Molecular Ecology (2006) **15**, 2767–2786 doi: 10.1111/j.1365-294X.2006.02972.x

Can common species provide valuable information for conservation?

ANDREW R. WHITELEY,* PAUL SPRUELL and FRED W. ALLENDORF
University of Montana, Division of Biological Sciences, Missoula, MT 59812, USA

Abstract

To demonstrate the importance of genetic data for multispecies conservation approaches, we examined the distribution of genetic variation across the range of the mountain whitefish (*Prosopium williamsoni*) at microsatellite and allozyme loci. The mountain whitefish is a common species that is particularly well suited for accurately revealing historical patterns of genetic structure and differs markedly from previously studied species in habitat requirements and life history characteristics. As such, comparing the population genetic structure of other native fishes to similar data from mountain whitefish could inform management and conservation strategies. Genetic variation for mountain whitefish was hierarchically distributed for both allozymes and microsatellites. We found evidence for a total of five major genetically differentiated assemblages and we observed subdivision among populations within assemblages that generally corresponded to major river basins. We observed little genetic differentiation within major river basins. Geographic patterns of genetic differentiation for mountain whitefish were concordant with other native species in several circumstances, providing information for the designation of conservation units that reflect concordant genetic differentiation of multiple species. Differences in genetic patterns between mountain whitefish and other native fishes reflect either differences in evolutionary histories of the species considered or differences in aspects of their ecology and life history. In addition, mountain whitefish populations appear to exchange genes over a much larger geographic scale than co-occurring salmonids and are likely to be affected differently by disturbances such as habitat fragmentation.

Keywords: genetic structure, landscape genetics, mountain whitefish, multispecies conservation, *Prosopium williamsoni*, salmonid

Received 15 November 2005; revision accepted 17 March 2006

Introduction

There is a growing consensus that single species conservation efforts do not adequately protect the biological and landscape needs of multiple species within threatened ecosystems (Lambeck 1997; Roberge & Angelstam 2004). Consequently, there has been a recent trend in conservation strategies towards shifting from single-species to multispecies approaches (Lambeck 1997; Freudenberger & Brooker 2004). These efforts consider the habitat requirements of multiple species to prioritize conservation efforts (Roberge & Angelstam 2004).

Correspondence: Andrew R. Whiteley, *Present address: Département de biologie, Université Laval, Sainte-Foy, Québec, Canada G1K 7P4. Fax: (418) 656-2043; E-mail: andrew.whiteley.1@ulaval.ca

Considering genetic data from multiple species in threatened ecosystems might be particularly informative for multispecies conservation approaches. To date, genetic comparisons among species have generally occurred among large-scale regional genetic groups in the context of comparative phylogeography (Bermingham & Moritz 1998; Froufe *et al*. 2003; Hoffmann & Baker 2003; Avise 2004; Dick *et al*. 2004; Satoh *et al*. 2004; Schoenswetter *et al*. 2004; Carstens *et al*. 2005; Michaux *et al*. 2005) or at very fine spatial scales (Turner & Trexler 1998; McDonald *et al*. 1999; King & Lawson 2001; Dawson *et al*. 2002; Brouat *et al*. 2004; Castric & Bernatchez 2004; Whiteley *et al*. 2004), but few attempts have been made to compare species at all hierarchical levels of biological organization (from populations through ecosystem and landscape levels). More detailed comparisons of patterns and geographic scale of genetic

differentiation at multiple hierarchical levels are needed to make genetic comparisons more informative for comprehensive conservation efforts. For example, species may exhibit similar large-scale patterns of genetic differentiation, but differ markedly at a finer geographic scale. Alternatively, similarity of patterns of genetic relationships at a fine scale may contrast with differences at large and intermediate spatial scales. Detailed genetic comparisons of multiple species at multiple hierarchical levels in a given landscape would allow us to more broadly consider conservation options and more accurately assign conservation priorities.

Historical genetic relationships among populations of threatened and endangered species often remain unclear. It can be difficult to obtain large enough samples from all relevant populations and regions due to the fact that these species may be extirpated in some areas, occur at low abundance where present, and sampling might put populations at even greater risk. In systems where genetic relationships among populations of threatened species are unclear, comprehensive hierarchical genetic analyses of additional species that accurately reveal historical relationships might be especially useful.

Species most likely to reveal historical population relationships are widely distributed across the range of the ecosystem of interest, have not been transplanted within their native range, do not hybridize with other species, and have large populations. Wide-ranging species allow the largest possible scope of comparison. Transplantation and hybridization can obscure historical genetic patterns (Allendorf *et al*. 2001), as can genetic drift in recently contracted or chronically small populations. In addition, for species with large populations, it is easier to collect adequate samples and these species are amenable to invasive techniques such as allozyme analysis, which may permit direct comparison to existing data. Consequently, we suggest that abundant, widely distributed species will often provide an informative complement to genetic studies of imperiled taxa, especially in systems where comparisons to previously analysed species are possible. Genetic data from abundant species may also provide an opportunity to disentangle the effects of historical and contemporary factors on genetic patterns of rare species.

River systems in western North America have been the focus of intense conservation efforts (Policansky & Magnuson 1998; McClure *et al*. 2003; Mebane *et al*. 2003). Conservation issues range from habitat fragmentation due to a variety of sources (e.g. dams and road building) to water-quality issues related to activities such as mining and forest use (e.g. Kareiva *et al*. 2000; Levin & Tolimieri 2001; Collins & Montgomery 2002). Genetic patterns for four salmonids with large distributions in inland freshwater systems (bull trout, *Salvelinus confluentus*; cutthroat trout, *Oncorhynchus clarki*; rainbow trout, *Oncorhynchus mykiss*; and Chinook salmon, *Oncorhynchus tschawytscha*) have been described in detail from this region and have been used in part to determine conservation and management priorities (e.g. NOAA 2003). Genetic variation is distributed hierarchically for these species across this region. At the largest geographic scale, major cohesive genetic groups have been observed for all four species (Fig. 1). These groups are likely to be largely the result of the historical effects of glaciation, where populations resided in several refugia from which they dispersed to colonize previously ice-covered regions (Fig. 1; McPhail & Lindsey 1986; Taylor *et al*. 1999, 2003; McCusker *et al*. 2000; Teel *et al*. 2000; Costello *et al*. 2003). In addition, all four species tend to be subdivided at a fine geographic scale, with significant genetic differences often occurring among tributaries within major river basins (Allendorf & Utter 1979; Allendorf & Leary 1988; Wenburg *et al*. 1998; Spruell *et al*. 1999, 2003; Taylor *et al*. 1999, 2003; McCusker *et al*. 2000; Teel *et al*. 2000; Costello *et al*. 2003; Waples *et al*. 2004).

Conservation efforts for fishes in western North America have proceeded largely in a single-species manner. For the four species mentioned, there has not been an attempt to compare and contrast patterns of genetic differentiation. In addition, for some of these species, transplantation and anthropogenic-induced hybridization may obscure historical genetic patterns (e.g. Allendorf & Leary 1988). Genetic data from a common species with the desirable attributes for comparative genetic analyses mentioned above may be valuable as a step towards a more comprehensive conservation approach. Furthermore, because all four species tend to be genetically subdivided on a small geographic scale, they offer a limited view of the geographic scale of genetic differentiation of all of the native fishes in this region. Genetic analysis of a species likely to differ in geographic scale of subdivision may offer an alternative perspective that can broaden the scope of conservation and management planning.

The mountain whitefish (*Prosopium williamsoni*) co-occurs with the four species mentioned above and fits our criteria for a useful common species for comparative genetic analysis. Mountain whitefish have not been translocated within their native range and do not occur sympatrically with other *Prosopium* species in most of their range, precluding hybridization with other species (with the exception of one population revealed during the course of this study, described below). Mountain whitefish are abundant and invasive sampling is unlikely to have a negative demographic influence. This species occurs throughout western North America in most major river basins (McPhail & Lindsey 1970) and has experienced the same geomorphological influences as other native fishes. Thus, it is likely that patterns of the genetic structure of mountain whitefish will reflect historical connectivity among river basins throughout western North America

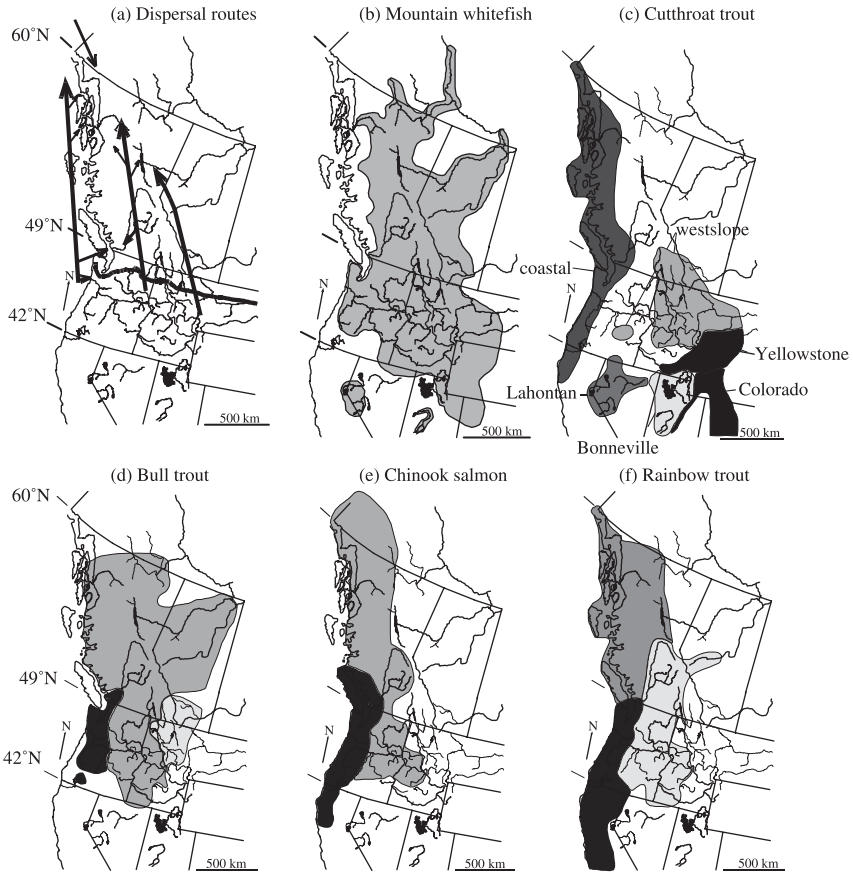

Fig. 1 Postglacial dispersal and range maps of five wide-ranging native salmonids in western North America. Map of hypothesized dispersal routes (a) is adapted from McPhail & Lindsey (1986). The southern extent of glaciation is shown with a solid line in (a). Range maps are from Behnke (2002). A summary of existing genetic data for large-scale genetic groups within species' ranges are shown in panels c–f. Cutthroat trout genetic data (c) are from Allendorf & Leary (1988) and Taylor *et al*. (2003) and common names of six currently recognized cutthroat trout subspecies mentioned in the text are shown. Bull trout genetic data (d) are from Taylor *et al*. (1999) and Spruell *et al*. (2003). Chinook salmon genetic data (e) are summarized in Waples *et al*. (2004). Rainbow trout genetic data (f) are from Allendorf & Utter (1979) and McCusker *et al*. (2000). In (f), northern populations tend to be a mix of coastal (dark grey area) and inland (light grey area) genetic groups. This mixture is portrayed by an intermediate shade of grey in the northern region, which does not represent a third genetic group. Note that actual ESUs for both rainbow trout and Chinook salmon reflect fine-scale genetic subdivision within the groups shown here (e.g. Waples *et al*. 2004).

(McPhail & Troffe 2001). Furthermore, mountain whitefish differ in ecological aspects from previously analysed species because they reside and spawn primarily in larger rivers, they appear to have less habitat specificity throughout their life cycle, and they have larger N_e (Whiteley *et al*. 2004). Thus, the geographic scale of genetic differentiation may differ between mountain whitefish and these other species. Genetic patterns for mountain whitefish, while they will not solely define conservation units for native fishes in western North America, could provide complementary information that would improve conservation prioritization and provide an example of the advantages of multispecies conservation genetic approaches.

