The influence of Wisconsinan glaciation and contemporary stream hydrology on microsatellite DNA variation in rainbow trout (*Oncorhynchus mykiss*)

P. Tamkee, E. Parkinson, and E.B. Taylor

Abstract: Microsatellite DNA variation was examined in rainbow trout (*Oncorhynchus mykiss*) populations from throughout British Columbia, Canada, to address the roles of historical isolation, postglacial dispersal, and contemporary geomorphology in structuring genetic variation and differentiation. We detected signatures of historical isolation and postglacial recolonization in the form of "interior" and "coastal" population groupings, a decline in genetic variation as distance increased from putative glacial refugia, and different extents of isolation-by-distance in different regions. Rainbow trout populations were structured genetically into major regions and into smaller watersheds and then into drainages. Within drainages, high levels of dispersal and gene flow were inferred between geographically proximate and contiguous lakes. Elevation, stream branching points (nodes), fluvial distance, migration barriers, and stream and lake order influenced genetic diversity within, and differentiation among, populations. Habitat characteristics, particularly lake surface area and perimeter, were poor predictors of genetic variation. Although founder events and postglacial dispersal influenced broadscale patterns of genetic diversity in rainbow trout, our results suggest that contemporary factors can strongly modulate historical patterns.

Résumé : L'étude de la variation de l'ADN des microsatellites chez des populations de truites arc-en-ciel (*Oncorhynchus mykiss*) dans l'ensemble de la Colombie-Britannique nous permet de déterminer les rôles de l'isolement passé, de la dispersion postglaciaire et de la géomorphologie contemporaine sur la structuration et la différenciation de la variation génétique. Nous décelons des signatures de l'isolement passé et de la recolonisation postglaciaire sous forme de groupements de populations « intérieures » et « côtières », de déclin de la variation génétique en fonction de la distance croissante des refuges glaciaires putatifs et d'isolements par la distance d'importances diverses dans les différentes régions. Les populations de truites arc-en-ciel se structurent génétiquement en régions majeures, puis en réseaux hydrographiques plus petits et finalement en bassins de drainage. Dans les bassins, on concluon un haut niveau de dispersion et de flux génique entre les lacs contigus et rapprochés géographiquement. L'altitude, les points (nœuds) de branchement des cours d'eau, la distance fluviale, les barrières à la migration et l'ordre des cours d'eau et des lacs influencent la diversité génétique au sein des populations et la différenciation entre les populations. Les caractéristiques de l'habitat, particulièrement la superficie et le périmètre des lacs, sont de mauvaises variables prédictives de la variation génétique. Bien que les événements du fondateur et la dispersion postglaciaire influencent les patrons de diversité génétique à grande échelle chez la truite arc-en-ciel, nos résultats indiquent que les facteurs actuels peuvent fortement moduler les patrons du passé.

[Traduit par la Rédaction]

Introduction

Understanding the influences of environmental and geographical factors, whether they be historical or contemporary in nature, on the distribution of genetic variation in populations is an important aspect of evolutionary and conservation biology (e.g., Burridge et al. 2008; Guy et al. 2008). Genetic diversity within species often reflects processes that occurred during historical times (e.g., Hewitt 1996; Lees et al. 1996; Bernatchez and Wilson 1998) as well as processes and environmental changes over more contemporary timescales (e.g., Banks et al. 2005). For example, glaciation and postglacial recolonization have markedly shaped the distribution of species and their attributes (e.g., McPhail and Lindsey 1986; Hewitt 1996; Wilson and Hebert 1998). Across small spatial scales, the influence of geography is also likely to

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impact a species' ability to colonize habitats, extend its distribution, and consequently influence the degree of intraspecific variability. Phylogeographic analyses can help assess the extent to which large-scale historical events have influenced contemporary genetic and geographic structure of species (e.g., Bernatchez and Wilson 1998; Avise 2000; Harris and Taylor 2010). Contemporary environments and landscape features, however, may also influence gene flow, demographic processes, and selection, which could influence the expression of historical signatures of genetic structure (Templeton et al. 1995; Sork et al. 1999; Costello et al. 2003). Studies of landscape genetics (Manel et al. 2003; Storfer et al. 2007) investigate how landscape features influence processes such as gene flow, genetic drift, and selection and their effect on contemporary genetic variability. Landscape genetics and phylogeography are similar in that they both try to understand the factors that shape patterns of genetic diversity, yet each tends to focus on different spatial and temporal scales. Although ongoing research investigates patterns of genetic diversity on large and small geographic scales, there have been relatively few studies that have simultaneously investigated genetic variability both at different geographic and temporal scales (Angers et al. 1999; Castric et al. 2001; Costello et al. 2003).

Salmonid fishes (salmon, trout, char, grayling, and whitefish) depend on freshwater for dispersal for all or part of their life cycle. The spatial complexity of habitats that they occupy coupled with life history features that promote isolation among local populations, such as natal stream philopatry ("homing"), minimize the potential for gene flow to constrain genetic changes caused by genetic drift and natural selection (Meffe and Vrijenhoek 1988; Riginos and Nachman 2001). Consequently, salmonids display a rich variety of geographic variability (e.g., Taylor 1991; Hendry et al. 2004). *Oncorhynchus mykiss* is native to the North Pacific Ocean and occurs in a variety of life history types, including an anadromous (sea-run) form known as steelhead and a nonanadromous freshwater resident form known as rainbow trout. The native distribution of the species encompasses the coastal drainages of North America in the Pacific Ocean basin from Alaska to Mexico largely west of the continental divide in North America and from the Sea of Okhotsk to the Kamchatka Peninsula in the western Pacific Ocean (Behnke 1992). For both life history types, opportunities for migration are influenced by patterns of hydrographic networks, particularly for the freshwater-resident rainbow trout (e.g., Currens et al. 1990; Narum et al. 2008).

The last glacial event in North America influenced the distribution of freshwater fishes and, therefore, also played a significant role in the shaping of intraspecific genetic diversity (McPhail and Lindsey 1986; Bernatchez and Wilson 1998). As a consequence of the glacial history of *O. mykiss* that involved repeated cycles of isolation, divergence, and recolonization as well as their current occupancy of subdivided and diverse aquatic habitats (lakes, streams, and marine environments), this species is an excellent model organism to assess the influences of historical and contemporary geographic factors that influence genetic diversity (cf. Keeley et al. 2007). Indeed, several genetic analyses conducted on a macrogeographic scale suggest that a major subdivision exists among *O. mykiss* populations between those located on the east and the west sides of the Cascade-Coast mountains (allozymes: Okazaki 1984; Parkinson 1984; minisatellite DNA: Taylor 1995; mitochondrial DNA: McCusker et al. 2000). McCusker et al.'s (2000) study also suggested that there were perhaps three putative glacial refugia (Queen Charlotte Islands, South Coast, and Interior) from which *O. mykiss* later colonized British Columbia, Canada, after the last glacial retreat. Therefore, rainbow trout and steelhead have likely expanded their range postglacially from the south and west of the Wisconsinan glaciers and dispersed north and east into British Columbia (McCusker et al. 2000). In contrast with this broad study of the phylogeographic history of *O. mykiss* across its range, except for a few specific watersheds (e.g., Narum et al. 2008), comparatively little information exists on the role of contemporary hydrology in shaping genetic diversity in this widespread species.

