus pairs across populations and none of the differences was significant after sequential Bonferroni correction (0.05/21 comparisons per population sample with seven loci).

Variation in allele frequencies and thus genetic differentiation among bull trout sample locations was pronounced (F_{ST} = 0.304, 95% CI 0.212–0.382; Tables 2; see Table 4). The high mountain lake (Trout Lake, B7) was the most genetically differentiated site (Fig. 2a). Even with this site excluded, bull trout had a large proportion of genetic variation partitioned among sites. The F_{ST} for the six river sites (B1–B6) was 0.176 (95% CI 0.131–0.213; see Table 4). For tests of homogeneity of population allele frequencies at the seven loci analysed, on average 5.2 loci were statistically significantly different at the $P < 0.05$ level and on average 3.6 loci were significantly different at the $P < 0.001$ level. When the Trout Lake sample (B7) was removed, an average of 5.1 loci were statistically significantly different at the $P < 0.05$ level and 3.4 loci were statistically significantly different at the $P < 0.001$ level. When we combined P-values for the exact tests for population differentiation from all seven loci, all pairwise comparisons were highly significant ($P < 0.0001$).

The average geographical distance (± SE) between sites B1 and B6 was 261.4 ± 26.9 km and the average distance (± SE) between these six sites and Trout Lake was 559.8 ± 44.7 km (Fig. 3a). We found a significant relationship between pairwise CSE values and geographical distance for bull trout (r = 0.80, P = 0.001) when all comparisons were considered (Fig. 3a). When Trout Lake was removed, the relationship between pairwise CSE values and geographical distance was not significant (r = 0.39, P = 0.19). Results were similar if pairwise F_{ST} was used as the genetic distance metric (r = 0.74, P = 0.04 for all comparisons; r = 0.26, P = 0.25 when Trout Lake was removed) or when geographical distances were log transformed (data not shown).

Mountain whitefish

We used eight microsatellites to analyse mountain whitefish from 10 locations (Table 3). There were nine river sites (W1–W9; Fig. 1, Table 3) and one high-mountain lake site (Doctor Lake, W10; Fig. 1). We detected greater genetic variation within populations of mountain whitefish than bull trout. H_S ranged from 0.403 to 0.580 and mean allelic richness per population ranged from 2.5 to 5.2 (Table 3). Doctor Lake had the lowest allelic richness and the lowest H_S. We detected the greatest heterozygosity at site W7 (0.580) and the greatest allelic richness at the site W5 (5.2).

All mountain whitefish population samples conformed to Hardy–Weinberg proportions ($P > 0.05$ for all exact tests). Five tests for linkage disequilibrium had P-values less than 0.05. When we corrected (Rice 1989) for the 28 comparisons made for each population (0.05/28 comparisons per population sample with eight loci) none of the tests was significant. In addition, no pattern was evident for genotypic disequilibrium either within a sample or for a pair (or pairs) of loci.

Allele frequencies were relatively homogeneous among mountain whitefish sample sites (Table 3) and genetic differentiation among sites was low ($F_{ST} = 0.042$, 95% CI 0.028–0.061; Table 4). As was observed in bull trout, the high mountain lake (Doctor Lake, W10) was the most genetically divergent site (Fig. 2). Differentiation among sites was reduced when Doctor Lake was excluded ($F_{ST} = 0.018$, 95% CI 0.012–0.028; Table 4).

Theoretical (Hedrick 1999) and empirical studies (O'Reilly *et al*. 2004; Olsen *et al*. 2004) have shown that estimates of genetic differentiation among populations using F-statistics might be biased low when highly polymorphic loci are used. We calculated estimates of $F2_{ST}$ to determine if the greater number of alleles and higher heterozygosity we observed for mountain whitefish relative to bull trout might have contributed to the lower F_{ST} estimates we observed for mountain whitefish. With $F2_{ST}$, all loci are treated as biallelic by using the frequency of the most common allele and pooling the frequencies of all others (McDonald 1994; Allendorf & Seeb 2000). Estimates of $F2_{ST}$ for mountain whitefish were only slightly higher than estimates of F_{ST}. For all sites, $F2_{ST}$ was 0.046 (95% CI 0.037–0.058) and for sites W1–W9, $F2_{ST}$ was 0.019 (95% CI 0.009–0.032). *BT73*, in particular, was highly variable in mountain whitefish (mean $H_E = 0.89$). To determine if this locus had a disproportionate effect on our estimates of F_{ST}, we also treated this locus as biallelic, without doing so for the remaining loci. This measure led to a slight increase in overall F_{ST} for all mountain whitefish sites ($F_{ST} = 0.048$ (95% CI 0.033–0.065) but not for the F_{ST} estimate for sites W1–W9 ($F_{ST} = 0.019$, 95% CI 0.011–0.029).