In this study, we used allozymes and microsatellites to examine the hierarchical distribution of genetic variation across the range of the mountain whitefish. We answered the following questions: What is the genetic structure of mountain whitefish in western North America? How do patterns of genetic differentiation compare among native fish species in western North America? How does the geographic scale of genetic differentiation compare among species? Finally, do these data provide additional insight for management of native fishes in western North America?

Methods

Samples

We obtained samples from throughout the range of the mountain whitefish (Table 1; Fig. 2). Where possible, we obtained whole fish for tissues for both allozyme analysis and for DNA extraction and subsequent microsatellite analysis. For each population sample, care was taken to include fish from multiple size classes to maximize the probability of analysing unrelated individuals. Most sites included fish from multiple nearby collection locations within a river. We were able to obtain samples from a wider geographic range for microsatellite analysis than for allozyme analysis, partly due to problems with international transport of whole frozen fish from Canadian sites.

Allozymes

We performed horizontal starch gel electrophoresis according to the procedures of Leary & Booke (1990) on fish collected from 29 locations (Table 1). We screened products of 32 loci coding for enzymes from muscle, liver,

Table 1 Genetic diversity and sample statistics for each mountain whitefish population. Populations are arranged from north to south and within major rivers, from downstream to upstream. Alphanumerical codes correspond to Fig. 2. *N* is sample size. H_S is average expected heterozygosity within sites

			Allozymes		Microsatellites	
Location	*N*	Latitude (N)/ longitude (W)	H_S	Mean number of alleles	H_S	Mean number of alleles
Mackenzie River						
A. Liard River, BC						
1. Fort Nelson River						
a. Prophet River	19	57.7/123.4	—	—	0.215	1.83
2. Kechika River	27	59.2/127.6	—	—	0.268	3.00
a. Gataga River	21	58.6/126.9	—	—	0.220	3.00
B. Peace River						
1. Smoky River, AB						
a. Wapiti River	29	55.7/118.8	—	—	0.237	2.00
b. Kakwa River	20	54.3/119.5	—	—	0.230	2.00
2. Finlay River, BC						
a. Thutade Lake	19	56.8/127.0	—	—	0.091	1.83
3. Parsnip River, BC	18	55.2/123.1	—	—	0.310	3.50
Stikine River						
C. Klappan River, BC	15	58.0/129.7	—	—	0.186	1.67
Fraser River						
D. Chilliwack, BC	17	49.2/121.9	—	—	0.454	3.33
E. Siska Fish Wheel, BC	10	50.2/121.6	—	—	0.481	3.17
F. Thompson River, BC						
1. Bonaparte River						
a. Machete Lake	20	51.4/120.6	—	—	0.212	2.00
2. North Thompson River						
a. Eagle Creek	10	51.9/120.9	—	—	0.482	3.33
3. South Thompson River						
a. Oliver Creek	12	51.1/120.1	—	—	0.356	2.67
G. Bridge River, BC						
1. Carpenter Reservoir	25	50.9/122.5	—	—	0.329	3.50
Olympic Peninsula						
H. Hoh River, WA	23	47.8/124.2	—	—	0.165	1.83
I. N. F. Skokomish River, WA	30	47.5/123.4	—	—	0.138	1.33
Columbia River Basin						
J. Lewis River, WA						
1. Swift Reservoir	32	46.1/122.2	—	—	0.389	4.33
K. Willamette River, OR	34	46.7/123.2	0.030	1.36	0.343	4.17
L. Deschutes River, OR						
1. Warmsprings River	32	44.9/121.1	—	—	0.413	4.50
M. Walla Walla River, WA						
1. Touchet River	17	46.1/118.7	—	—	0.303	2.33
N. Snake River						
1. Clearwater River, ID						
a. Lolo Creek	21	46.4/116.2	—	—	0.190	2.17
b. S. F. Clearwater River	23	45.8/115.5	—	—	0.272	3.00
c. Lochsa River	20	46.5/114.8	—	—	0.339	3.83
2. Grande Ronde River, OR						
a. Lostine River	27	45.5/117.4	0.040	1.36	0.301	3.50
3. Salmon River, ID						
a. S. F. Salmon River	36	44.7/115.7	—	—	0.324	3.83
b. Pahsimeroi River	28	44.6/113.9	0.031	1.21	0.350	3.33
c. Salmon River at Chalis	25	44.5/114.2	0.045	1.43	0.392	4.00
4. Malhuer River, OR	26	43.9/117.0	—	—	0.361	2.83

Table 1 *Continued*

Location	*N*	Latitude (N)/ longitude (W)	Allozymes		Microsatellites	
			H_S	Mean number of alleles	H_S	Mean number of alleles
5. Boise River, ID						
a. South Fork Boise River	20	43.4/115.6	0.031	1.21	0.460	3.33
6. Big Wood River, ID	20	43.5/114.3	0.002	1.07	0.000	1.00
7. Snake River-Menan, ID	41	43.8/112.0	0.014	1.21	0.394	4.83
8. South Fork Snake River, ID	32	43.7/111.8	0.021	1.29	0.402	4.33
9. Teton River, ID	33	43.8/111.2	0.008	1.07	0.291	2.67
10. Henry's Fork Snake River, ID	41	44.4/111.4	0.002	1.07	0.288	2.17
O. Big Lost River, ID						
1. Lower Big Lost River	26	43.4/113.5	0.026	1.14	0.000	1.00
2. Upper Big Lost River	32	44.2/113.9	0.022	1.14	0.000	1.00
P. Yakima River, WA	22	47.2/120.9	0.038	1.29	0.350	4.17
Q. Clark Fork River, MT						
1. Cabinet Gorge Dam	16	48.1/116.1	0.030	1.21	0.462	3.50
2. Flathead River						
a. Mainstem Flathead River	30	48.4/114.2	0.024	1.21	0.501	3.17
b. Doctor Lake	22	47.2/113.5	—	—	0.343	2.17
3. Ninemile Creek	30	47.0/114.4	—	—	0.497	3.83
4. Rattlesnake Creek	91	46.9/114.0	—	—	0.522	4.17
5. Milltown Dam	20	46.9/113.9	—	—	0.522	3.33
6. Blackfoot River						
a. North Fork Blackfoot River	50	47.0/113.1	—	—	0.538	4.50
7. Rock Creek	42	46.6/113.7	—	—	0.511	3.67
8. Bitterroot River	143	46.3/114.1	0.029	1.21	0.528	4.67
R. Pend Oreille River, BC						
1. Confluence with Columbia River	20	49.5/117.7	0.037	1.21	0.431	3.50
S. Beaver Creek, BC	25	49.7/117.7	—	—	0.411	3.67
T. Kootenay River, BC						
1. Kootenay Lake	21	49.5/116.8	0.033	1.21	0.395	3.83
2. Bull River	20	49.7/115.2	0.015	1.14	0.085	1.17
Saskatchewan River						
U. Bow River, AB	24	50.0/111.7	0.025	1.14	0.214	2.17
Missouri River						
V. Yellowstone River, MT	40	45.5/110.6	0.021	1.29	0.356	3.33
W. Judith River, MT						
1. Big Spring Creek	20	47.1/109.5	—	—	0.412	2.67
2. South Fork Judith River	22	46.8/110.3	0.048	1.29	0.428	2.17
X. Gallatin River, MT	21	45.9/111.5	0.030	1.36	0.490	4.17
Y. Madison River, MT	30	45.0/111.6	0.027	1.29	0.465	3.67
Z. Jefferson River, MT						
1. Bighole River	30	45.9/113.2	0.019	1.14	0.509	4.17
Bonneville Basin						
AA. Logan River, UT	34	41.8/111.8	—	—	0.269	3.50
AB. Weber River, UT	31	41.9/111.5	0.000	1.00	0.361	3.33
AC. Bear River, UT	31	40.9/110.5	0.000	1.00	0.320	2.00
Lahontan Basin						
AD. Walker River, CA, NV	33	38.2/119.1	0.013	1.21	0.090	1.33
AE. Truckee River, NV	12	39.6/119.6	0.018	1.21	0.114	1.33

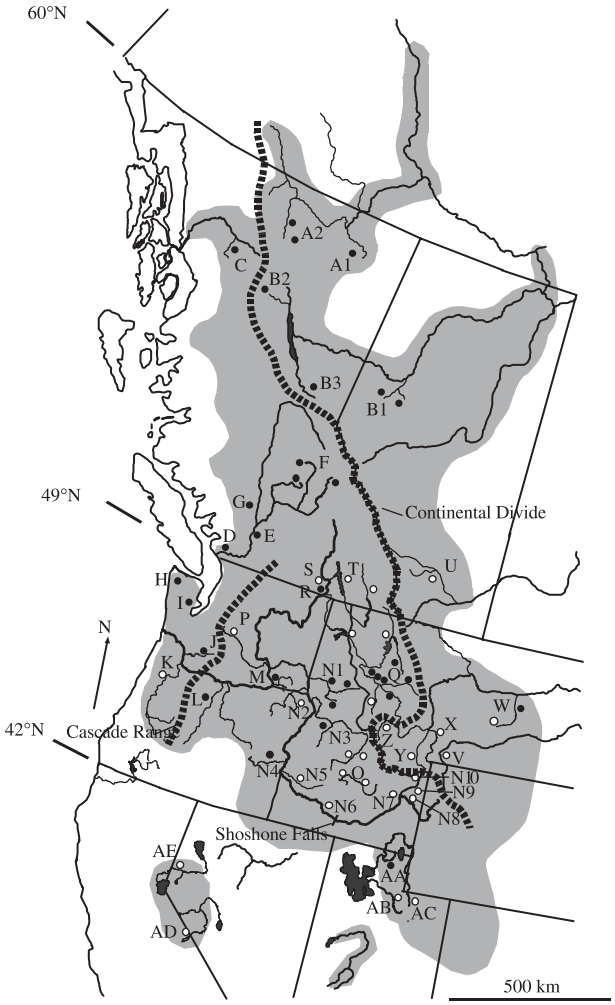

Fig. 2 Map of mountain whitefish range (shaded area). Black circles represent locations that were analysed with microsatellites, white circles represent sites that were analysed with both allozymes and microsatellites. Letter codes correspond to Table 1.

or eye tissue and found evidence of genetic variation at 14 loci. We followed Shaklee *et al*. (1990) for nomenclature of enzymes, loci, and alleles. Enzyme Commission (EC) numbers follow IUBMBNC (1992) and are as follows: adenylate kinase (EC 2.7.4.3; *AK-1,2**), alcohol dehydrogenase (EC 1.1.1.1; *ADH**), aspartate aminotranserase (EC 2.6.1.1; *sAAT-1**, *sAAT-2**, *sAAT-3**, *sAAT-4**), creatine kinase (EC 2.7.3.2; *CK*-A1*), cytosol nonspecific dipeptidase (EC 3.4.13.18; *PEPA-1**, *PEPA-2**), glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12; *GAPDH-3,4**); glycerol-3-phosphae dehyrogenase (EC 1.1.1.8; *G3PDH-1,2**); hexosaminidase (EC 3.3.1.52; *HEX**); isocitrate dehydrogenase (NADP+) (EC 1.1.1.42; *sIDHP-1,2**); L-lactate dehydrogenase (EC 1.1.1.27; *LDH-A1**, *LDH-A2**, *LDH-B1**, *LDH-B2**, *LDHC**); malate dehyrogenase (EC 1.1.1.37; *sMDH-A1,2**, *sMDH-B1,2**); malic enzyme (NADP+) (EC 1.1.1.40; *mMEP-1**, *sMEP-1**); phosphoglucomutase (EC 5.4.2.2.; *PGM-1**, *PGM-2**); superoxide dismutase (EC 1.15.1.1; *sSOD-1**) and tripeptide animopeptidase (EC 3.4.11.4; *PEPB**). Tissues were kept frozen until dissection. We used the electrophoresis buffers described in Leary *et al*. (1993). Stains used to reveal the position of enzymes in the gels after electrophoresis were from Harris & Hopkinson (1976) and Allendorf *et al*. (1977). An Arlee strain rainbow trout (*Oncorhynchus mykiss*) from the Jocko River State Fish Hatchery, Arlee, Montana (maintained by the Montana Department of Fish, Wildlife & Parks), was run on each gel as a mobility standard. We scored alleles at the malate dehydrogenase isolocus, *MDHB-1,2**, as products from two separate loci and assumed that all observed variation occurred at one locus (Leary & Book 1990).