In this study, our broad goal was to build on our understanding of the processes influencing genetic structure within salmonid fishes by investigating how history and contemporary hydrology both might contribute to patterns of genetic diversity. First, we revisited the phylogeographic history of rainbow trout and investigated the influence of historical isolation and postglacial recolonization on genetic (microsatellite DNA) diversity in *O. mykiss* in British Columbia, an area spanning a major portion of the species' range. Next, we focussed on the role of contemporary geographic features in influencing patterns of genetic diversity in *O. mykiss* and, in particular, those that influence connectivity and accessibility of dispersal corridors (Castella et al. 2000; Gerlach and Musolf 2000; Costello et al. 2003). To achieve these ends, we tested three major ideas. First, we tested whether clines in genetic diversity are likely to occur as a result of postglacial dispersal in *O. mykiss* (cf. Sage and Wolff 1986; Turgeon and Bernatchez 2001; Costello et al. 2003). We predicted that a genetic signature of postglacial dispersal from refugia would occur in the form of a progressive decline in genetic diversity with increasing distance from putative refugia. Second, the relative strength of isolation-by-distance (IBD) may differ between regions due to the differences in time since colonization and the degree to which dispersal is inhibited within regions (cf. Hutchison and Templeton 1999). Recently founded populations may not have had sufficient time to reach equilibrium such that regional IBD is not apparent (McCauley 1993; but see Crispo and Hendry 2005). Given the different proximity of current populations to putative refugia, we predicted that populations closest to their putative refuge would be closer to IBD equilibrium than those with more peripheral distributions (recently colonized). Third, we gathered information on watershed characteristics, habitat area, and the presence of impassable migration barriers to test if there were any associations between these aspects of the physical environment and genetic diversity of populations inhabiting these areas. We predicted that watershed characteristics that reduce connectivity would promote genetic differentiation among localities and that variation in features that might influence effective population sizes (such as habitat area) would be associated with differences in genetic variation within localities.

Materials and methods

Sampling design and localities

During 1999–2002, a total of 2867 *O. mykiss* tissue samples were collected from 69 localities throughout British Columbia (Fig. 1; Supplementary Table S1[2]). Most of our samples consisted of adult or subadult fish (i.e., >20 cm total length) in some cases supplemented with smaller fish (10–20 cm total length); none of our samples consisted of fish less than 10 cm long or less than 1 year in age. Sample sizes ranged from 14 to 60 with all but six localities having at least 30 fish sampled. We sampled populations from throughout British Columbia to test for genetic signatures of postglacial dispersal and that varied in the extent of their connectivity with each other to address the influence of contemporary geography on genetic diversity. We sampled only populations that have had no history of artificial supplementation, and samples came from lake-, stream-, freshwater-resident and anadromous populations. Of the total, 50 localities (2141 tissue samples) represented interconnected habitats within watersheds and are hereafter referred to as "population chains". We studied seven such population chains from tributaries of the upper Columbia, Thompson, and upper Fraser rivers' drainages (Fig. 1; Supplementary Table S2[2]). All localities from the upper Columbia River population chain, upper Fraser River lake chain, and the Thompson River chain were sampled across comparable within-drainage pairwise fluvial distances: 0.8–130, 3.6–165, and 10–144 km, respectively. There was greater variability in elevation among the sample sites in the upper Columbia River chain (128–338 m), upper Fraser River chain (304–464 m), and Thompson River watershed (92–484 m). All fish samples were collected by electroshocking, gillnetting, angling, or seining. Tissue was collected and stored in 95% ethanol until DNA could be isolated from approximately 5 mg of tissue using the PureGene DNA isolation kit (Gentra).

Microsatellite DNA analysis

Ten microsatellite loci were assayed in paired polymerase chain reactions (PCRs) (i.e., "diplexes") as follows: *Oneu14* and *Ssa197*, *Oneu8* and *Ssa85*, *Ssa456* and *Omy77*, *Ots3* and *Okia3*, and *Ots100* and *Ots103* (Supplementary Table S3[2]). PCRs for each microsatellite diplex were carried out with ^{32}P-labeled primers in 10 μL volumes containing 100 ng of DNA template, 10× reaction buffer (Gibco/BRL), 0.4 mmol/L dNTP, 0.5 μmol/L reverse (both) primers, 0.25 μmol/L forward-labeled (both) primers, 1.5 mmol/L $MgCl_2$, and 0.5 unit of *Taq* polymerase. Each PCR diplex profile consisted of 5× 95 °C/1 min, T_a/1 min, and 72 °C/1 min, 30× 94 °C/1 min, T_a/1 min, and 72 °C/1 min, and 1× 72 °C/5min, where T_a is the annealing temperature(s). Prior to running the PCR products on a 7% polyacrylamide gel, 7 μL of loading buffer was added to the PCR product and denatured for 5–15 min before 5 μL of PCR product mix was loaded onto a 7% polyacrylamide gel in 1.2× Tris–Borate–EDTA buffer. To determine accurate measurements of alleles, an M13 mp19 control DNA sequencing ladder was electrophoresed with all of the samples and the alleles were scored by eye. All gels contained two to four "standard" fish to ensure consistency of scoring.

Genetic data analysis

The following tests were performed using GENEPOP version 3.4 (updated from Raymond and Rousset 1995). Tests for deviations from Hardy–Weinberg equilibrium were performed for each locus–population combination. Tests for genotypic linkage disequilibrium were performed for all combinations of locus pairs within populations. Tests for population differentiation between all pairs of populations were performed over all loci combined using log-likelihood (G) based exact tests (Goudet et al. 1996). All critical significance levels for simultaneous tests were evaluated at a table-wide α level of 0.05 using sequential Bonferroni adjustment (Rice 1989). We calculated an estimate of F_{ST} from Weir and Cockerham's (1984) θ using FSTAT version 2.9.3.2 (Goudet 2001). We also calculated R_{ST}, incorporating allelic size differences, using SPAGeDi version 1.2 (Hardy and Vekemans 2002) and tested for significant differences between F_{ST} and R_{ST} using the allele size permutation test of Hardy et al. (2003). Rejection of the null hypothesis of $F_{ST} = R_{ST}$ is considered evidence that mutation, in addition to genetic drift, plays a role in generating the allelic frequency differences observed (Hardy et al. 2003).