The mean number of loci at which population allele frequencies were statistically significantly different between population pairs for the eight loci analysed was 3.3 ($P < 0.05$) and 2.0 ($P < 0.001$). When Doctor Lake was excluded, an average of 2.6 loci were statistically significantly different between population pairs at the $P < 0.05$ level and an average of 1.2 loci were statistically significantly different at the $P < 0.001$ level. We were unable to reject the null hypothesis of identical allele frequency distributions for 15 of the 55 pairwise comparisons when all loci were combined ($P > 0.05$).

Despite the low level of genetic differentiation, the mountain whitefish dendrogram shows evidence of spatial structure (Fig. 2b). The Flathead River site (W9) and the lake site (W10) were genetically divergent from sites W1–W8 and these eight sites clustered closely together (Fig. 2b). There were no statistically significant differences in allele frequencies among samples W2a, W2b and W3 or among sites W5, W6 and W7. These sites were pooled into two groups for Fig. 2b. The mountain whitefish dendrogram (Fig. 2b) showed a similar overall topology as the bull trout dendrogram (Fig. 2a), although, on average, CSE distances were substantially less for mountain whitefish (see below).

The geographical scale of the population comparisons for mountain whitefish was similar to the scale for bull trout. The average geographical distance between sites W1 and W9 was 202.8 km ± 26.4 km SE. The average pairwise distance (± SE) between sites W1 and W9 and Doctor Lake was 651.5 ± 22.6 km. We found a significant relationship between pairwise CSE values and geographical distance ($r = 0.88$, $P = 0.003$) when all comparisons were considered for mountain whitefish (Fig. 3b). When Doctor Lake was removed, the relationship remained significant ($r = 0.83$, $P = 0.039$). There was a break in geographical distance between sites W1–W8 and site W9 (Fig. 3B). The relationship between pairwise CSE values and geographical distance was not significant when only considering sites W1–W8 ($r = 0.08$, $P = 0.35$). Results were highly similar if pairwise F_{ST} was used as the genetic distance metric ($r = 0.79$, $P = 0.005$ for all comparisons; $r = 0.79$, $P = 0.009$ when Doctor Lake was removed; $r = 0.15$, $P = 0.24$ among sites W1–W8 only) or when geographical distances were log transformed (data not shown).

Comparisons of species

Average CSE distances were approximately five times greater for bull trout than for mountain whitefish (Fig. 3; mean (± SE) CSE for mountain whitefish was 0.035 ± 0.004 and 0.192 ± 0.025 for bull trout). With the high mountain lakes excluded, mean (± SE) CSE for mountain whitefish was 0.024 ± 0.002 and 0.129 ± 0.014 for bull trout. Results for pairwise F_{ST} were similar. Mean (± SE) pairwise F_{ST} for mountain whitefish was 0.059 ± 0.012, while mean (± SE) pairwise F_{ST} for bull trout was 0.284 ± 0.045. With the high

Table 3 Allele frequencies for mountain whitefish in the Clark Fork River. Sample size (n), average expected heterozygosity (H_S), and mean allelic richness are shown. W2a and W2b are from the same location but were collected in successive years