Microsatellites

The general methods used for polymerase chain reaction (PCR) and visualization of subsequent PCR products followed Spruell *et al*. (1999), Neraas & Spruell (2001), and Whiteley *et al*. (2004). DNA was extracted from either fin clips or liver tissue by standard methods. We visualized fluorescently labelled PCR products on acrylamide gels using a Hitachi FMBIO II fluorescent imager and used a MapMarker LOW (BioVentures) ladder along with individuals of known genotypes as standards for scoring. We used six of the eight loci (*COCL4*, *SSA14*, *SSA456*, *ONE8*, *SFO8-1*, and *SFO8-2*) from Whiteley *et al*. (2004) because these six loci could be scored reliably across the range of the mountain whitefish. PCR reagent concentrations varied among loci and are available from the authors upon request, as are allele frequencies and all individual genotypes for both marker types.

Data analysis

Allele frequencies, deviations from Hardy–Weinberg expectations, linkage disequilibrium, observed (H_O) and expected (H_E) heterozygosities per locus and population, mean within-population expected heterozygosity (H_S), mean number of alleles per population, pairwise exact tests for genic differentiation, *F*-statistics and pairwise F_{ST}'s were calculated using GENEPOP 3.4 (Raymond & Rousset 1995) and FSTAT 2.9.3.2 (Goudet 1995, 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 the sequential Bonferroni procedure to adjust multiple tests for linkage disequilibrium within populations (Rice 1989). We tested to determine if the amount of within-population genetic variation (arcsine transformed H_S and untransformed mean number of alleles) detected by

allozymes and microsatellites was correlated using a Spearman rank correlation test.

We calculated $F2_{ST}$ for both microsatellites and allozymes to determine if the greater heterozygosity observed with microsatellites might have contributed to a downward bias in our estimate of population differentiation. With $F2_{ST}$, all loci are treated as bi-allelic by using the frequency of the most common allele and pooling the frequencies of all others (McDonald 1994; Allendorf & Seeb 2000). We used SPAGEDI (Hardy & Vekemans 2002) to calculate R_{ST} for microsatellites and to test for significant differences between R_{ST} and F_{ST}. R_{ST} values significantly greater than F_{ST} values suggest that stepwise-like mutation processes have occurred at a locus (Hardy *et al*. 2003). We used standard error estimates from SPAGEDI to calculate 95% confidence intervals for R_{ST}.

In the Kechika River sample, we observed microsatellite alleles outside the normal size range for alleles at several loci for three individuals. This population lies within a zone of sympatry with the round whitefish (*Prosopium cylindraceum*; McPhail & Lindsey 1970). We used paired interspersed nuclear element-PCR (PINE-PCR) (Spruell *et al*. 2001) to test the hypothesis that these three fish were hybrids between mountain whitefish and round whitefish. These three fish appeared to be F_1's because all fragments diagnostic for both mountain whitefish and round whitefish were present in each fish. We removed these three fish from subsequent analyses (Allendorf *et al*. 2001). We did not detect any further evidence of hybridization between mountain whitefish and other species.

Principal components analysis (PCA) based on sample allele frequencies was used to examine range-wide patterns of population differentiation and to define cohesive genetic assemblages (equivalent to major phylogeographic groups). We excluded one allele at each locus to account for non-independence among alleles within loci for both marker types. For allozymes, the PCA is based only on loci that were polymorphic (frequency < 0.99) in at least one population. We used SPSS 11 (SPSS Inc.) for PCA analyses.

We used an analysis of molecular variance (AMOVA, Excoffier *et al*. 1992), performed with ARLEQUIN 2.001 (Schneider *et al*. 2000) to investigate how genetic variation was partitioned based on several geographical arrangements. Initially we pooled sites within regions defined by PCA for both allozymes (three PCA groups) and microsatellites (five PCA groups). We also tested alternative arrangements, including pooling sites based on the four primary glacial refugia proposed by McPhail & Lindsey (1986) and observations from other native fishes in this region (Figs 1 and 2; coastal 'Cascadia', all sites west of the Cascade and Coastal Mountains; inland 'Cascadia', all sites east of the Cascade and Coastal Mountains and west of the Continental Divide; upper Missouri River; and the upper Snake River, all sites upstream of Shoshone Falls and from the Bonneville and Lahontan Basins). In addition, we pooled sites based on genetic patterns observed for the cutthroat trout (Allendorf & Leary 1988). We used the cutthroat trout because it occupies the most similar range to that of the mountain whitefish (Fig. 1; Behnke 2002). We used four geographical groups to partition mountain whitefish genetic data that corresponded to sites within the range of the coastal cutthroat trout (*Oncorhynchus clarki clarki*), the west-slope cutthroat trout (*Oncorhynchus clarki lewisi*), the Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*), and we conservatively combined the Yellowstone (*Oncorhynchus clarki bouvieri*) and Bonneville cutthroat trout (*Oncorhynchus clarki utah*) into the fourth group (Fig. 1). Finally, we pooled sites within major watersheds (Table 1, Fig. 2). Each letter in Fig. 2 represents a similarly sized watershed that was used as a separate group for this analysis. We treated sites T1 and T2 from the Kootenay River as separate groups because they are separated by a waterfall barrier to fish passage. Sites R and S were considered together as one group because they are close together in the upper mainstem Columbia River. Sites N7 and N8 were also considered together as one group because they are close together in the Snake River. For the watershed AMOVA, there were 24 allozyme groups and 43 microsatellite groups (Table 2).

To further describe the scale and patterns of genetic differentiation among mountain whitefish populations, we constructed dendrograms based on microsatellite and allozyme allele frequencies. We used PHYLIP 3.5 (Felsenstein 1993) to calculate Cavalli-Sforza & Edwards's (1967) genetic distance (CSE) with the GENDIST module. We used the NEIGHBOUR module to construct a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram. 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 and does not assume any models of mutation (Takezaki & Nei 1996).

We used BAPS 2.0 (Bayesian Analysis of Population Structure, Corander *et al*. 2003, 2004) for defining fine-scale population relationships and as a complementary approach for defining population groups within PCA and dendrogram-determined cohesive genetic assemblages. We used the group method, where the geographic location of each population sample was used as a prior and individuals were not allowed to move among samples sites, for clustering predefined populations based on multilocus tests of allele frequency differences. We chose the group-clustering method because mountain whitefish populations are confined to river basins and it is not possible for gene flow to directly occur among many of the populations we analysed. We ran BAPS five times for 10^5 iterations with a burn-in period of 20 000. Panmictic population groups defined by the data partitions with the highest posterior probability for

Table 2 Analysis of molecular variance (AMOVA) for allozymes and microsatellites. Arrangements pooled samples by either PCA-defined groups, the four primary glacial refugia hypothesized by McPhail & Lindsey (1986), patterns observed for subspecies of the cutthroat trout, or major watersheds (see text for details)

Geographical arrangement	Number of groups	Variance component	Percentage of variation*
Allozymes			
PCA groups	3	Among groups	65.9
		Among sites within groups	10.8
		Within sites	23.3
Four refugia	4	Among groups	51.0
		Among sites within groups	23.2
		Within sites	25.8
Cutthroat trout subspecies	4	Among groups	37.5
		Among sites within groups	36.6
		Within sites	25.9
Major watersheds	24	Among watersheds	64.4
		Among sites within watersheds	5.5
		Within sites	30.1
Microsatellites			
PCA groups	5	Among groups	31.3
		Among sites within groups	14.6
		Within sites	54.1
Four refugia	4	Among groups	22.7
		Among sites within groups	19.8
		Within sites	57.5
Cutthroat trout subspecies	4	Among groups	23.2
		Among sites within groups	21.2
		Within sites	55.6
Major watersheds	42	Among watersheds	33.6
		Among sites within watersheds	4.1
		Within sites	62.3

*$P < 0.0001$ for all variance components.

microsatellites and allozymes separately were plotted onto the map of western North America.

Pairwise genetic distances were plotted against pairwise geographic distances to further analyse the geographic scale of gene flow for mountain whitefish. We limited our analysis to populations in the contiguous Columbia River basin and to those within the Inland Cascadia genetic group (see below). Furthermore, we limited our analysis to microsatellite data because they provided much stronger resolution of fine-scale genetic differentiation. We measured river channel distances among sites using a geographic information system (GIS). We analysed patterns with and without two sites located above impassable waterfalls, the Big Wood (N6) and Bull (T2) Rivers, and one site from a high mountain lake, Doctor Lake (Q2b), because genetic differentiation of these sites from all others was clearly not due to geographic distance alone. We used Mantel tests implemented by the program IBD (Isolation by Distance, Bohonak 2003) to test the significance of the relationship between genetic and geographic distance matrices. Tests were performed with and without log-transformation of geographic distances and using both F_{ST} and $F_{ST}/(1 - F_{ST})$ (Rousset 1997).

We compared the relationship between genetic and geographic distances for mountain whitefish to bull trout data collected over the same geographic scale within the Columbia River (mean geographic distance among sample locations ± SE was 1091 ± 38.0 km for mountain whitefish and 1065.9 ± 19.3 km for bull trout). We used bull trout for this comparison because this is the only other native species for which microsatellite data from throughout the Columbia River were available. We used genetic data from Spruell *et al*. (2003) and included all sites that occurred in the equivalent of the Inland Cascadia group but excluding sites in the lower Columbia River east of the Continental Divide ($N = 45$). Lower Columbia River sites were excluded for both species because they belong to a different major genetic group (see below) and were clearly not isolated from other populations by distance alone. We collected geographic distances between the midpoints of sampling reaches using a GIS. The same methodology described above was used to analyse patterns of isolation by distance.

Results

Variation within populations

Allozymes. Fourteen of the 32 allozyme loci screened were polymorphic. We found a total of 37 alleles for the 794 individuals analysed from 29 sites. Mean within-population expected heterozygosity (H_S) ranged from zero to 0.048 and mean number of alleles ranged from 1.00 to 1.43 (Table 1). The Weber (AB) and Bear (AC) Rivers from the Bonneville Basin in Utah had no genetic variation and the Big Wood River (N6) and Henry's Fork of the Snake River (N10), both upstream of barrier waterfalls, had highly reduced genetic variation.

None of the polymorphic allozyme loci showed evidence of significant departures from Hardy–Weinberg proportions. Of 159 tests for linkage disequilibrium, seven were significant ($P < 0.05$), where eight significant tests were expected by chance ($\alpha = 0.05$). There was no pattern of significant linkage disequilibrium within any of the population samples or for any of the locus pairs across populations.

Microsatellites. For microsatellites, we observed a total of 142 alleles at six loci for the overall sample of 1769 individuals from 62 sites. The mean number of alleles per locus was 19.8 (range: 7–61 alleles). H_S ranged from 0 to 0.538 (Table 1). The mean number of alleles per population ranged from 1.00 to 4.83 (Table 1). The Big Lost (O) and Big Wood (N6) Rivers, both of which are isolated populations, had no genetic variation. The Bull River (T2) and Thutade Lake (B2a), both isolated above waterfalls, and the high mountain Doctor Lake (Q2b) also had reduced genetic variation (Table 1).

Seventeen of 275 tests showed evidence for significant deviations from Hardy–Weinberg proportions with microsatellites (14 significant tests were expected by chance at $\alpha = 0.05$). No consistent patterns within loci across populations or within populations across loci were observed, except in the case of *ONE8* in populations from the upper Missouri River. Four of six sites from the upper Missouri River had significant departures from Hardy–Weinberg proportions at this locus. In each of these four cases there was a deficit of heterozygotes (positive F_{IS}), suggesting that a null allele might occur in this geographic region at this locus. However, we did not observe any potential null homozygotes and assumed that a null allele, if present, was at low frequency and would not have a large influence on genetic patterns.

Of 643 tests for linkage disequilibrium, 33 were significant, where 32 were expected by chance ($\alpha = 0.05$). There was no significant pattern of linkage disequilibrium within any of the population samples or between any locus pairs across populations. Only two tests remained significant after correcting for multiple tests within a population, *SSA14* and *COCL4* for Lolo Creek (N1a) and *COCL4* and *SFO8-1* for Big Spring Creek (W1).