Microsatellite data for all sample sites within a watershed, e.g., for each population chain, were analyzed using the program STRUCTURE (Pritchard et al. 2000), which employs a Bayesian model-based clustering method for inferring population structure from genotypic data and estimates K candidate source populations. We tested the probability of K = 1–12, 1–10, 1–8, 1–7, 1–5, 1–4, and 1–4 candidate source populations (final numbers were chosen as the upper limit, as it represented the number of sampling sites within each specific population chain) within the Columbia River, Deadman River, lower north Thompson River, Glatheli Lake, Blanchet Lake, and both Nutli and Skinny Lake chains, respectively. The admixture with correlated allele frequencies model was used with a burn-in of 25 000 iterations followed by 75 000 replications of the Monte Carlo Markov Chain procedure (Pritchard et al. 2000).

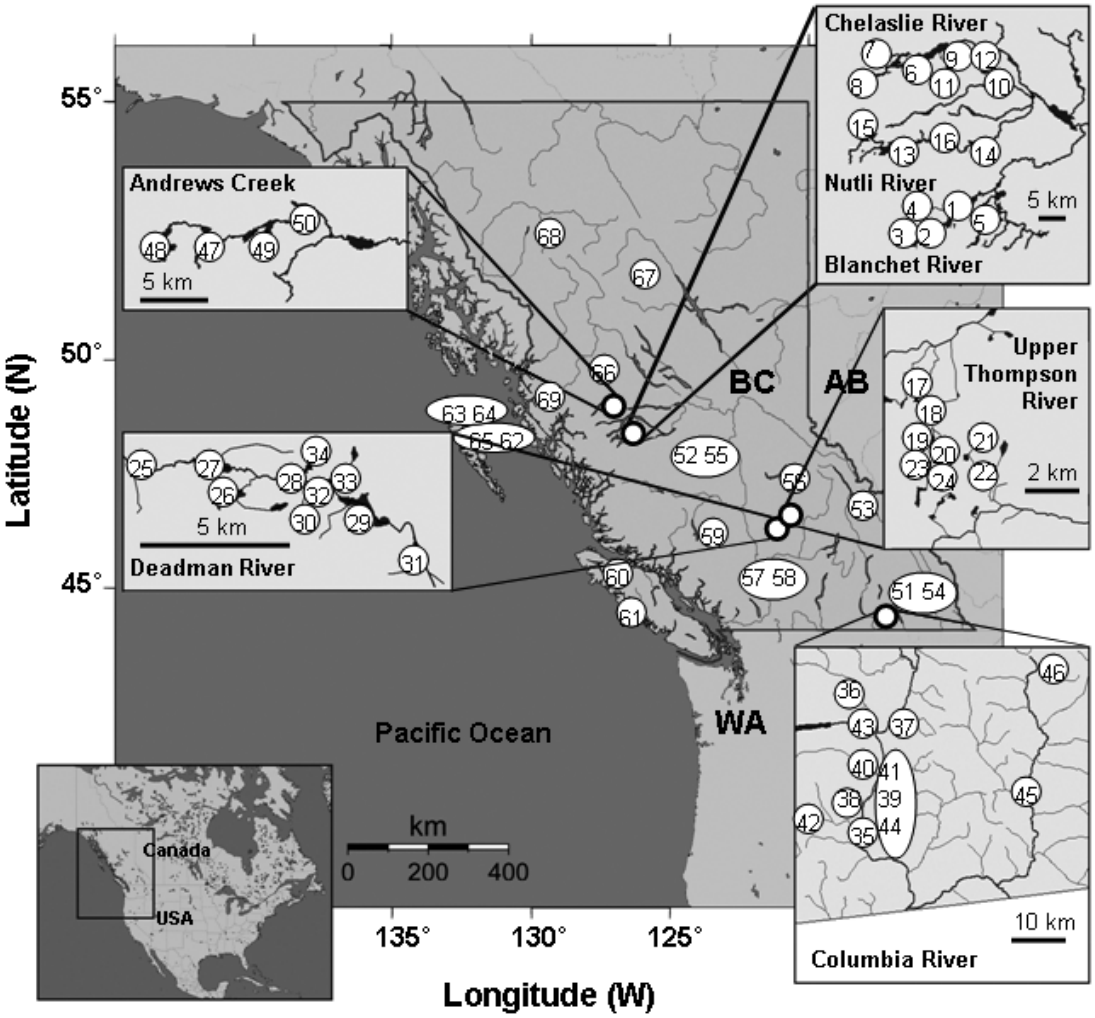
To visualize the genetic relationships among localities, the Cavalli-Sforza and Edwards (1967) chord distance was calculated and an unrooted neighbour-joining (NJ) tree was built using the PHYLIP software package (Felsenstein 1995). A principal components analysis (PCA) was also conducted on allele frequency data using PCA-GEN (Goudet 1999) to summarize genetic differentiation among all samples other than through the use of a bifurcating tree. The PCA summarized all of the variation across the 10 loci (171 alleles) and orients samples along major axes of allele frequency variation (Pimentel 1979).

Historical impacts on contemporary genetic diversity

We tested for clines in genetic diversity outwards from the two major putative glacial refugia inferred for rainbow trout, the Columbian and coastal refugia (McCusker et al. 2000), by first grouping our samples into coastal (i.e., lo-

[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca).

Fig. 1. Map of rainbow trout (*Oncorhynchus mykiss*) localities examined in the study. Sample sites are identified by the circled numbers (sites 38, 42, and 46 are above migration barriers). 1, Blanchet Lake; 2, Blanchet 2 Lake; 3, Blanchet 3 Lake; 4, Grizzly Lake; 5, Tlutlias Lake; 6, Glatheli Lake; 7, Unnamed 1 Lake; 8, Michel Lake; 9, Unamed 2 Lake; 10, Theleteban Lake; 11, Unamed 3 Lake; 12, Ghitzeli Lake; 13, Fenton Lake; 14, Nutli Lake; 15, Goodrich Lake; 16, Morgan Lake; 17, 01157LNTH Lake; 18, 01166LNTH Lake; 19, 01179LNTH Lake; 20, 01184LNTH Lake; 21, 01176LNTH Lake; 22, 01189LNTH Lake; 23, 01193LNTH Lake; 24, 01201LNTH Lake; 25, 00376DEAD Lake; 26, 00422DEAD Lake; 27, 00357DEAD Lake; 28, 00409DEAD Lake; 29, 00439DEAD Lake; 30, 00447DEAD Lake; 31, 00466DEAD Lake; 32, 00416DEAD Lake; 33, 00410DEAD Lake; 34, 00369DEAD Lake; 35, lower Murphy Creek; 36, lower Norns Creek; 37, Kootenay River; 38, upper Sullivan Creek; 39, China Creek; 40, lower Blueberry Creek; 41, Sand Bar Eddy; 42, upper Murphy Creek; 43, Norns Creek fan; 44, Columbia River at Genelle; 45, Salmo River; 46, Clearwater Creek; 47, Skinny Lake; 48, Twinkle Lake; 49, Needle Lake; 50, Horseshoe Lake; 51, Fry Creek; 52, Blackwater River; 53, Kinbasket Reservoir; 54, Lardeau River; 55, Kuyakuz Lake; 56, Clearwater River; 57, Murray Creek; 58, Coldwater River; 59, Fish Lake; 60, Nimpkish River; 61, Gold River; 62, Cooper Creek; 63, Mamin River; 64, Yakoun River; 65, Riley Creek; 66, Canyon Creek; 67, Moosevale Creek; 68, Ealue Lake; 69, Khtada Lake. Inset at the bottom left depicts the study area within North America; all others depict population chains.