Sample number	Sample location	Basin	*ONE8*						*SSA14*					*FGT25*				*SFO8-1*			*SFO8-2*		*COCL4*		
			*178	*180	*182	*184	*186	*190	*167	*169	*171	*173	*175	*170	*176	*178	*180	*158	*162	*164	*195	*197	*146	*150	*152
W1	Ninemile Creek	Clark Fork	—	0.050	0.567	0.383	—	—	—	0.050	0.467	0.217	0.267	0.083	—	0.117	0.800	0.317	0.517	0.167	0.917	0.083	0.810	0.155	0.034
W2a	Rattlesnake Creek 2000	Clark Fork	—	0.108	0.578	0.304	—	0.010	0.080	0.020	0.540	0.070	0.290	0.067	—	0.048	0.885	0.385	0.442	0.173	0.875	0.125	0.647	0.255	0.098
W2b	Rattlesnake Creek 2001	Clark Fork	—	0.192	0.564	0.244	—	—	0.026	0.066	0.513	0.171	0.224	0.064	—	0.038	0.897	0.372	0.462	0.167	0.923	0.077	0.641	0.295	0.064
W3	Clark Fork River-Milltown Dam	Clark Fork	—	0.150	0.725	0.125	—	—	0.100	0.100	0.450	0.175	0.175	0.050	—	0.050	0.900	0.289	0.395	0.316	0.806	0.194	0.775	0.175	0.050
W4	Rock Creek	Clark Fork	—	0.155	0.571	0.274	—	—	0.060	0.036	0.429	0.071	0.405	0.134	—	0.061	0.805	0.464	0.452	0.083	0.866	0.134	0.679	0.190	0.131
W5	Bitterroot River-Hamilton, MT	Bitterroot	—	0.083	0.708	0.208	—	—	0.014	0.125	0.431	0.153	0.278	0.167	—	0.069	0.764	0.386	0.514	0.100	0.871	0.129	0.736	0.167	0.097
W6	W.F. Bitterroot River	Bitterroot	—	0.085	0.659	0.256	—	—	0.024	0.061	0.537	0.134	0.244	0.085	—	0.037	0.878	0.293	0.500	0.207	0.878	0.122	0.732	0.207	0.061
W7	E.F. Bitterroot River	Bitterroot	—	0.116	0.616	0.267	—	—	0.047	0.058	0.523	0.128	0.244	0.151	—	0.093	0.756	0.291	0.512	0.198	0.780	0.220	0.651	0.279	0.070
W8	N.F. Blackfoot River	Blackfoot	0.010	0.184	0.643	0.153	0.010	—	0.160	0.080	0.440	0.130	0.190	0.020	—	0.060	0.920	0.350	0.510	0.140	0.860	0.140	0.720	0.220	0.060
W9	Flathead River	Flathead	—	—	0.700	0.300	—	—	0.050	0.033	0.700	0.083	0.133	0.367	0.017	0.067	0.550	0.700	0.133	0.167	0.650	0.350	0.517	0.433	0.050
W10	Doctor Lake	Flathead	—	—	1.000	—	—	—	—	0.364	0.114	—	0.523	—	—	0.432	0.568	0.136	0.045	0.818	0.773	0.227	0.364	0.636	—