Comparison of markers. Within-population genetic variation (both H_S and mean number of alleles) was significantly correlated between allozymes and microsatellites. The Spearman rank correlation ρ value for H_S was 0.395 ($P = 0.037$) and for mean number of alleles, $\rho = 0.583$ ($P = 0.002$). Only in several populations were amounts of within-population genetic variation highly dissimilar between marker types. These include the Big Lost River (O), which had no microsatellite variation but moderate allozyme variation and the three Bonneville Basin sites (AA–AC), which had no allozyme variation but moderate microsatellite variation (Table 1).

We did not find evidence for significant linkage disequilibrium between microsatellite and allozyme loci. Of 396 total tests, 10 were significant ($P < 0.05$), where 20 were expected by chance ($\alpha = 0.05$). There was no pattern of significant linkage disequilibrium within any of the population samples or for any of the locus pairs across populations.

Divergence among populations

Broad geographic subdivisions

Allozymes. There was a large degree of genetic subdivision across the range of mountain whitefish with allozymes ($F_{ST} = 0.689$, 95% C.I. 0.340, 0.863; $F2_{ST} = 0.698$, 95% CI 0.343, 0.867). For the 29 sites analysed, principal components analysis revealed what appear to be three primary clusters of populations (cohesive genetic assemblages; Fig. 3a). These assemblages corresponded to the upper Missouri River (V–Z; Table 1, Fig. 2), the upper Snake River (including two sites from Utah; N7-10, O, AB, AC), and the Cascadia region (*sensu* McPhail & Lindsey 1986; K, N2, N3b & c, N5, N6, P, Q1, 2a & 8, R, T, U). The most genetically divergent upper Snake sites came from the Big Lost River (O). The one site analysed with allozymes west of the Cascade Mountains (K) was not differentiated from other Cascadia sites. A dendrogram based on CSE genetic distances depicted the same three cohesive genetic assemblages as were revealed by PCA (data not shown).

The AMOVA partitioned a greater proportion of variation among groups and less variation among sites within groups when sites were pooled based on PCA results than when they were pooled based on the four refugia arrangement (Table 2). Only a slightly greater proportion of variation was partitioned among PCA groups as among watersheds when sites were pooled by major watersheds. Less variation was partitioned among sites within watersheds for the watershed geographical arrangement than among sites within the PCA groups (PCA groups arrangement; Table 2). The arrangement based on cutthroat trout subspecies had the least amount of variation partitioned among groups and the most partitioned among sites with groups (Table 2).

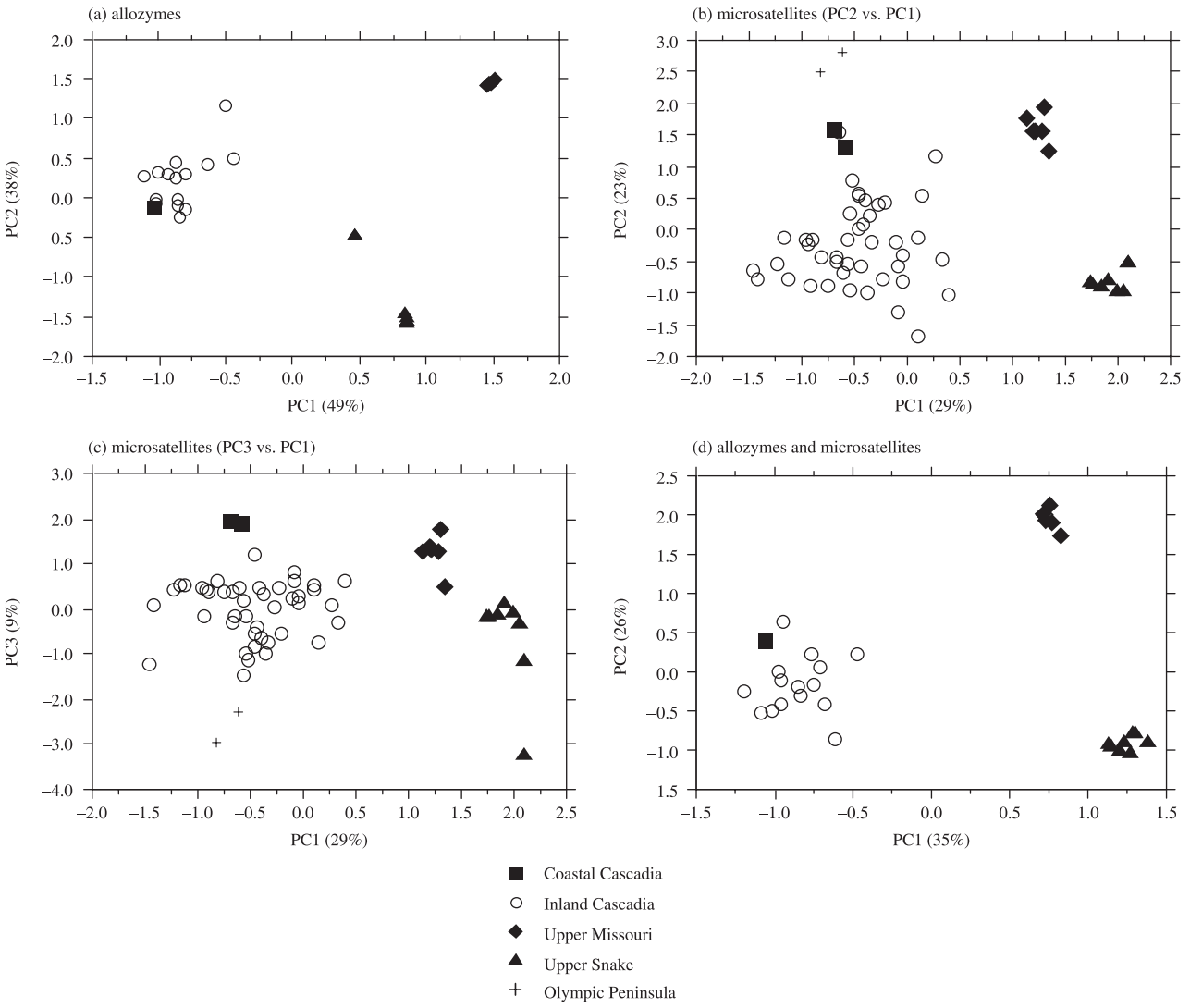

Fig. 3 Principal components analysis of (a) allozymes, (b, c) microsatellites, and (d) microsatellites and allozymes combined. Numbers in parentheses are the proportion of variation attributable to each component. Symbols correspond to major genetic assemblages mentioned in text. In (a), the Coastal Cascadia site (solid square) that clusters with Inland Cascadia sites (open circles) is shown to allow comparison to microsatellite results (panels b and c), despite the lack of genetic differentiation observed for the coastal site with allozymes.

Microsatellites. The mean global F_{ST} for microsatellites was 0.369 (95% CI 0.343, 0.393). The mean global $F2_{ST}$ was slightly greater (0.434, 95% CI 0.386, 0.466). The mean global R_{ST} estimate for microsatellites (0.237) had an extremely large 95% confidence interval (–0.538, 1.012). R_{ST} was significantly greater than F_{ST} at *ONE8* (0.849 > 0.388; one-sided $P = 0.003$) and at *SFO8-1* (0.727 > 0.379; one-sided $P = 0.003$) indicating that stepwise-like mutations contributed to among-population differentiation at these two loci. The large variation in overall R_{ST} was primarily due to the low value (0.165) observed for *SSA456*.

Principal components analysis of microsatellite allele frequencies revealed five cohesive genetic assemblages for the 62 sites analysed (Fig. 3b, c). The five major assemblages contained populations found in (i) the upper Snake River, including sites in Utah (N7-10, O, AA–AC; Table 1, Fig. 2); (ii) the upper Missouri River (V–Z); (iii) rivers that lie between the Cascade Mountains and the Continental Divide and extend from Nevada to northern British Columbia and Alberta ('Inland Cascadia'; A–G, L, M, N1-6, P–U); (iv) rivers to the west of the Cascade Mountains, excluding the Olympic Peninsula ('Coastal Cascadia'; J & K); and (v) rivers of the Olympic Peninsula (H & I). The UPGMA dendrogram based on CSE distances and microsatellite allele frequencies depicted the same large-scale genetic groups as our principal components analysis and provided better resolution of genetic differentiation within groups (see below; Fig. 4). The AMOVA based on major watersheds partitioned

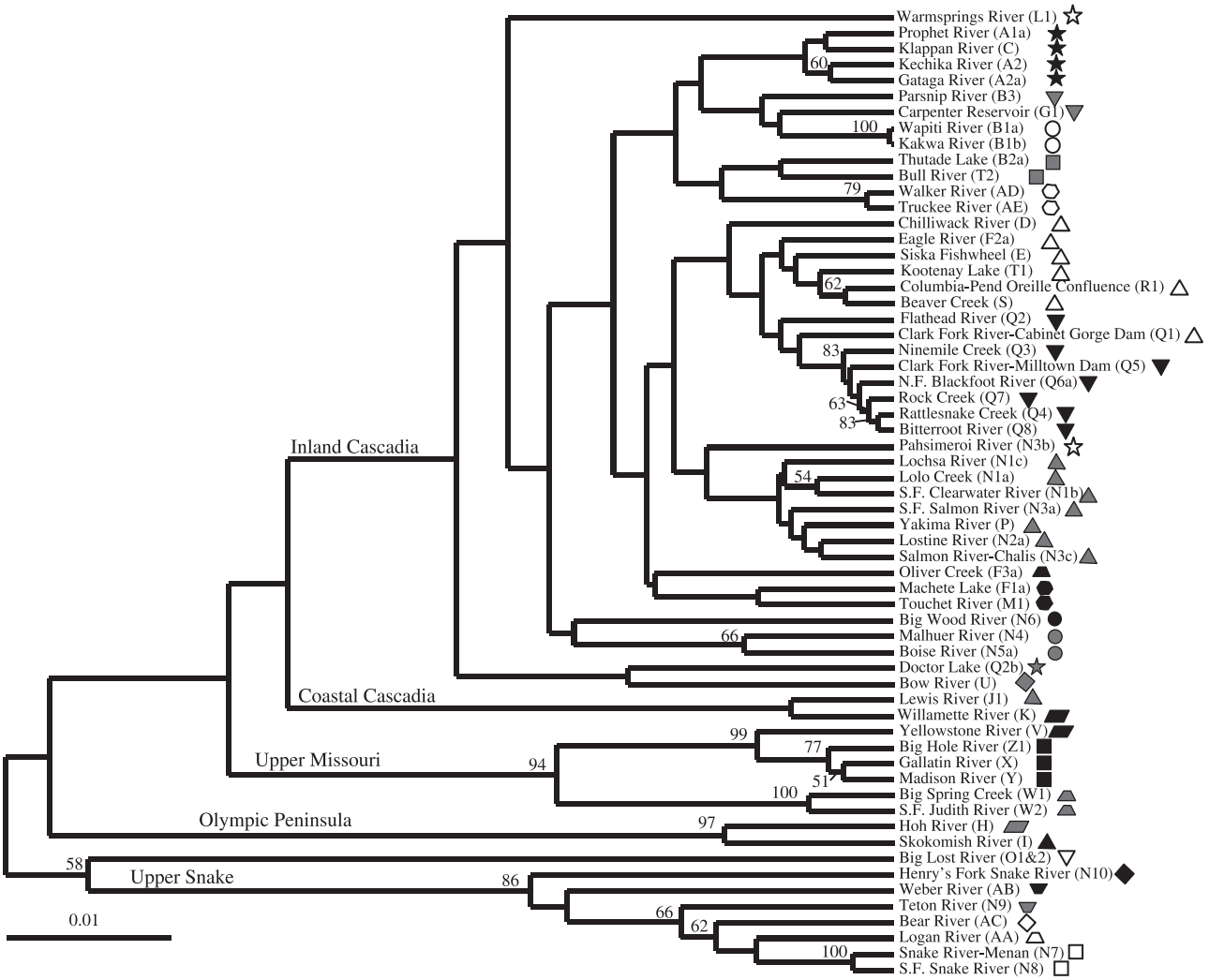

Fig. 4 UPGMA dendrogram based on microsatellite allele frequencies and CSE distances. Bootstrap values greater than 50% are shown. Identities of major genetic assemblages are shown on their respective branches. Symbols are the same as shown in Fig. 4 (b) and are meant to allow direct comparison between population groupings in this dendrogram and from BAPS.

more variation among groups and less variation among sites within groups than the other three arrangements (Table 2). The AMOVA arrangement based on PCA groups partitioned slightly less variation among groups than the watershed arrangement (Table 2).