cated on coastal islands or draining directly to the ocean west of the Coast Mountain Range) or interior (i.e., located east of the Coast Mountain Range) lineages (Supplementary Table S1[2]). Within each lineage, populations were organized by latitude, longitude, and straight-line geographic distance from the geographic centre of these putative glacial refugia and we tested for correlations between these geographic variables and measures of microsatellite DNA variation (expected heterozygosity (H_E), number of alleles per locus (A), and allelic richness (A_R)) using the JMPin software package version 3.2.1 (Sall and Lehman 1996). We also tested if the coastal and interior groupings accounted for a significant component of the total variation in allele frequencies using an analysis of molecular variance (AMOVA) in ARLEQUIN (Excoffier et al. 2006). Within each of the putative refugial groups, the significance of correlations between geographic distance (fluvial distance) and genetic distance (F_{ST}) was tested to determine if the observed genetic structure could be explained by an IBD model (Wright 1943; Slatkin 1993) and to determine whether populations had reached drift–migration equilibrium using the approach of Hutchison and Templeton (1999) independently for all population chains. Fluvial distances between sample localities were determined using the Geographic Information System program ArcView

version 3.1 (ESRI, Redlands, California) and genetic and geographic distances were compared using the Mantel test (Mantel 1967) option in the software program R-Package version 4.0 (Casgrain and Legendre 2001). Partial Mantel tests were also conducted to examine how each of absolute elevation, numbers of nodes, and geographic distance individually influenced genetic differentiation among localities, i.e., effects of one variable on genetic differentiation were examined while holding the effects of the other two variables constant. The absence of any pattern of IBD suggests that the species is far from equilibrium and may have only recently invaded the area that it now occupies (Slatkin 1993; Hutchison and Templeton 1999). Subsequent to a significant Mantel test result between genetic and geographic distances (significant IBD), a second Mantel test was performed using residuals from the initial fitted line (calculated using JMPin) against geographic distance; at equilibrium, the scatter of the residuals should increase with greater geographic separation as drift supersedes gene flow at larger distances (Hutchison and Templeton 1999).

Contemporary environmental variables and genetic diversity

To assess the influence of contemporary geographic drainage pattern on genetic structure, we conducted a further AMOVA on divisions into major regions (coastal, upper Fraser River, upper Columbia River, and Thompson River). We also tested the extent to which the distribution of genetic variation was explained by the separation into above- and below-barrier populations from the same locality.

A variety of geographical variables at watershed- and locality-specific scales were examined to determine if contemporary physical environment was related to observed patterns of genetic diversity. These variables included fluvial and geographic distance among sites, number of stream nodes (i.e., branching points between streams), absolute elevation, migration barriers, and measurements of habitat size: lake perimeter, lake surface area, and connectivity (stream and lake order). Nodes were located at each directional turn as one traced a path (as a dispersing fish might) moving from the root (starting point) to each locality. The significance of correlations between particular contemporary geographic features and genetic diversity (A, A_R, and H_E) and distance (F_{ST}) was also tested by using partial Mantel tests. The effect of geographic distance on genetic structure was also assessed using results from the assignment tests. First, GENECLASS version 5.1 (Cornuet et al. 1999) was used to assess the accuracy with which individual fish could be classified to their known sample population to provide another measure of the distinctiveness of populations. We employed the likelihood-based method based on their composite 10 locus microsatellite genotypes. The "leave one out" option was used to avoid biasing likelihoods and assignment tests were calculated with a Bayesian method (Rannala and Mountain 1997) using a simulation procedure with 10 000 randomly generated genotypes. Rainbow trout with less than a 5% probability of belonging to their sampled population were not assigned to that locality. To be conservative, the few individuals that were assayed at fewer than seven loci were also not used (Bernatchez and Duchesne 2000). The proportion of "misassigned" genotypes at each site was then compared with the geographic distance between sites. Castric and Bernatchez (2004) found that in brook trout (*Salvelinus fontinalis*), this approach was a more sensitive one for detecting IBD in postglacial populations.

Results

Genetic structure and subsequent pooling of localities

Ten out of a possible 690 (10 loci × 69 populations) tests showed statistically significant deviations from Hardy–Weinberg equilibrium and all were heterozygote deficits. These deviations were found at several separate loci in 10 different populations. Tests for linkage disequilibrium resulted in significant departures in four out of a possible 3150 tests. The statistically significant results were not concentrated on particular locus pairs or within specific populations (Supplementary Table S4[2]).

There were 2346 pairwise comparisons made between populations for differences in allele frequencies summed across all 10 loci and only 60 of these comparisons were not statistically significant after adjusting for multiple comparisons. Nonsignificant comparisons occurred only between localities within population chains, i.e., Grizzly Lake and Blanchet Lake (Blanchet Lake chain); China Creek and Kootenay River, Sand Bar Eddy and lower Norns Creek, and Kootenay River and Genelle (upper Columbia River chain); Twinkle Lake and Needle Lake (Skinny Lake chain); 11 pairwise comparisons in the lower north Thompson River lake chain, 10 pairwise comparisons in the Glatheli Lake chain, and 34 pairwise comparisons in the Deadman Lake chain. These general results of the G tests were similar to those for pairwise F_{ST} (Tamkee 2005). Both the F_{ST} (0.18) and R_{ST} (0.13) calculated across all localities were significantly greater than zero (both $p < 0.001$), but they were not significantly different from one another ($p > 0.1$).

The STRUCTURE analyses indicated that the most likely number of genetic populations (K) given our data set for the upper Fraser River population chains, Thompson River population chains, and Columbia River population chain were 10, 3, and 10, respectively (Supplementary Table S5[2]). Accordingly, sample localities within interconnected lake chains (Fig. 1) were pooled as a single population according to the results from STRUCTURE, which resulted in a total of 42 genetically distinct populations being used in subsequent analyses, including the 19 other nonchain localities (out of the total of 69 localities sampled). The populations that were pooled were generally those that also showed no significant genetic differentiation by G tests (see above) (Supplementary Table S4[2]). Following pooling of localities, virtually all were in Hardy–Weinberg equilibrium with 19 out of a possible 420 (10 loci × 42 sample sites) tests showing statistically significant heterozygote deficits (Supplementary Table S6[2]). These exceptions were not concentrated within particular populations or at particular loci and there was no evidence of locus-specific artifacts such as null alleles or population-specific factors such as inbreeding. Tests for linkage disequilibrium resulted in significant departures in five out of a possible 1953 tests. The statistically significant results were not concentrated on particular locus pairs or within specific localities. The number of alleles observed across 10 loci and 2867 individuals from

the 42 genetic populations ranged from two (*Ssa197*) to 38 (*Oki3a*) with an average of 17.1 alleles per locus (Supplementary Table S6[2]). Observed heterozygosity averaged 0.42 across all loci and localities and ranged from 0.42 (*Ots103*) to 0.93 (*Okia3*). Genetic variation within sample sites ranged widely; expected heterozygosity, averaged across the 10 loci, ranged from a low of 0.05 (Clearwater Creek) to highs of 0.62–0.68 (Gold River, China Creek, Kootenay River, and lower Murphy Creek).