Sample number	Sample location	Basin	*SSA456*															*BT73*					Mean allelic richness
			*138	*158	*160	*162	*166	*186	*200	*206	*210	*220	*222	*224	*228	*230	*232	*206	1-*206	n	H_S		
W1	Ninemile Creek	Clark Fork	0.283	0.083	0.483	—	0.017	0.017	—	—	0.033	0.067	—	—	—	—	0.017	0.204	0.796	30	0.533		5.1
W2a	Rattlesnake Creek 2000	Clark Fork	0.317	0.067	0.567	—	0.019	—	—	—	0.019	—	—	0.010	—	—	—	0.177	0.823	51	0.533		4.7
W2b	Rattlesnake Creek 2001	Clark Fork	0.355	0.039	0.513	—	—	—	—	0.013	0.013	0.066	—	—	—	—	—	0.205	0.795	39	0.532		4.9
W3	Clark Fork River-Milltown Dam	Clark Fork	0.500	0.050	0.425	—	0.025	—	—	—	—	—	—	—	—	—	—	0.147	0.853	20	0.530		4.3
W4	Rock Creek	Clark Fork	0.155	0.095	0.667	—	—	—	—	—	0.036	0.024	—	0.024	—	—	—	0.207	0.793	42	0.539		4.9
W5	Bitterroot River-Hamilton, MT	Bitterroot	0.306	0.069	0.500	—	—	0.014	0.014	—	0.014	0.014	0.056	0.014	—	—	—	0.097	0.903	36	0.547		5.2
W6	W.F. Bitterroot River	Bitterroot	0.378	0.171	0.402	0.012	—	—	0.012	—	—	0.024	—	—	—	—	—	0.134	0.866	41	0.531		5.1
W7	E.F. Bitterroot River	Bitterroot	0.329	0.122	0.488	—	—	—	—	—	—	0.049	—	0.012	—	—	—	0.119	0.881	43	0.580		4.9
W8	N.F. Blackfoot River	Blackfoot	0.390	0.090	0.400	—	—	—	—	—	—	0.040	0.010	0.040	0.010	0.010	0.010	0.151	0.849	50	0.538		5.1
W9	Flathead River	Flathead	0.367	0.017	0.517	—	0.100	—	—	—	—	—	—	—	—	—	—	0.217	0.783	30	0.555		4.2
W10	Doctor Lake	Flathead	0.182	—	0.818	—	—	—	—	—	—	—	—	—	—	—	—	0.523	0.477	22	0.403		2.5

For presentation purposes, the most common allele at *BT73* (*206) is shown and the frequencies of all other alleles at this locus were combined

Table 4 Genetic differentiation of bull trout and mountain whitefish populations. The high mountain lake site excluded for bull trout was Trout Lake (B7) and for mountain whitefish was Doctor Lake (W10). The exact tests column contains results of tests for genic differentiation and is presented as the percentage of loci at which allele frequencies are statistically significantly different ($P < 0.05$). See text for 95% confidence intervals for estimates of F_{ST}

	Bull trout		Mountain whitefish	
Population groups	F_{ST}	Exact tests (%)	F_{ST}	Exact tests (%)
All sites	0.304	74.3	0.042	41.3
High mountain lake excluded	0.176	72.9	0.018	32.5

mountain lakes excluded mean (± SE) pairwise F_{ST} for mountain whitefish was 0.023 ± 0.005, while mean (± SE) pairwise F_{ST} for bull trout was 0.179 ± 0.029.

Discussion

We used ecological and life history characteristics of bull trout and mountain whitefish to predict that bull trout would have greater population substructure in the same river system. We were able to control for the effects of historical factors by analysing both species in the same river system. We found substantial differences in neutral divergence, suggesting that ecological and life history factors, through their effects on the probability of dispersal, are responsible for these results. Reduced gene flow and perhaps reduced population size and founder effects in high-mountain lakes served as a proximate factor shaping the distribution of genetic variation in a similar manner for each species.

Based on the genetic differentiation observed we predicted that bull trout would have greater among-population adaptive divergence than mountain whitefish. The same ecological and life history characteristics that affect neutral divergence for these species might also affect adaptive differences among populations. We combined our results for neutral divergence with predicted differences in adaptive divergence in a framework where ecological and life history characteristics are the driving factors.

Neutral divergence

Bull trout. We found large differences in allele frequencies among bull trout populations. The degree of genetic differentiation among bull trout populations found in this study is similar to that found in previous studies of bull trout performed at similar geographical scales (within river basins). For example, Costello *et al*. (2003) estimated F_{ST} values of 0.24 and 0.23 for two river systems in British Columbia, Canada. Our F_{ST} was also similar to what has been found for other bull trout populations in Montana and Idaho (Spruell *et al*. 1999; Kanda & Allendorf 2001; Neraas & Spruell 2001). The large F_{ST} we observed is also similar to other inland salmonid species that tend to use headwater habitats (Currens *et al*. 1990; Angers *et al*. 1999; Bouza *et al*. 1999; Carlsson & Nilsson 1999; Taylor *et al*. 2003).