Comparison of markers. The PCA of combined microsatellite and allozyme allele frequencies for the 29 sites analysed with both marker types clearly revealed three large-scale genetic assemblages. The combined PCA showed a marked separation of the upper Missouri, upper Snake, and Cascadia genetic groups (Fig. 3d). The Coastal Cascadia site (K) was not separated from Inland Cascadia sites for principal components axes 1 and 2 but was separated on PC 4 (which explained 6% of the variation; data not shown), probably due to the fact that this site was genetically differentiated with microsatellites but not allozymes.

Variation among populations within assemblages

Allozymes. For allozymes, mean pairwise F_{ST} and CSE values were significantly greater in the upper Snake River group than the Cascadia or upper Missouri groups (Table 3). We used BAPS to further analyse the geographic distribution of genetic variation at this geographic scale. BAPS revealed a total of eight clusters for the allozyme data (marginal posterior probability = 0.91; Table 3). All of the most likely data partitions contained eight population clusters. Of these, the two most likely data partitions (probability of 0.34 vs. 0.24) differ only by the placement of the Bow River (U). In the most likely data partition, the Bow River was placed with sites from the Columbia River (N2, N5, P, Q2a, S, T1; Fig. 5a). In the second most likely partition, the Bow River was placed with a different BAPS-defined cluster that consisted of sites from the Columbia River (N6, Q8, T2) and the Lahontan

Table 3 Genetic differentiation within cohesive genetic assemblages. Dashes are due to the discrepancy in resolution of major genetic assemblages between allozymes and microsatellites in the case of the Cascadia groups (allozymes revealed a general Cascadia group, while microsatellites revealed both a Coastal and an Inland Cascadia group). For the Olympic Peninsula group, the dashes were used to indicate that the two sites were not analysed with allozymes. Mean pairwise F_{ST} and CSE values are given with standard deviations in parentheses. Superscripts denote significant differences ($\alpha = 0.05$) with Tukey's posthoc tests. Estimates of the standard deviation for mean microsatellite genetic distances for the Olympic Peninsula and Coastal Cascadia groups were not possible because only two sites were analysed. The number of BAPS groups are the same as shown in Fig. 4

	Allozymes				Microsatellites			
PCA group	No. of sites	Pairwise F_{ST}	CSE	No. of BAPS groups	No. of sites	Pairwise F_{ST}	CSE	No. of BAPS groups
Olympic Peninsula	—	—	—	—	2	0.337	0.210	2
Cascadia	16	0.194 (0.147)[a]	0.050 (0.034)[a]	4	—	—	—	—
Coastal Cascadia	—	—	—	—	2	0.020	0.120	1
Inland Cascadia	—	—	—	—	43	0.215 (0.140)[a]	0.033 (0.014)[a]	16
Upper Missouri	5	0.109 (0.096)[a]	0.037 (0.032)[a]	2	6	0.120 (0.094)[a]	0.027 (0.017)[a]	3
Upper Snake	8	0.376 (0.345)[b]	0.081 (0.083)[b]	2	9	0.317 (0.267)[b]	0.049 (0.032)[b]	7

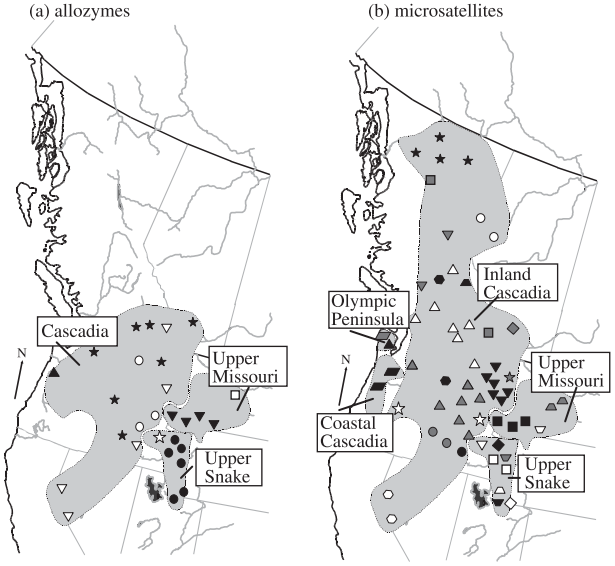

Fig. 5 Results from Bayesian Analysis of Population Structure (BAPS) across the range of mountain whitefish for (a) allozymes and (b) microsatellites. The geographic locations of cohesive genetic assemblages identified with principal components analysis are labelled and shaded grey. Each BAPS-defined group has a separate symbol and/or shading. Symbols in (a) are independent of those in (b).

Basin in Nevada (AD & AE). Each PCA group had a similar number of BAPS-defined clusters (Table 3, Fig. 5a).

Microsatellites. For microsatellites, pairwise F_{ST} and CSE values were greatest for the Olympic Peninsula group and lowest for the Coastal Cascadia group (Table 3), but statistical tests of significance were not possible because only two sites were analysed for each of these groups. Mean pairwise F_{ST} and CSE values were significantly greater in the upper Snake River group than in the Inland Cascadia and upper Missouri groups (Table 3).

When we applied BAPS to the 62 sample locations for the microsatellite data set, a total of 29 population clusters had the highest marginal posterior probability (0.83). The two most likely partitions of the data (probabilities of 0.54 vs. 0.28) only differed by the placement of the lower Clark Fork River site (Q1) with (1) sites from the Fraser and Columbia Rivers in British Columbia (D, E, R, S, T) or (2) other sites from the Clark Fork River (Q2-8). The Inland Cascadia PCA group had the greatest number of BAPS-defined clusters (Table 3) but also had the greatest number of sites (Table 3) and occurred over the largest geographic area (Fig. 5b). The upper Snake River group had a large number of BAPS groups (Table 3) within a small geographic area (Fig. 5b). There was strong concordance between groups defined by BAPS and groups in the UPGMA dendrogram (Figs 4 and 5b).

Comparison of markers. Microsatellites provided greater resolution of genetic relationships within major genetic assemblages than allozymes. The number of BAPS-defined groups was greater for microsatellites in each geographic assemblage (Table 4). In addition, geographically proximate populations tended to cluster together with microsatellites (Figs 4 and 5b) but this relationship was weaker for allozymes (Fig. 5a), most probably because relatively few allozyme loci were variable among sites within cohesive genetic assemblages (data not shown).

Geographic scale of genetic differentiation

For mountain whitefish, genetic and geographic distances were significantly correlated for microsatellites for sites in the Columbia River and also within the Inland Cascadia

Table 4 Mean pairwise genetic differentiation for within-basin and among-basin comparisons within the Columbia River. Number in parentheses is the standard deviation. Comparisons within basins correspond to filled circles in Fig. 6; comparisons among basins correspond to open circles in Fig. 6

	Allozymes		Microsatellites	
Population comparisons	F_{ST}	CSE	F_{ST}	CSE
All populations				
Comparisons within basins	0.170 (0.142)	0.035 (0.026)	0.056 (0.071)	0.012 (0.009)
Comparisons among basins	0.186 (0.151)	0.050 (0.033)	0.194 (0.119)	0.033 (0.014)
Above barrier and small lake populations excluded				
Comparisons within basins	0.130 (0.133)	0.023 (0.015)	0.037 (0.034)	0.010 (0.005)
Comparisons among basins	0.119 (0.095)	0.035 (0.025)	0.150 (0.078)	0.029 (0.012)

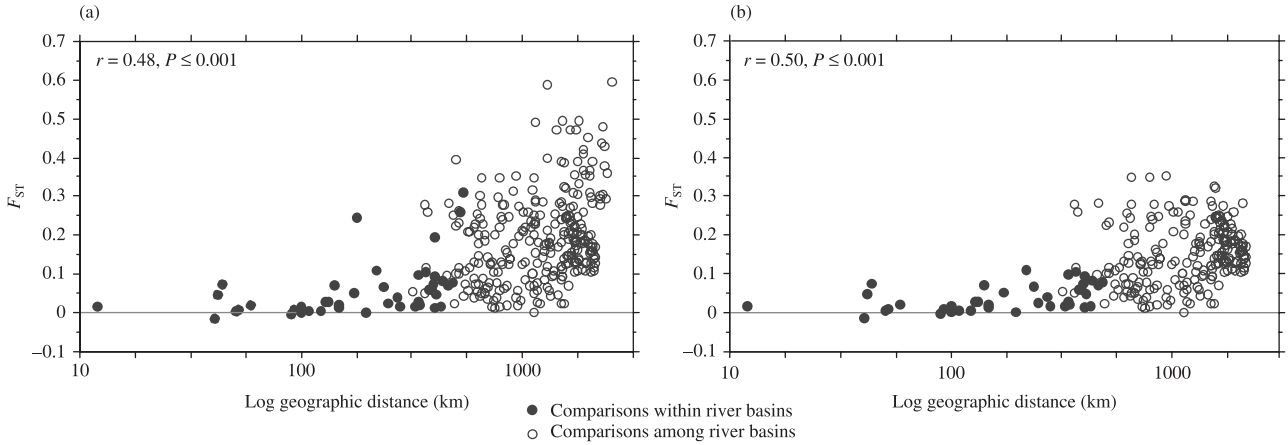

Fig. 6 Genetic vs. geographic distance for mountain whitefish populations in the Columbia River basin and within the Inland Cascadia genetic group. Within river basin comparisons are shown as filled circles and among river basin comparisons are shown as open circles. In (a), all populations in the Columbia River basin within the Inland Cascadia group are shown. In (b), two above barrier sites (B2a and T2) and one high mountain lake site (Q2b) were removed.

group (Fig. 6; for pairwise F_{ST}, $r = 0.50$, $P \leq 0.001$ for all sites and $r = 0.46$, $P \leq 0.001$ with the two above barrier and one high mountain lake sites removed, data not shown for CSE). Log-transformation of geographic distances or analysis of $F_{ST}/(1 - F_{ST})$ did not have a significant influence on these results (Fig. 6b; $F_{ST}/(1 - F_{ST})$ (data not shown)). The mean and variance of pairwise genetic distance values increased between approximately 300 and 500 km (Fig. 6; CSE data not shown), which corresponded approximately to comparisons among sites within river basins (mean geographic distance ± SE = 242 ± 23 km; solid circles Fig. 6) and comparisons among sites in separate river basins (mean = 1313 ± 32 km; open circles Fig. 6). Means and standard deviations of genetic distance values (pairwise F_{ST} and CSE) were generally lower for comparisons within basins than for comparisons among basins (Table 4).

For bull trout, genetic and geographic distances were also significantly correlated within the Columbia River system (for pairwise F_{ST}, $r = 0.58$, $P < 0.001$). The relationship between genetic and geographic distance differed markedly between bull trout and mountain whitefish (Fig. 7). The range of pairwise F_{ST} values was very large (from zero to approximately 0.8) for bull trout and remained large until approximately 1400 km (Fig. 7).

Discussion

What is the genetic structure of mountain whitefish in western North America?