Historical influences on genetic diversity and divergence

Among sample sites located in the interior lineage (e.g., populations east of the Coast Mountains), there was a decrease in genetic variation with an increase with latitude (all $p \leq 0.05$) and an increase in genetic variation with an increase in longitude (H_E, $p = 0.02$; A_R, $p = 0.03$; A, $p = 0.06$) (Supplementary Table S7[2]). The relationships of straight-line geographic distance between each sample locality and the most southern locality (assumed to be closest to the heart of the glacial refuge in the Columbia River south of the ice sheet) to A, A_R, and H_E were also statistically significant within the interior lineage ($r = 0.26$ and $p = 0.003$, $r = -0.26$ and $p = 0.003$, and $r = -0.25$ and $p = 0.003$, respectively). Among coastal lineage populations, there were general trends of decreasing genetic variation with increasing straight-line geographic distance eastwards, increasing latitude, and decreasing longitude from the presumed centre of the glacial refuge (northeast tip of Queen Charlotte Islands); however, these results were not statistically significant (Supplementary Table S7[2]).

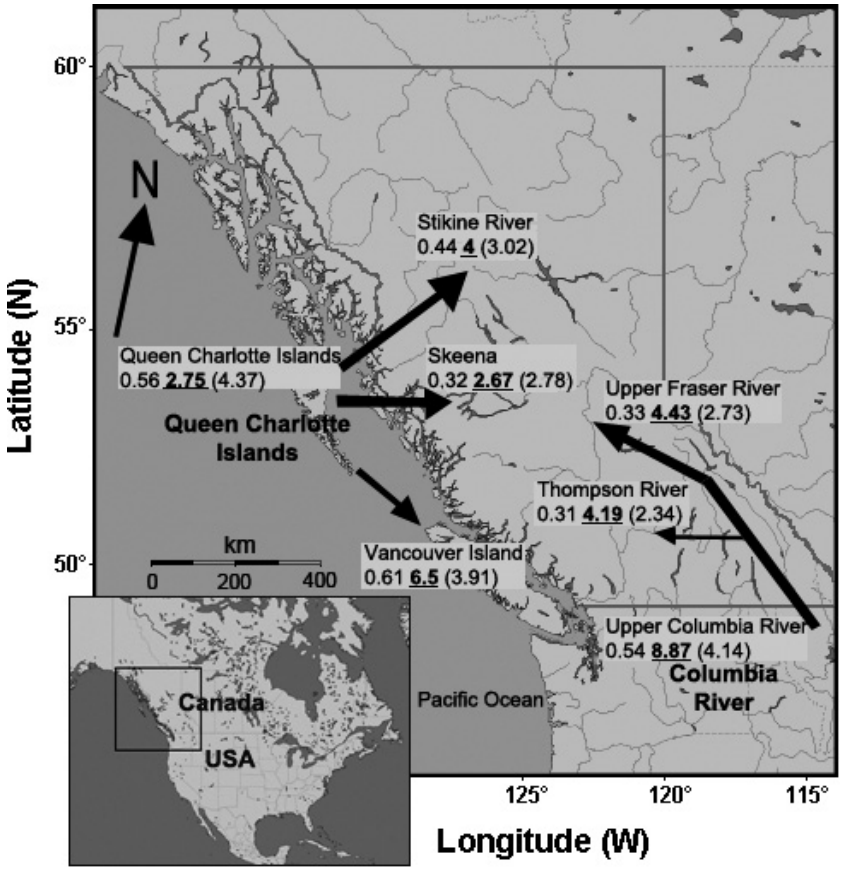
When populations within the interior lineage were grouped by watersheds and compared, those that are closest to the south (e.g., closest to glacial refuge) exhibited higher levels of genetic variation compared with watersheds at the periphery of the interior lineage (Fig. 2). For example, populations from the Columbia River watershed had significantly higher levels of allelic richness and gene diversity than populations from the Thompson River and upper Fraser River watersheds ($p = 0.03$ and $p = 0.005$ and $p = 0.001$ and $p = 0.003$, respectively). Among the coastal lineage, populations from the Queen Charlotte Islands and Vancouver Island contained higher levels of A_R ($p = 0.03$ and $p = 0.03$, respectively) and gene diversity ($p = 0.04$ and $p = 0.05$, respectively) compared with Skeena River populations. Comparisons between Vancouver Island populations and Queen Charlotte Islands populations, however, demonstrated no significant differences.

Projection of populations in PC space (Fig. 3) suggested substantial differentiation among four major sets of populations: most samples from the upper Fraser River drainage, other interior-drainage populations from the Columbia River drainage, a heterogeneous group of populations from the lower North Thompson and Deadman rivers' lake chains located in the Thompson River drainage system, and all coastal populations (Nimpkish River, Gold River, Mamin River, Riley Creek, Copper Creek, Yakoun River, Canyon Creek, Moosevale Creek, Ealue Lake, and Khtada Lake). Above-barrier populations (e.g., upper Sullivan Creek, upper Murphy Creek, and Clearwater Creek) were highly distinct both among themselves and from all other populations (Fig. 3).

Populations hypothesized to have originated from the interior lineage (e.g., east of the Coast Mountains) did not form a single unique distinct cluster in the NJ tree (Fig. 4). The NJ tree did, however, reveal interior population subclusters with high bootstrap values at the drainage level (e.g., Columbia River subcluster, upper Fraser River subcluster, and Thompson River subcluster) (Fig. 4). In addition, populations hypothesized to have originated from a coastal refuge clustered together and included the Queen Charlotte Island cluster of populations (Mamin River, Riley Creek, Yakoun River, and Copper River), Vancouver Island populations (Gold River and Nimpkish River), and populations located in northwestern British Columbia (Ealue Lake, Moosevale Creek, and Canyon Creek) (Fig. 4). Most populations clustered together by drainages and then into the expected coast (e.g., Stikine River drainage, Skeena River drainage, Queen Charlotte Islands, and Vancouver Island) or interior group (Columbia River drainage, Thompson River drainage, and upper Fraser River drainage). By contrast, Khtada Lake was expected to group with the coastal populations but clustered with the interior populations such as Fry Creek (upper Columbia River drainage) and Coldwater River (Thompson River drainage) (Fig. 4). Among the interior populations, there were a few populations that grouped with populations from drainages other than their own, e.g., the grouping of Fish Lake (upper Fraser River) with Murray Creek and Coldwater River (Thompson River drainage) or the grouping of Clearwater River (Thompson River drainage) with Blackwater River (upper Fraser River drainage).