The pronounced differentiation observed between bull trout populations is likely due to the fact that individuals occur in small subpopulations that are prone to drift. In addition, gene flow is reduced because bull trout home with high precision (McPhail & Baxter 1996; Spruell *et al*. 1999; Neraas & Spruell 2001). Ecological and life history characteristics also apparently contribute to neutral divergence in this species. Dispersal probabilities for bull trout are probably low because of the location of spawning sites far upstream in heterogeneous locations that can be difficult to access (both in time and space). It is the product of the proportion of individuals in each subpopulation that are from outside the subpopulation (m) and the effective population size (N_e) that determine F_{ST} (Mills & Allendorf 1996). Small population size will enhance the effect of low individual bull trout dispersal probability on F_{ST} because both N_e and m will be small.

Mountain whitefish. For mountain whitefish, we found that the vast majority of genetic variation occurs within populations with little differentiation occurring among populations. Genetic differentiation among mountain whitefish populations was substantially lower than that observed for bull trout and the reduced differentiation did not appear to be caused by greater within-population variation that we observed for mountain whitefish. Two nonmutually exclusive hypotheses could explain the genetic patterns we observed for this species: (1) reduced gene flow and little drift due to large N_e or (2) at least moderate gene flow among spawning groups.

We were able to address the first hypothesis because habitat fragmentation by a dam allowed us to estimate N_e for mountain whitefish in this system. Milltown Dam has been a barrier to upstream fish movement in the mainstem of the Clark Fork River since 1907 (Schmetterling 2001). In addition, very few mountain whitefish are able to pass downstream because of the turbines and high abundance of predatory fish in the upstream reservoir. We observed very little genetic differentiation among sites located on

either side of this dam (among sites W1–W8 $F_{ST} = 0.006$; 95% CI 0.002–0.010). The N_e consistent with the observed neutral divergence (F_{ST}) of isolated populations separated for t generations can be determined with the approximation:

$$F_{ST} \approx 1 - e^{-t/2N_e}$$

(Waples 1998). We used $t = 25$ because we assumed that the average generation length of mountain whitefish is 4 years and that no gene flow has occurred for approximately 100 years (since the dam was installed). Our assumption of complete isolation might be violated, but gene flow should at least be very close to zero over this time frame. For our observed $F_{ST} = 0.006$, our estimate of N_e is approximately 2000. These data are consistent with large populations that do not diverge at neutral markers because of drift and thus, hypothesis 1 is consistent with the low neutral divergence observed.

However, elevated gene flow also appears to be an important factor that prevents allele frequencies from diverging among sites W1–W8 (hypothesis 2). There is very little genetic divergence among sites W1–W8 (mean pairwise F_{ST} for the 28 comparisons among sites W1–W8 is 0.008 ± 0.002 SE) but increased genetic divergence between these sites and the more geographically distant Flathead River site (Fig. 3b; mean pairwise F_{ST} for the eight comparisons between sites W1–W8 and site W9 is 0.076 ± 0.004 SE). N_e probably does not differ between each of the sites W1–W8 and the Flathead River site (W9). In contrast, gene flow is likely reduced by geographical distance and the presence of Flathead Lake, a 495 km^2 natural lake. Therefore, N_e is not apparently large enough to prevent divergence among mountain whitefish populations when gene flow is reduced over what are likely longer periods of time. If there were little to no gene flow among sites W1–W8 (hypothesis 1), we would expect as much differentiation among these eight sites as we observed between these sites and site W9. Thus, it appears that reduced drift due to large N_e contributes to the lack of neutral divergence observed for mountain whitefish but high gene flow also prevents genetic divergence.

The combined effects of the ecological and life history factors we have considered (proximity of spawning locations, low complexity of intervening habitat, relative environmental homogeneity of spawning sites, large N_e, and group spawning behaviour) appear to lead to the substantial differences in among-population divergence we observed between bull trout and mountain whitefish. Dispersing mountain whitefish are more likely to successfully spawn at non-natal sites (because of the proximity of sites, low complexity of intervening habitat and their group spawning behaviour). In addition, for a given m, F_{ST} will be lower in mountain whitefish than bull trout because of the greater N_e of the former. Thus, even if mountain whitefish home at the same rate as bull trout (i.e. m is equal), we would expect to see less differentiation among populations of mountain whitefish.