Distribution of genetic variation. The distribution of genetic variation we observed across the range of the mountain whitefish was influenced by historical factors at the range-wide scale while aspects of the ecology and life history of this species appeared to interact with landscape features at a smaller geographic scale (within cohesive genetic assemblages). We observed a large proportion of genetic variation partitioned among large-scale genetic assemblages

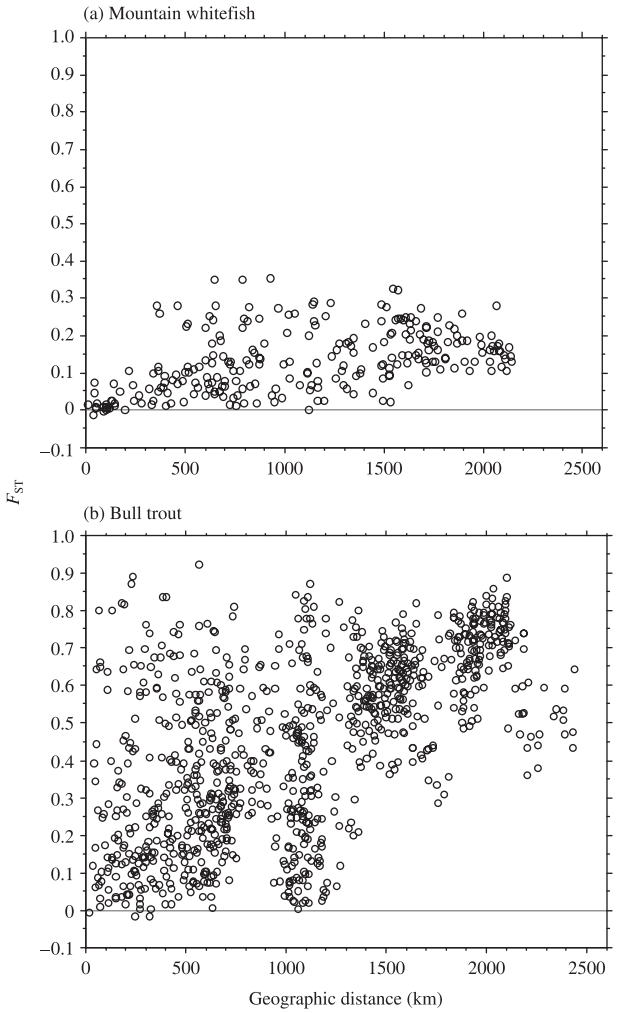

Fig. 7 Genetic vs. geographic distance for (a) mountain whitefish and (b) bull trout in the Columbia River basin and within the Inland Cascadia genetic group. Bull trout data are from Spruell *et al*. (2003).

and a large proportion of genetic variation within populations. Relative to other salmonid species, we observed fairly low levels of differentiation among populations within assemblages.

We observed a large range of values of within-population genetic variation (H_S and mean number of alleles; Table 1). Populations in the Clark Fork (Q) and Missouri Rivers (V–Z) consistently had the highest values. Populations with low values were usually from sites known to be physically isolated. For example, the Big Lost River (O) is part of the isolated 'sinks' basins in southeastern Idaho that flow underground before joining with the Snake River. The Big Wood River (N6) in Idaho and the Bull River (T2) and Thutade Lake (B2a) in British Columbia are all isolated by barrier waterfalls.

The correlation we observed between amounts of within-population genetic variation (H_S) for both marker types suggests that this variation reflects the effects of evolutionary and demographic factors on the entire mountain whitefish genome. The exception to this general pattern in the Big Lost River (O) may be due to large N_e at the *MDHB-1,2** isolocus (Allendorf & Thorgaard 1984). In the Bonneville Basin, Utah (sites AA–AC), microsatellite alleles may have been retained and/or mutations may have subsequently restored variation at microsatellite loci following the putative founding event between approximately 10 000–30 000 years ago (McPhail & Lindsey 1986; Johnson 2002).

Among-population divergence. Genetic differentiation was distributed in a hierarchical manner across the range of the mountain whitefish, most likely in response to historical factors associated with glaciation. At the broadest geographic scale, we found evidence for substantial genetic differentiation among regions (three cohesive assemblages for allozymes and five cohesive assemblages for microsatellites). The genetic groups depicted are consistent with the multiple glacial refugia hypothesis of McPhail & Lindsey (1986). Mountain whitefish may have resided in the upper Missouri, upper Snake, and Columbia Rivers. Within the Columbia River there may have been multiple refugia, including the Chehalis River on the Olympic Peninsula. These genetic assemblages correspond to several important geological features (Fig. 2). The upper Missouri and Cascadia groups are separated by the Continental Divide, which forms a biogeographical break for other fish species in this region (McPhail & Lindsey 1986; Wilson & Hebert 1998; Stamford & Taylor 2004). Within the Cascadia group, the coastal and interior populations are separated by the Cascade and Coastal Mountains (Fig. 2). In addition, the upper Snake group resides above an approximately 60 000-year-old barrier waterfall (Shoshone Falls; Fig. 2) that has served as another biogeographical break (McPhail & Lindsey 1986; Johnson 2002). Ancient connections between the upper Snake River and the Bonneville Basin are hypothesized causes of biogeographical similarity among these systems (McPhail & Lindsey 1986; Minckley *et al*. 1986; Johnson 2002).

Within major genetic assemblages, the landscape template and hierarchical organization of river basins appears to have influenced the geographic scale and patterns of genetic differentiation. Sites within the same or adjacent river basins tended to cluster together with microsatellites (Figs 4 and 5b); however, this pattern was not as strong with allozymes (Fig. 5a). The lack of geographic signal in the allozyme data set at this scale is probably a result of low resolution of this marker type within genetic assemblages. In addition, the AMOVAs for both microsatellites and allozymes where sites were pooled by major watersheds had less variation partitioned among sites within watershed than other arrangements had among sites within their

respective groupings (Table 2). Furthermore, the increase in mean and variance of pairwise F_{ST} and CSE values that corresponded approximately to within vs. among river basins in the relationship between genetic and geographic distance is consistent with this hypothesis (Fig. 6; Table 4); however, this pattern was again more apparent with microsatellites than allozymes (Table 4). This strong pattern observed with the more highly resolving microsatellites suggests that genes are exchanged among populations within river basins much more often than among populations in separate river basins. We also observed increased genetic differentiation among sites located within river basins but separated by geomorphic barriers. These isolated sites tended to be as genetically differentiated from other populations located in the same river basin as populations in different basins were from each other (Fig. 6). This suggests that barriers within river basins reduce gene flow to a similar extent as gene flow is reduced among river basins.

In general, we found little evidence of differentiation among sites within major river basins (Figs 3 and 4), which is consistent with our observations for a single river basin in Montana (Whiteley *et al*. 2004) and predictions based on the ecology and life history of this species. An exception to this pattern occurred in the upper Snake River and on the Olympic Peninsula, where mountain whitefish populations were more finely subdivided than elsewhere (Table 3). The most likely cause of this increased subdivision is natural restrictions of gene flow, either due to geomorphological discontinuities or to saltwater barriers to dispersal. The upper Snake River Plateau has a complex geomorphological history (McPhail & Lindsey 1986; Johnson 2002). In addition to the isolation of the Big Lost (O), the Henry's Fork site (N10) is above an impassable waterfall (Mesa Falls), and Bonneville Basin sites (AA–AC) are currently isolated from the upper Snake River. Thus, population isolation due to the fragmented physical template might be responsible for the high genetic differentiation observed in this region. On the Olympic Peninsula, gene flow among sites may be limited because mountain whitefish apparently are not saltwater tolerant (McPhail & Lindsey 1986).

Intolerance to saltwater may explain genetic patterns for mountain whitefish in two other instances. First, we observed significant differentiation of Olympic Peninsula sites (H & I) from other Columbia River sites west of the Cascade Mountains (J & K). These rivers are geographically close together and we would expect greater genetic similarity if oceanic dispersal were possible. Second, the site we analysed from the lower Fraser River (D) grouped with other Fraser River and Columbia River sites (Figs 3 and 4) instead of grouping with coastal sites. This pattern is consistent with dispersal through inland freshwater dispersal routes rather than an oceanic route (McPhail & Lindsey 1986).

How do patterns of genetic differentiation compare among native fish species in western North America?

Concordance of patterns. We observed several examples of concordant patterns of genetic differentiation between mountain whitefish and other species. These examples highlight evolutionary divergence that warrants increased conservation attention and will aid in defining conservation units. The first example involved mountain whitefish and bull trout populations in the Snake River upstream from Hells Canyon and downstream from Shoshone Falls. Bull trout populations from this region (from the Malhuer, Boise, and Jarbidge Rivers) lie within the Inland Cascadia group but are genetically differentiated from other sites (Spruell *et al*. 2003). However, it is not clear whether these bull trout populations deserve heightened conservation status. Mountain whitefish populations from this region, from the Malhuer (N4), Boise (N5), and Big Wood Rivers (N6), also lie within the Inland Cascadia group but are differentiated from other sites (Figs 3 and 4). Three dams in this section of the Snake River (constructed between 1958 and 1967) might be responsible for these observations. However, it seems unlikely that these dams are the sole cause of these patterns, given this short timescale. The differentiation observed for each species probably predates the construction of these dams and may be due to historically reduced gene flow through Hells Canyon. The striking similarity in patterns between these two species suggests that threatened bull trout populations in this region warrant greater protection and that this region may contain distinct conservation units of other native fishes.

Several salmonid species in the Pahsimeroi River (N3b) provide another example of parallel patterns of genetic divergence. The Pahsimeroi River is spring dominated and differs environmentally from the Salmon River and adjacent tributaries. Populations of steelhead and Chinook salmon in the Pahsimeroi River are genetically differentiated from other populations in the Salmon River (NOAA 2003), but a history of hatchery stocking potentially confounds these among-population genetic relationships. The spring-dominated nature of this system has led others to suggest that the genetic signal of among-population differentiation of both species at this site might reflect local adaptation and historically reduced gene flow (NOAA 2003). The genetic differentiation we observed between mountain whitefish from the Pahsimeroi River and other sites in the Salmon River (Figs 4 and 5) provides an unusual example of genetic differentiation at a small geographic scale for the mountain whitefish. It is possible that environmental characteristics of this site have led to genetic differentiation of Pahsimeroi River populations of steelhead, Chinook salmon, and mountain whitefish from other nearby populations for each species. In this case, data from the mountain whitefish, because it has not been transferred or influenced

by hatchery practices, clarify genetic data from species that have been influenced by hatchery stocking.

Differences in patterns. Differences among species in patterns of genetic differentiation may reflect species-specific biological differences in responses to factors that can reduce gene flow. For example, an inland/coastal genetic split corresponding to the Coastal Mountains in British Columbia and the Cascade Mountains in Oregon and Washington (Fig. 1) has been observed in studies of rainbow trout (Allendorf & Utter 1979; McCusker *et al*. 2000), bull trout (Taylor *et al*. 1999; Spruell *et al*. 2003), cutthroat trout (Allendorf & Leary 1988), Chinook salmon (Teel *et al*. 2000), coho salmon (*Oncorhynchus kisutch*; Small *et al*. 1998), and longnose suckers (*Catostomus catostomus*; McPhail & Taylor 1999) as well as amphibians (e.g. Good 1989; Nielson *et al*. 2001; Carstens *et al*. 2005). For fishes, there are some species-specific differences in where this split occurs (Fig. 1; Spruell *et al*. 2003). Patterns for mountain whitefish from coastal sites differ in two ways from previously studied species. First, populations in the lower Fraser River belong to the coastal assemblage for other fishes (Fig. 1; Small *et al*. 1998; McPhail & Taylor 1999; Taylor *et al*. 1999; Teel *et al*. 2000) rather than the inland assemblage as we observed for mountain whitefish (Fig. 4b). Second, we observed greater differentiation between sites on the Olympic Peninsula and other coastal sites than has been observed for other species (e.g. Spruell *et al*. 2003). Both of these observations may be due to the absence of oceanic dispersal for mountain whitefish. In both cases, biological aspects of mountain whitefish may be responsible for differences in genetic patterns and these differences have implications for conserving historical relationships among populations. For example, mountain whitefish in the lower Fraser River would belong to an inland ESU, while other species in the same river would belong to coastal ESUs. [In this paper we use ESU in its most generic sense to describe groups of populations that have a shared evolutionary history and are sufficiently genetically differentiated from other such groups to merit separate conservation efforts (*sensu* Ryder 1986; Waples 1991). We do not presume any specific functional definition (e.g. Moritz 1994). Nor are we advocating legal status for the ESUs we discuss.]

Overall patterns of genetic differentiation for mountain whitefish differed from those of cutthroat trout subspecies, as indicated by the AMOVA (Table 2). This lack of concordance is largely due to three instances where populations of cutthroat subspecies are more genetically differentiated than sympatric mountain whitefish populations. However, we also found one striking example where mountain whitefish populations are more genetically differentiated than those of a cutthroat trout subspecies.