Hierarchical analysis of the distribution of genetic diversity (AMOVA) did not provide strong support for a single major "coastal"–"interior" bifurcation; comparatively little microsatellite variation was found between these two phylogeographic regions across the study area (Table 1*a*). AMOVA results based on grouping populations into regions representing two putative refugia, coastal populations (Skeena River watershed, Stikine River watershed, Queen Charlotte Islands, and Vancouver Island) and interior populations (all remaining populations), accounted for a small, but significant proportion of the total variation (5.4%, $p < 0.01$). An even smaller proportion of the total variation (4.3%, $p = 0.05$) was attributable to major geographic area when imposing a third hypothesized refuge: north coast (Stikine River, Skeena River, and Queen Charlotte Island samples), south coast (Vancouver Island samples), and interior origin (upper Fraser River, Thompson River, and upper Columbia River samples). By contrast, the AMOVA revealed that the grouping of populations that explained the greatest amount of variation across the sampling area was the comparison between the four groups of populations identified from PCA at 19% ($p < 0.01$): a group of coastal populations and three groups of interior populations (Fig. 4; Table 1*a*). The comparison between major watersheds (Stikine River, Skeena River, Queen Charlotte Island, Vancouver Island, upper Fraser River, Thompson River, and upper Columbia River) resolved 18.9% of the total variation ($p < 0.001$). At the subregional scale, there was no significant variability among watersheds within the coastal group ($p > 0.1$) (Table 1*b*) or within the upper Columbia River groups of populations ($p > 0.1$). By contrast, there was significant subregional differentiation within the upper Fraser River and

Fig. 2. Geographic distribution of genetic variation at microsatellite loci among populations of rainbow trout (*Oncorhynchus mykiss*) from major watersheds. Expected heterozygosity, mean number of alleles per locus (in bold and underlined), and allelic richness (in parentheses) are shown. Arrows show inferred postglacial dispersal routes from glacial refugia, each of which is labeled in the largest bold font. Inset at the bottom left depicts the study area within North America.



Thompson River population groups (both $p < 0.01$) (Table 1*b*) and a comparison between below and above migration barrier samples within watersheds resolved 17.8% of the total variation ($p < 0.01$).

There were no fixed allele differences that were associated with putative glacial refugial groups of *O. mykiss* in British Columbia. There were a small number of alleles, occurring at a frequency of at least 5%, that were unique in both interior and coastal populations. In the coastal assemblage of populations, there was one unique allele at *Ssa85* and one at *Omy77*; in the interior assemblage, there was one unique allele at *Oneu8*, two at *Ots100*, and two at *Okia3*, all of which were found in the Columbia River drainage populations, whereas at least two were absent from the Fraser River and Thompson River areas (Tamkee 2005).

Contemporary habitat size, elevation, and genetic diversity

Measures of habitat size characteristics across all 42 localities, particularly lake area and perimeter, showed many weak positive, but nonsignificant, relationships with H_E and A (lake area: $r = 0.45$ and $p = 0.06$ and $r = 0.33$ and $p = 0.18$, respectively; lake perimeter: $r = 0.42$ and $p = 0.08$ and $r = 0.35$ and $p = 0.16$, respectively) (Table 2). By contrast, A_R showed significant positive relationships with habitat area and perimeter ($r = 0.54$ and $p = 0.02$ and $r = 0.53$ and $p = 0.02$, respectively). Stream and lake order demonstrated strong, positive significant relationships with A and A_R ($r = 0.32$ and $p = 0.04$ and $r = 0.32$ and $p = 0.04$, respectively) but not with H_E, although the trend was in the same direction ($r = 0.28$ and $p = 0.07$). Elevation had a strong impact on all measures of genetic diversity; among all watersheds, higher elevation localities had lower variation (Table 3). When the Columbia watershed localities were grouped into those residing above migration barriers ($N = 3$) and those below barriers ($N = 10$), permutation tests demonstrated that above-barrier localities had significantly lower A_R (2.3 versus 4.6, respectively) and H_E (0.37 versus 0.61, respectively) but significantly greater pairwise F_{ST} (0.46 versus 0.08, respectively) than localities below upstream migration barriers (all $p < 0.05$).

IBD and geographic features

Oncorhynchus mykiss displayed a pattern of IBD throughout the total range of interior and coastal areas that we sampled as well as within the interior lineage ($r = 0.23$ and $p = 0.001$ and $r = 0.62$ and $p = 0.0001$, respectively); however, no significant pattern of IBD was found among the coastal lineage populations when they were examined separately ($r = 0.06$ and $p = 0.4$) (Supplementary data Fig. S1[2]).

Geographic features had variable effects on genetic differentiation across the different watersheds. Among the Fraser

Fig. 3. Results of PCA of allele frequency variation among localities of rainbow trout (*Oncorhynchus mykiss*) assayed at 10 microsatellite loci for the reduced sample set of 42 populations depicted as plots of mean component scores for each population along axes 1 and 2. Ellipses enclose samples from major watersheds. Locality.: 1, Blanchet Lake; 2, Blanchet 2 Lake; 3, Blanchet 3 Lake; 4 , Tlutlias Lake; 5, Khtada Lake; 6, Fry Creek; 7, Nimpkish River; 8, Gold River; 9, Blackwater River; 10, Clearwater Creek; 11, Salmo River; 12, Kinbasket Reservoir; 13, Lardeau River; 14, Glatheli Lake; 15, Ghitzeli Lake; 16, Goodrich Lake; 17, Morgan Lake; 18, 01179 LNTH Lake; 19, 01189 LNTH Lake; 20, 00466 DEAD Lake; 21, Murray Creek; 22, Kuyakuz Lake; 23, Coldwater River; 24, Clearwater River; 25, Fish Lake; 26, lower Murphy Creek; 27, upper Sullivan Creek; 28, China Creek; 29, lower Blueberry Creek; 30, lower Norns Creek; 31, upper Murphy Creek; 32, Norns Creek fan; 33, Kootenay River; 34, Copper Creek; 35, Mamin River; 36, Yakoun River; 37, Riley Creek; 38, Canyon Creek; 39, Moosevale Creek; 40, Ealue Lake; 41, Twinkle Lake; 42, Horseshoe Lake.