Nonequilibrium conditions

An alternative explanation for the differences in F_{ST} observed between bull trout and mountain whitefish is that neither species has reached equilibrium between drift and gene flow. Most natural groups of populations are probably not at equilibrium (McCauley 1993; Hutchison & Templeton 1999; Turgeon & Bernatchez 2001; Kinnison *et al*. 2002; Ramstad *et al*. 2004). If nonequilibrium conditions prevail, values of F_{ST} could fluctuate, leading to misguided interpretations about the relative values of F_{ST}. However, given the substantial differences we found, it is highly unlikely that the F_{ST} distributions for these two species would overlap.

In addition, populations of each species might not be at equilibrium, but both species should be at a similar point in their progression to equilibrium. It is likely that the Clark Fork basin either served as a glacial refugium for both species or was founded by both species approximately 10 000 years ago, after the continental glaciers receded (McPhail & Lindsey 1986). Thus, both species would have had equal time in which to proceed toward equilibrium. Differences in population size affect time to equilibrium, with larger populations taking longer to achieve equilibrium (Crow & Aoki 1984). This factor is of little consequence within the realistic ranges of N_e for these species in this basin (1000 or more). Thus, even if mountain whitefish exist at an N_e that is an order of magnitude larger than that of bull trout, the effect of this larger population size on time to equilibrium is negligible (Crow & Aoki 1984). Finally, it is unlikely that unusual population dynamics have occurred in this particular river basin because our results are consistent with those observed in other regions for bull trout (Spruell *et al*. 1999; Kanda & Allendorf 2001; Neraas & Spruell 2001; Costello *et al*. 2003) and mountain whitefish (A. R. Whiteley unpublished).

Additional factors

Physical barriers interact with biological factors to influence amounts of gene flow. Fragmentation caused by dams can reduce gene flow and cause neutral divergence in stream systems (Neraas & Spruell 2001). Milltown Dam has reduced movement of bull trout and whitefish in this system for approximately 100 years but has not served as a proximate factor shaping the distribution of genetic variation of either species, probably because these two species lie at opposite ends of the spectrum of population genetic structure. Drift appears to be the dominant factor shaping bull trout genetic structure and overwhelms any reduction in gene

flow caused by the dam. Mountain whitefish populations appear to be too large to have increased neutral divergence due to dams over this time scale.

Founding events and reduced gene flow in high mountain lakes appear to act as proximate factors with similar impacts on the genetic structure of each species. The high-mountain lake sites of both species have reduced genetic variation (Tables 2 and 3) and are genetically divergent (Figs 2 and 3). Bull trout site B7 is separated by one dam and mountain whitefish site W10 is separated by two dams and thus, increased geographical distance and anthropogenic-induced fragmentation by dams might be responsible for these results. However, fragmentation by dams is probably not the only responsible factor, given our results for Milltown Dam. In addition, it is possible that the genetic patterns observed for these lake sites result from past stocking events. However, bull trout and mountain whitefish are not typically the focus of stocking efforts and for these two species there are no records of stocking either of the lakes considered in this study. Anthropogenic intervention does not appear to be a likely explanation for these data. Other studies of salmonids have found that small, high-mountain lakes can influence genetic structure (e.g. Castric *et al*. 2001). Both high-mountain sites in our study share characteristics of founding effects (a reduced number of alleles that are a subset of the alleles present in nearby populations). It is likely that historical events associated with the founding of these lakes and subsequent reduced gene flow due the high probability of geomorphological discontinuities at high elevation have contributed to our observations.