First, the westslope cutthroat trout (*Oncorhynchus clarki lewisi*) occurs in the Columbia River basin west of the Continental Divide and in the upper Missouri basin to the east, with the exception of the Yellowstone River (Fig. 1; Allendorf & Leary 1988). With allozymes, populations of westslope cutthroat trout are generally highly genetically differentiated from each other on each side of the Continental Divide, such that populations tend to be as differentiated from one another on the same side of the Divide as they are on opposite sides of the Divide (Leary *et al*. 1988). It is unclear if there should be one or two ESUs for this subspecies because, with allozymes, the genetic signal of regional differentiation on opposite sites of the Divide may have been obscured by genetic drift in small populations. Regional differentiation reflecting two ESUs may be observed if additional molecular data were collected. In the absence of such data, the genetic differentiation of mountain whitefish populations separated by the Divide suggests that hierarchical genetic differentiation may occur for the westslope cutthroat trout and two ESUs may exist.

Second, the Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) in the Great Basin is also a genetically differentiated subspecies (Fig. 1; Allendorf & Leary 1988), while mountain whitefish in the Great Basin (AD & AE) are part of the Inland Cascadia genetic assemblage. It is possible that populations of both species have been isolated from other Inland Cascadia sites for the same amount of time but differentiation of mountain whitefish populations has not occurred as rapidly due to larger N_e. Thus, mountain whitefish populations might provide a better reflection of historical relationships in this case as well.

Third, the similarity we observed between mountain whitefish populations in the Yellowstone River (V) and the remainder of the upper Missouri River (W–Z) contrasts markedly with the genetic divergence of cutthroat trout subspecies in these two rivers (Yellowstone cutthroat trout, *Oncorhynchus clarki bouvieri* in the Yellowstone River and westslope cutthroat trout in the remainder of the upper Missouri River, Fig. 1; Allendorf & Leary 1988). In this case, two distinct cutthroat trout subspecies lie within what would be one upper Missouri mountain whitefish ESU. Biological differences, including the possibility of greater historical movement, as well as larger N_e of mountain whitefish are probably responsible for this lack of concordance.

In contrast, populations of mountain whitefish in the Yellowstone River are more genetically differentiated from population in the upper Snake River than are populations of Yellowstone cutthroat trout in these same river systems. Yellowstone cutthroat trout in these two river basins are only slightly genetically differentiated at allozyme loci (Allendorf & Leary 1988). The large degree of genetic differentiation we observed for mountain whitefish populations in these two river basins suggests that Yellowstone cutthroat trout in the Yellowstone River and upper Snake River may be more genetically divergent than indicated by

allozymes and perhaps mtDNA or microsatellites would provide further resolution of population relationships.

How does the geographic scale of genetic differentiation compare among species?

Mountain whitefish populations appear to exchange genes over a much larger geographic scale than other native inland fishes studied to date. For these other species, genetic variation is often partitioned among regions, among river basins within regions, among large rivers within river basins, and among tributaries within large rivers (Allendorf & Utter 1979; Allendorf & Leary 1988; Taylor *et al*. 1999, 2003; McCusker *et al*. 2000; Teel *et al*. 2000; Waples *et al*. 2001, 2004; Costello *et al*. 2003; Spruell *et al*. 2003). Thus, relative to mountain whitefish, these other salmonid species are subdivided on a finer geographic scale and gene flow appears to extend over smaller portions of the landscape. These species tend to have one, if not two, additional levels of hierarchical subdivision relative to the mountain whitefish. For example, bull trout and westslope cutthroat trout populations tend to be more genetically differentiated among tributaries within river basins than mountain whitefish populations are among river basins (Costello *et al*. 2003; Taylor *et al*. 2003; Whiteley *et al*. 2004). Our comparison of the isolation-by-distance relationship between mountain whitefish and bull trout (Fig. 7) is consistent with this interpretation.

Mountain whitefish populations in entire river systems may be part of one large metapopulation (*sensu* Hanski 1999). For example, the entire Columbia River Basin (with the exception of locations in the upper Snake River) might be one large metapopulation of mountain whitefish, while this river system probably contains many metapopulations of other salmonids. With respect to salmonid fishes, metapopulation dynamics have only been considered over much smaller geographic scales for trout, charr, and salmon (e.g. Rieman & Dunham 2000). The same principles that have emerged from studies of other salmonids (Rieman & Dunham 2000; Dunham *et al*. 2003; Neville *et al*. in press) may apply to the mountain whitefish, only over much larger temporal and spatial scales.

Do these data provide additional insight for management of native fishes in western North America?

Delineating conservation units requires an understanding of evolutionary relationships among populations (Moritz 1994; Waples 1995; Crandall *et al*. 2000; Fraser & Bernatchez 2001; McKay & Latta 2002). Following this first step, it must then be determined which populations, or groups of populations, should be the focus of conservation efforts. Regions where genetic patterns for the mountain whitefish were concordant with other species, as we observed for the Snake River upstream from Hells Canyon, warrant conservation designations that reflect the independent evolutionary trajectories of the species in those regions. Regions where genetic patterns for the mountain whitefish were not concordant with other species highlight important evolutionary relationships that might not be currently recognized by conservation efforts. For example, mountain whitefish would belong to different ESUs than other species in the same river systems in several cases. These differences in genetic patterns must be considered to conserve historical relationships among populations of different species in the same systems.

Mountain whitefish populations appear to exchange migrants over a larger geographic scale than other salmonids. Management and conservation efforts should focus at the scale of river basins for this species because this is the scale at which evolutionary processes are likely to be most influential. Co-occurring salmonids should generally be managed at a finer geographic scale (i.e. tributaries within basins). Ideally, effective conservation efforts will work to protect populations of multiple species at all of these levels. Important questions to consider with respect to the geographic scale of genetic differentiation include the following: What demographic and evolutionary effects will habitat fragmentation (e.g. dams) have on different species? And, how much connectivity is needed for different species and at what scale? These questions are important for more than mountain whitefish conservation because the scale of genetic differentiation for this species may be similar to other unexamined native fishes in this region.

Our work provides a case study of the importance of considering genetic data from multiple species across the same landscape and including common species in those comparisons for a more comprehensive approach to conservation. We demonstrated how similarities and differences among species in the scale and patterns of genetic differentiation can be used to highlight important evolutionary relationships, to help define species' habitat requirements, and to determine where single-species management is most likely to provide inadequate conservation of other species in an ecosystem. Appreciating these differences in the pattern and scale of genetic differentiation and evolutionary dynamics can enhance the efficacy of region-wide management and conservation plans.

Acknowledgements

J. Benzie, E. Crone, L. Eby, D. Emlen, W. C. Funk, B. Rieman, and two anonymous reviewers provided helpful comments on this manuscript. The following people helped with sample collection: D. Allen, B. Antcliffe, K. Apperson, D. Becker, R. Bison, K. Bott, S. Brenkman, K. Brewin, J. Brostrom, P. Brown, J. Burrows, D. Bustard, P. Byorth, J. Byrne, R. Christian, C. Clancy, P. Clancey, S. Corbett, C. Corsi, D. Cremins, B. Culling, J. De Gisi, C. Downs, K. Frank, B. Frater, B. Gamett, P. Giroux, G. Grisak, G. Haas, M.

Hanson, L. Hildebrand, N. Horner, P. Hvenegaard, P. James, L. Janssen, R. Johnson, B. Jonasson, D. Karl, L. Knotek, J. Laddell, J. Magee, G. Martin, J. Martin, S. Masse, A. Maxwell, K. McDonald, G. Mendel, C. Muhlfeld, L. Neraas, M. Neufeld, D. O'Brien, C. Parken, F. Partridge, R. Peterson, K. Pratt, L. Rempel, B. Rieman, B. Roper, T. Salow, H. Schaller, C. Schell, D. Schmetterling, B. Schrader, L. Schwabe, M. Sevon, D. Shiozawa, D. Sneep, S. Spalding, A. Tews, G. Tisdale, J. Tohtz, J. Walters, C. Warren, R. Wehnke, A. Weissman, T. Whitesel, R. Wildman, and R. Zemlak. D. Holzer provided geographic distances among sites in the Columbia River. J. Powers assisted with data collection. This project was funded in part by the US Forest Service, Montana Department of Fish, Wildlife & Parks, the American Fisheries Society (Eugene Maughan Scholarship), the Sustainable Fisheries Foundation (William Trachtenberg Memorial Scholarship) and the American Society of Ichthyologists and Herpetologists (Edward Raney Scholarship).

References

Allendorf FW, Leary RF (1988) Conservation and distribution of genetic variation in a polytypic species: the cutthroat trout. *Conservation Biology*, **2**, 170–184.

Allendorf FW, Seeb LW (2000) Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. *Evolution*, **54**, 640–651.

Allendorf FW, Thorgaard G (1984) Polyploidy and the evolution of salmonid fishes. In: *Evolutionary Genetics of Fishes* (ed. Turner BJ), pp. 1–53. Plenum Press, New York.

Allendorf FW, Utter FM (1979) Population genetics. In: *Fish Physiology*, Vol. 8 (eds Hoar WS, Randall DJ, Brett JR), pp. 407–454. Academic Press, New York.

Allendorf FW, Mitchell N, Ryman N, Stahl G (1977) Isozyme loci in brown trout (*Salmo trutta* L.): detection and interpretation from population data. *Hereditas*, **86**, 179–190.

Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. *Trends in Ecology & Evolution*, **16**, 613–622.

Avise JC (2004) *Molecular Markers, Natural History, and Evolution*. Sinauer Associates, Sunderland, Massachusetts.

Behnke RJ (2002) *Trout and Salmon of North America*. The Free Press, New York.

Bermingham E, Moritz C (1998) Comparative phylogeography: concepts and applications. *Molecular Ecology*, **7**, 367–369.

Bohonak AJ (2003) IBD (Isolation By Distance): a program for analyses of isolation by distance. *Journal of Heredity*, **92**, 153–154.

Brouat C, Chevallier H, Meusnier S, Noblecourt T, Rasplus JY (2004) Specialization and habitat: spatial and environmental effects on abundance and genetic diversity of forest generalist and specialist *Carabus* species. *Molecular Ecology*, **13**, 1815–1826.

Carstens BC, Brunsfeld SJ, Demboski JR, Good JM, Sullivan J (2005) Investigating the evolutionary history of the Pacific Northwest mesic forest ecosystem: hypothesis testing within a comparative phylogeographic framework. *Evolution*, **59**, 1639–1652.

Castric V, Bernatchez L (2004) Individual assignment test reveals differential restriction to dispersal between two salmonids despite no increase of genetic differences with distance. *Molecular Ecology*, **13**, 1299–1312.

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *Evolution*, **21**, 550–570.

Collins BD, Montgomery DR (2002) Forest development, wood jams, and restoration of floodplain rivers in the Puget lowland, Washington. *Restoration Ecology*, **10**, 237–247.

Corander J, Waldmann P, Sillanpaa MJ (2003) Bayesian analysis of genetic differentiation between populations. *Genetics*, **163**, 367–374.

Corander J, Waldmann P, Marttinen P, Sillanpaa MJ (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics*, **20**, 2363–2369.

Costello AB, Down TE, Pollard SM, Pacas CJ, Taylor EB (2003) The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, *Salvelinus confluentus* (Pisces: Salmonidae). *Evolution*, **57**, 328–344.

Crandall KA, Binindaemonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution*, **15**, 290–295.

Dawson MN, Louie KD, Barlow M, Jacobs DK, Swift CC (2002) Comparative phylogeography of sympatric sister species, *Clevelandia ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition Zone. *Molecular Ecology*, **11**, 1065–1075.

Dick CW, Roubik DW, Gruber KF, Bermingham E (2004) Long-distance gene flow and cross-Andean dispersal of lowland rainforest bees (Apidae: Euglossini) revealed by comparative mitochondrial DNA phylogeography. *Molecular Ecology*, **13**, 3775–3785.

Dunham JB, Young KA, Gresswell RE, Rieman BE (2003) Effects of fire on fish populations: landscape perspectives on persistence of native fishes and non-native fish invasions. *Forest Ecology and Management*, **178**, 183–196.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Felsenstein J (1993) PHYLIP *(Phylogeny Inference Package)*, version 3.5c. Department of Genetics,University of Washington, Seattle, Washington.