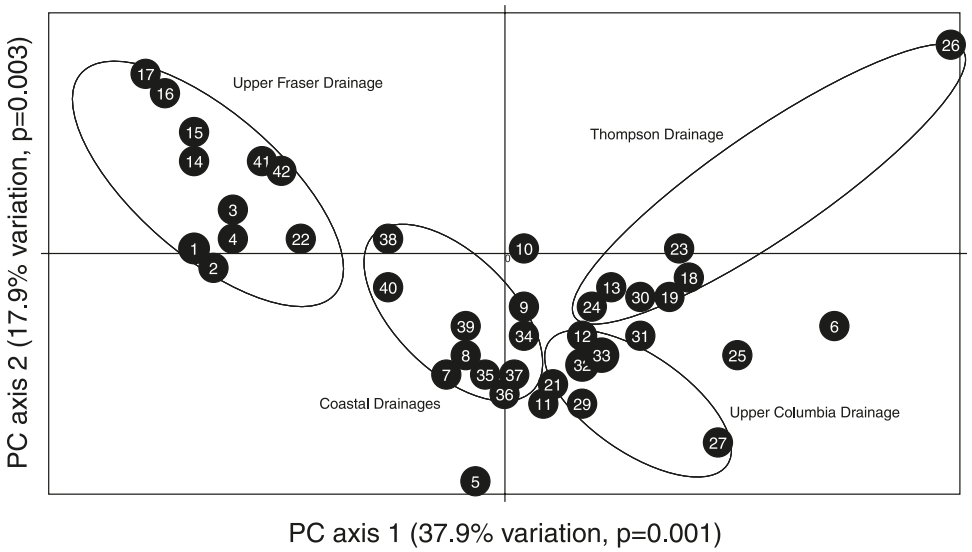
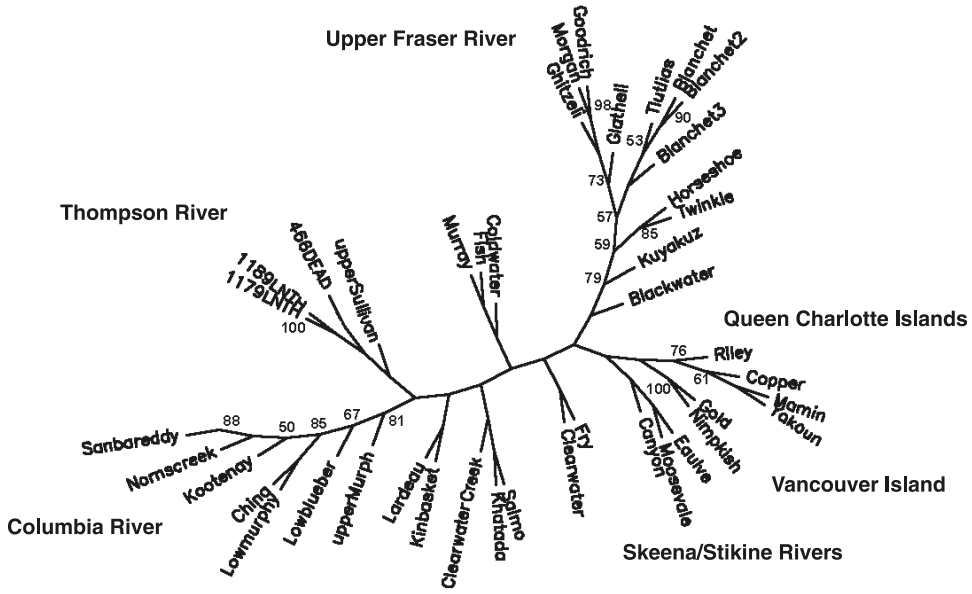


Fig. 4. Neighbor-joining tree of relationships among populations of rainbow trout (*Oncorhynchus mykiss*) in British Columbia from the reduced data set of 42 populations. Clustering was based on Cavalli-Sforza and Edwards' (1967) chord distances derived from allelic variation at 10 microsatellite loci. Numbers at branch points represent bootstrap percentages from 1000 replicates (only those values ≥50% are shown). Locality names follow those in Fig. 3 except for the following: Lowmurphy, lower Murphy Creek; Lowblueber, lower Blueberry Creek; upperMurph, upper Murphy Creek; Nornscreek, Norns Creek fan.



River lake chain localities, geographic distance was not significantly related to genetic differentiation (F_{ST}) after controlling for the effects of elevation and the number of nodes between streams (Table 4). Both elevation and presence of nodes, however, did impact the genetic structure of Fraser River lake chain localities after controlling for geographic distance (Table 4). Within the Columbia River chain localities, IBD was significant even after controlling for the effects of elevation and nodes (Table 4). Elevation also played an important role in shaping the genetic structure of upper Columbia River populations, but it was not as influential as geographic distance. When above-barrier localities

Table 1. Hierarchical analysis of the distribution of genetic diversity in rainbow trout (*Oncorhynchus mykiss*) populations under various (*a*) regional and (*b*) subregional grouping (V_{bg}) hypotheses.

Comparison	V_{bg}	V_{ap}	V_{wp}	p
(*a*) Regional				
North coast vs. south coast vs. interior	4.28	35.63	60.09	0.0430
Coast vs. interior	5.41	35.07	59.52	0.0088
Among watersheds	18.87	21.94	59.19	0.0001
Among PCA groups	19.10	21.86	59.05	0.0001
(*b*) Subregional				
Coastal	0.30	23.90	75.80	0.5083
Upper Fraser	26.40	21.60	52.00	<0.0001
Thompson	50.00	1.40	48.60	0.0049
Upper Columbia	2.10	9.50	88.40	0.1750
Upper Columbia without barrier populations	4.4	3.7	91.8	0.0528

Note: Regional groupings: north coast (Stikine River, Skeena River, and Queen Charlotte Islands), south coast (Vancouver Island), and interior (Thompson River, upper Fraser River, and upper Columbia River). Watershed groupings: Columbia River (upper Columbia River, mid-Columbia River, Salmo River, and Duncan River), Thompson River (Clearwater River, upper Thompson River, Bonaparte River, Nicola River, and mid-Thompson River), upper Fraser River (Nechako River, Blackwater River, and Chilcotin River), Queen Charlotte Islands, Vancouver Island, Skeena River, and Stikine River. PCA groups refer to those population groups shown in Fig. 3. Subregional analysis population groups: Queen Charlotte Islands, Vancouver Island, Skeena River, Stikine River, upper Fraser River, Thompson River, Columbia River, and component populations. Probability values for all observed variation among populations within groups (V_{ap}) and within populations (V_{wp}) were <0.001.

were removed ($N = 3$), there was a stronger relationship between geographic and genetic distance, but the presence of nodes and elevation had limited effects on genetic differentiation (Table 4). Across all six Thompson River watershed localities, there was no signature of IBD; however, elevation was found to be an important variable in shaping population structure (Table 4).