Neutral vs. adaptive divergence

Reduced gene flow provides conditions favourable to local adaptation if selective differences occur among populations (Lenormand 2002) and both theoretical (Haldane 1948; Slatkin 1973; Felsenstein 1976; Endler 1977; Slatkin 1978; García-Ramos & Kirkpatrick 1997; Hendry *et al*. 2001) and empirical data (King & Lawson 1995; Storfer *et al*. 1999; Hendry *et al*. 2002) suggest that gene flow can constrain adaptive divergence. In addition, empirical results suggest that estimates of neutral divergence from molecular markers (F_{ST}) provide conservative estimates of Q_{ST}, or among-population divergence in adaptive traits (Pfrender *et al*. 2000; Morgan *et al*. 2001) Based on our microsatellite data, we would predict that bull trout populations are more locally adapted than mountain whitefish populations in the Clark Fork River, as long as selection acting on bull trout populations is strong enough to overwhelm drift. Conversely, selection would not need to be strong to overwhelm drift in large mountain whitefish populations, but high gene flow could prevent local adaptation from occurring at this geographical scale. Thus, while mountain whitefish might be adapted at a larger geographical scale (among river basins), within river basins we predict that neutral divergence estimates from molecular markers are correlated with adaptive divergence among populations for these two species.

This system offers some additional insights into the relationship between neutral and adaptive divergence. Neutral divergence and adaptive divergence will be positively correlated in some circumstances. However, adaptive divergence can occur in the absence of neutral divergence (Mopper *et al*. 2000). It is possible that both types of divergence are actually covariates of other factors and instead of focusing directly on the relationship between neutral and adaptive divergence, we might increase our understanding by focusing on other factors that actually cause differences in both types of divergence. Causal factors might lead to a reduced probability of dispersal and therefore increased neutral divergence. In addition, the same factors might lead to increased adaptive divergence. In this case, neutral and adaptive divergence would be positively correlated. This general framework could explain why adaptive and neutral divergence are negatively correlated in some instances. For example, a factor or set of factors might lead to increased adaptive divergence and increased dispersal and gene flow, and thus reduced neutral divergence.

For bull trout and mountain whitefish, the same ecological and life history characteristics (mating location, mating system, length and extent of stage-specific migrations and population size) that we used to predict neutral divergence for these species might cause both neutral and adaptive differences among populations. Bull trout have more extensive migrations than mountain whitefish, migrating from rearing to adult feeding habitats and back to spawning habitats in headwater portions of streams. There are more opportunities for disruptions that prevent the completion of this life cycle for bull trout than in the comparatively simple migration and life history pattern of mountain whitefish. In addition to their effects on dispersal potential and thus neutral divergence, these ecological and life history aspects should lead to greater local adaptation of bull trout populations. Once neutral divergence and adaptive divergence arise as a result of the ecology and life history of an organism, these two elements of genetic structure can interact. For example, increased adaptive divergence might lead to further increases in neutral divergence owing to reduced success of migrant genotypes (Ehrlich & Raven 1969; Futuyma & Peterson 1985; Endler 2000; Mopper *et al*. 2000).

Empirical evidence for an association between local adaptation with ecological and life history factors such as mating system, migration, and/or population size is required to test this framework, as are more data on genetics and life history for a wider variety of species. This framework should apply to a wide array of taxa, and mountain

whitefish and bull trout offer just one opportunity to test these predictions. Our framework appears to be consistent with observations for other salmonids where there is evidence for local adaptation (Wood 1995; Koskinen *et al*. 2002). For example, Allendorf & Waples (1996) suggested that the high degree of local adaptation observed among populations of sockeye salmon (*Oncorhynchus nerka*) results from the number of habitats they occupy at various life stages and the complexity and length of migrations between these habitats. Thus, complexity of migration patterns and of the overall life cycle might lead to adaptive differences among populations of this species. These same factors might lead to reduced probability of dispersal and subsequent gene flow and thus the high F_{ST} commonly observed for this species (Wood 1995). Finally, adaptive differences among populations might contribute to reduced reproductive success of migrant individuals, acting to ratchet populations to greater neutral divergence.

Much recent debate has centred on whether adaptive or neutral differences among populations should be used for the purpose of defining conservation units (Crandall *et al*. 2000; McKay & Latta 2002). To understand the relationship between adaptive and neutral divergence, we suggest that more effort should be placed on the identification of factors that directly influence both types of divergence. Variation in ecological and life history factors, when causally associated with adaptive and neutral divergence, might be valuable both as a predictor of neutral divergence and a surrogate for measures of adaptive variation. Understanding the association between ecological and life history variation and neutral and adaptive divergence might allow us to define conservation units more effectively for a broad array of taxa.

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