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology*, **10**, 2741–2752.

Freudenberger D, Brooker L (2004) Development of the focal species approach for biodiversity conservation in the temperate agricultural zones of Australia. *Biodiversity and Conservation*, **13**, 253–274.

Froufe E, Alekseyev SS, Knizhin I, Alexandrino P, Weiss S (2003) Comparative phylogeography of salmonid fishes (Salmonidae) reveals late to post-Pleistocene exchange between three now-disjunct river basins in Siberia. *Diversity and Distributions*, **9**, 269–282.

Good DA (1989) Hybridization and cryptic species in *Dicamptodon* (Caudata: Dicamptodontidae). *Evolution*, **43**, 728–744.

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.

Goudet J, Raymond JM, Demeeus T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1933–1940.

Goudet J (2001) FSTAT *(Version 2.9.3): A program to estimate and test gene diversities and fixation indices*. Available from www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Hanski I (1999) *Metapopulation Ecology*. Oxford University Press, New York.

Hardy OJ, Vekemans X (2002) SPAGEDI: a versatile computer program to analyze spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618.

Hardy OJ, Charbonnel N, Freville H, Heuertz M (2003) Microsatellite allele size: a simple test to assess their significance. *Genetics*, **163**, 1467–1482.

Harris H, Hopkinson DA (1976) *Handbook of Enzyme Electrophoresis in Human Genetics*. Elsevier, New York.

Hoffmann FG, Baker RJ (2003) Comparative phylogeography of short-tailed bats (Carollia: Phyllostomidae). *Molecular Ecology*, **12**, 3403–3414.

IUBMBNC (International Union of Biochemistry and Molecular Biology NC) (1992) *Enzyme Nomenclature, 1992*. Academic Press, San Diego, California.

Johnson JB (2002) Evolution after the flood: phylogeography of the desert fish Utah chub. *Evolution*, **56**, 948–960.

Kareiva P, Marvier M, McClure M (2000) Recovery and management options for spring/summer chinook salmon in the Columbia River Basin. *Science*, **290**, 977–979.

King RB, Lawson R (2001) Patterns of population subdivision and gene flow in three sympatric natricine snakes. *Copeia*, **2001**, 602–614.

Lambeck RJ (1997) Focal species: a multi-species umbrella for nature conservation. *Conservation Biology*, **11**, 849–856.

Leary RF, Booke HE (1990) Starch gel electrophoresis and species distinctions. In: *Methods for Fish Biology* (eds Schreck CB, Moyle PB), pp. 140–170. American Fisheries Society, Bethesda, Maryland.

Leary RF, Allendorf FW, Phelps SR (1988) Population genetic structure of westslope cutthroat trout: genetic variation within and among populations. *Proceedings of the Montana Academy of Sciences*, **48**, 57–70.

Leary RF, Allendorf FW, Forbes SH (1993) Conservation genetics of bull trout in the Columbia and Klamath River drainages. *Conservation Biology*, **7**, 856–865.

Levin PS, Tolimieri N (2001) Differences in the impacts of dams on the dynamics of salmon populations. *Animal Conservation*, **4**, 291–299.

McClure MM, Holmes EE, Sanderson BL, Jordan CE (2003) A large-scale, multispecies status assessment: anadromous salmonids in the Columbia River Basin. *Ecological Applications*, **13**, 964–989.

McCusker MR, Parkinson E, Taylor EB (2000) Mitochondrial DNA variation in rainbow trout (*Oncorhynchus mykiss*) across its native range: testing biogeographical hypotheses and their relevance to conservation. *Molecular Ecology*, **9**, 2089–2108.

McDonald JH (1994) Detecting natural selection by comparing geographic variation in protein and DNA polymorphisms. In: *Non-Neutral Evolution: Theories and Molecular Data* (ed. Golding B), pp. 88–100. Chapman & Hall, New York.

McDonald DB, Potts WK, Fitzpatrick JW, Woolfenden GE (1999) Contrasting genetic structures in sister species of North American scrub-jays. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **266**, 1117–1125.

McKay JK, Latta RG (2002) Adaptive population divergence: markers, QTL and traits. *Trends in Ecology & Evolution*, **17**, 285–291.

McPhail JD, Lindsey CC (1970) *Freshwater Fishes of Northwestern Canada and Alaska*. Bulletin of the Fisheries Research Board of Canada, Number 173, Ottawa, Ontario.

McPhail JD, Lindsey CC (1986) Zoogeography of the freshwater fishes of Cascadia (the Columbia System and rivers north to the Stikine). In: *The Zoogeography of North American Freshwater Fishes* (eds Hocutt CH, Wiley EO), pp. 615–637. John Wiley & Sons, New York.

McPhail JD, Taylor EB (1999) Morphological and genetic variation in northwestern longnose suckers, *Catostomus catostomus*: the Salish sucker problem. *Copeia*, **4**, 884–893.

McPhail JD, Troffe P (2001) The mountain whitefish (*Prosopium williamsoni*): a brief review of the distribution, biology, and life history of a neglected recreational species. In: *Bull Trout II* (eds Brewin MK, Paul AJ, Monita M). Trout Unlimited Canada, Calgary, Alberta.

Mebane CA, Maret TR, Hughes RM (2003) An index of biological integrity (MBI) for Pacific Northwest rivers. *Transactions of the American Fisheries Society*, **132**, 239–261.

Michaux JR, Libois R, Filippucci MG (2005) So close and so different: comparative phylogeography of two small mammal species, the Yellow-necked fieldmouse (*Apodemus flavicollis*) and the Woodmouse (*Apodemus sylvaticus*) in the Western Palearctic region. *Heredity*, **94**, 52–63.

Minckley WL, Hendrickson DA, Bond CE (1986) Geography of western North American freshwater fishes: description and relationships to intracontinental tectonism. In: *The Zoogeography of North American Freshwater Fishes* (eds Hocutt CH, Wiley EO). John Wiley & Sons, New York.

Moritz C (1994) Defining 'Evolutionarily Significant Units' for conservation. *Trends in Ecology & Evolution*, **9**, 373–375.

Neraas LP, Spruell P (2001) Fragmentation of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork River system. *Molecular Ecology*, **10**, 1153–1164.

Neville H, Dunham JB, Peacock MM (in press) Assessing connectivity in salmonid fishes with DNA microsatellite markers. In: *Connectivity Conservation: Maintaining Connections in Nature* (eds Crooks K, Sanjayan MA). Cambridge University Press, Boston, Massachusetts.

Nielson M, Lohman K, Sullivan J (2001) Phylogeography of the tailed frog (*Ascaphus truei*): implications for the biogeography of the Pacific Northwest. *Evolution*, **55**, 147–160.

NOAA (2003) Independent populations of Chinook, steelhead, and sockeye for listed evolutionary significant units within the interior Columbia River domain. In: *Interior Columbia Basin Technical Recovery Team*. NOAA, Seattle, Washington. http://www.nwfsc.noaa.gov/trt/trt_pop_id.cfm.

Policansky D, Magnuson JJ (1998) Genetics, metapopulations, and ecosystem management of fisheries. *Ecological Applications*, **8**, S119–S123.

Raymond M, Rousset F (1995) GENEPOP (version 3.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rieman BE, Dunham JB (2000) Metapopulations and salmonids: a synthesis of life history patterns and empirical observations. *Ecology of Freshwater Fish*, **9**, 51–64.

Roberge J-M, Angelstam P (2004) Usefulness of the umbrella species concept as a conservation tool. *Conservation Biology*, **18**, 76–85.

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology & Evolution*, **1**, 9–10.

Satoh A, Sota T, Ueda T, Enokido Y, Paik JC, Hori M (2004) Evolutionary history of coastal tiger beetles in Japan based on a

comparative phylogeography of four species. *Molecular Ecology*, **13**, 3057–3069.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN, version 2.001: A software for population genetic data analysis*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Schoenswetter P, Tribsch A, Stehlik I, Niklfeld H (2004) Glacial history of high alpine *Ranunculus glacialis* (Ranunculaceae) in the European Alps in a comparative phylogeographical context. *Biological Journal of the Linnean Society*, **81**, 183–195.

Shaklee JB, Allendorf FW, Morizot DC, Whitt GS (1990) Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fisheries Society*, **119**, 2–15.

Small MP, Beacham TD, Withler RE, Nelson RJ (1998) Discriminating coho salmon (*Oncorhynchus kisutch*) populations within the Fraser River, British Columbia, using microsatellite DNA markers. *Molecular Ecology*, **7**, 141–155.

Spruell P, Rieman BE, Knudsen KL, Utter FM, Allendorf FW (1999) Genetic population structure within streams: microsatellite analysis of bull trout populations. *Ecology of Freshwater Fish*, **8**, 114–121.

Spruell P, Bartron ML, Kanda N, Allendorf FW (2001) Detection of hybrids between bull trout (*Salvelinus confluentus*) and brook trout (*Salvelinus fontinalis*) using PCR primers complementary to interspersed nuclear elements. *Copeia*, **2001**, 1093–1099.

Spruell P, Hemmingsen AR, Howell PJ, Kanda N, Allendorf FW (2003) Conservation genetics of bull trout: geographic distribution of variation at microsatellite loci. *Conservation Genetics*, **4**, 17–29.

Stamford MD, Taylor EB (2004) Phylogeographic lineages of Arctic grayling (*Thymallus arcticus*) in North America: divergence, origins and affinities with Eurasian *Thymallus*. *Molecular Ecology*, **13**, 1533–1549.

Takezaki N, Nei M (1996) Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*, **144**, 389–399.

Taylor EB, Pollard S, Louie D (1999) Mitochondrial DNA variation in bull trout (*Salvelinus confluentus*) from northwestern North America: implications for zoogeography and conservation. *Molecular Ecology*, **8**, 1155–1170.

Taylor EB, Stamford MD, Baxter JS (2003) Population subdivision in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) at the northern periphery of its range: evolutionary inferences and conservation implications. *Molecular Ecology*, **12**, 2609–2622.

Teel DJ, Milner GB, Winans GA, Grant WS (2000) Genetic population structure and origin of life history types in Chinook salmon in British Columbia, Canada. *Transactions of the American Fisheries Society*, **129**, 194–209.

Turner TF, Trexler JC (1998) Ecological and historical associations of gene flow in darters (Teleostei: Percidae). *Evolution*, **52**, 1781–1801.

Waples RS (1991) Pacific salmon, *Oncorhynchus* spp. and the definition of 'species' under the Endangered Species Act. *Marine Fisheries Review*, **53**, 11–22.

Waples RS (1995) Evolutionary significant units and the conservation of biological diversity under the Endangered Species Act. In: *Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation* (ed. Nielsen JL), pp. 8–27. American Fisheries Society Symposium 17, Bethesda, Maryland.

Waples RS, Gustafson RG, Weitkamp LA *et al.* (2001) Characterizing diversity in salmon from the Pacific Northwest. *Journal of Fish Biology*, **59**, 1–41.

Waples RS, Teel DJ, Myers JM, Marshall AR (2004) Life-history divergence in Chinook salmon: historic contingency and parallel evolution. *Evolution*, **58**, 386–403.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wenburg JK, Bentzen P, Foote CJ (1998) Microsatellite analysis of genetic population structure in an endangered salmonid: the coastal cutthroat trout (*Oncorhynchus clarki clarki*). *Molecular Ecology*, **7**, 733–749.

Whiteley AR, Spruell P, Allendorf FW (2004) Ecological and life history characteristics predict population genetic divergence of two salmonids in the same landscape. *Molecular Ecology*, **13**, 3675–3688.

Wilson CC, Hebert PDN (1998) Phylogeography and postglacial dispersal of lake trout (*Salvelinus namaycush*) in North America. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 1010–1024.

This work was part of Andrew Whiteley's dissertation research at the University of Montana. Andrew is currently a Postdoctoral Researcher at Université Laval in Québec, Canada. His research interests include the areas of ecology, conservation, and evolution. Paul Spruell is a Research Assistant Professor at the University of Montana interested in combining ecological and genetic data to better understand aquatic systems. Fred Allendorf is a Professor at the University of Montana whose interests span the areas of conservation and evolution.