Among the upper Fraser River lake chains, 653 of 784 trout (83.3%) were assigned to the locality from which they were sampled (ranging from 62% to 96% of trout at each locality). Among Thompson River watershed localities, 506 of 551 trout (91.8%) were assigned to the locality from which they were sampled (ranging from 78.1% to 100% of trout at each locality). By contrast, among Columbia River localities, only 421 of 817 trout (51.5%) were assigned to the locality from which they were sampled (ranging from 22.4% to 100% of trout at each locality). Among coastal island populations (anadromous populations from the Queen Charlotte Island and Vancouver Island), 116 of 172 trout (67.4%) were assigned to the locality from which they were sampled (ranging from 40% to 80% of trout at each locality). Increasing fluvial distance separating localities was associated with lower levels of misassignment within the upper Fraser River lake chains ($r = –0.20$, $p = 0.06$), within the upper Columbia River chains ($r = –0.46$, $p < 0.0001$), among all coastal populations ($r = –0.64$, $p = 0.003$), and among all interior populations ($r = –0.32$, $p < 0.0001$).

Discussion

Understanding how historical events and contemporary factors shape intraspecific diversity are important aspects of evolutionary and conservation genetics (Bowen 1999; Guy et al. 2008; Scandura et al. 2008). Postglacial colonization events have markedly shaped the contemporary distribution of genetic variation in nature (e.g., see reviews by Bernatchez and Wilson 1998; Hewitt 2000). For example, two general results that emerge from phylogeographic analyses are identification of the location of specific glacial refugia (e.g., Stewart and Lister 2001; Latch et al. 2009) and a reduction in variation within populations distant from such refugia that was presumably the result of bottlenecks and founder events associated with dispersal into previously unoccupied areas (e.g., Sage and Wolff 1986; Bernatchez and Wilson 1998). McCusker et al. (2000) addressed the possible effects of glaciation and postglacial recolonization on contemporary mitochondrial DNA polymorphism in rainbow trout throughout the North Pacific basin. Their results suggested that there were at least two glacial refugia (Queen Charlotte Islands (“coastal”) and the lower Columbia River (“interior”)) that supported rainbow trout that subsequently colonized previously ice-covered areas. Our study extended these and earlier results by examining variation in microsatellite DNA and the extent to which these historical events may have been influenced by more recent events driven by local landscape features.

Zoogeography of microsatellite DNA

Our results detected genetic signatures of postglacial colonization and provided further support for the geographic origins of coastal and interior lineages of rainbow trout within British Columbia. We demonstrated distinct groupings of coastal and interior populations (and watershed groupings within these regions) as well as the presence of unique alleles within each of the lineages. Turgeon and Bernatchez (2001) documented distinct assemblages of microsatellite alleles as well as clinal variation in allele frequencies in a cisco (*Coregonus artedi*) from different areas of North America. Their data supported the existence of two glacial

Table 2. Relationship between habitat size and genetic variation in rainbow trout (*Oncorhynchus mykiss*). Genetic variation variables include average number of alleles, expected heterozygosity, observed heterozygosity, and allelic richness.

	Average number of alleles		Expected heterozygosity		Observed heterozygosity		Allelic richness	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Lake surface area (ha)	0.28	0.07	0.30	0.04	0.28	0.06	0.30	0.05
Perimeter (km)	0.30	0.05	0.32	0.04	0.30	0.06	0.32	0.04
Lake surface area (ha) log transformed	0.40	0.008	0.24	0.12	0.22	0.04	0.36	0.11
Perimeter (km) log transformed	0.37	0.02	0.22	0.16	0.39	0.18	0.14	0.18
Stream and lake order	0.48	<0.0001	0.40	0.0008	0.37	0.002	0.44	0.0002

Note: Habitat size variables include lake surface area, perimeter, and stream and lake order.

lineages of cisco and they were able to infer the direction of postglacial dispersal and areas of secondary contact between lineages. We detected, however, no noticeable clines in frequency of unique alleles in rainbow trout lineages, which may reflect the relatively small geographic distances involved. The maximum distance between localities within the coastal and interior lineages of rainbow trout was 1000 and 900 km, respectively, whereas the study on cisco spanned 3500 km across northern North America (Turgeon and Bernatchez 2001). Despite our evidence in support of coastal and interior lineages, AMOVA indicated that only a small fraction of the genetic variation observed could be explained by these geographic groupings. The time span of the most recent glaciation (perhaps up to 100 000 years) may have been too short to result in the accumulation of substantial differences between groups as assayed by microsatellites relative to within-group variation, especially if mutation has been a negligible contributor to differentiation as our SPAGeDi suggested. Further, headwater transfer between interior-draining and coastal-draining watersheds during glacial retreat may have eroded some distinctions between putative lineages (cf. McPhail and Lindsey 1986; Taylor and Costello 2006; Burridge et al. 2008). Our results, however, are generally congruent with those found using allozymes, minisatellites, and morphology that all resolved coastal and interior groups in rainbow trout (e.g., Okazaki 1984; Behnke 1992; Taylor 1995) and other fishes (e.g., Wehrhahn and Powell 1987; Taylor and Costello 2006) and taxa (see review by Brunsfeld et al. 2001) across the same general area of northwestern North America.

The existence of these areas as glacial refugia for rainbow trout was also supported by declines in genetic variation (H_E, A, and A_R) in samples more distant from the putative Columbian refuge. Rainbow trout probably colonized the Thompson and upper Fraser rivers via connections with the Columbia River (McPhail and Lindsey 1986; Behnke 1992) and the Thompson and upper Fraser River populations lacked some of the rare alleles that were present in more southern localities (e.g., upper Columbia River). Similarly, bull trout (*Salvelinus confluentus*) from northern British Columbia had lower levels of genetic variation and tended to possess a subset of alleles present in populations residing in areas closer to southern refugia (Costello et al. 2003). Finally, we documented stronger IBD nearest the presumed interior glacial refugium and lack of IBD in areas more distant (Hutchison and Templeton 1999; Costello et al. 2003). Populations at the periphery of the interior lineage chains (upper Fraser River and Thompson River populations) did not show any significant pattern of IBD. By contrast, the upper Columbia River population chain showed significant IBD. The upper Columbia River is currently closer to descendants of refugial populations of rainbow trout in the Columbia drainage south of the ice sheet and during deglaciation was recolonized directly from these source populations (McPhail and Lindsey 1986). The upper Fraser River (including the Thompson River), however, is not only more distant from the Columbian refuge but was colonized indirectly via transfer through glacial lakes that flooded divides between the extant Fraser and Columbia rivers (McPhail and Lindsey 1986; cf. Costello et al. 2003 for bull trout). These differences in historical connectivity probably resulted in later re-

Table 3. Relationship between measures of genetic variation and elevation in rainbow trout (*Oncorhynchus mykiss*) after statistically controlling for the effects of geographic distance.

	Allelic richness		Average number of alleles per locus		Expected heterozygosity	
	r	*p*	*r*	*p*	*r*	*p*
All populations	–0.67	<0.0001	–0.71	<0.0001	–0.66	<0.0001
Upper Fraser River lake chains	–0.65	0.002	–0.84	<0.0001	–0.57	0.01
Upper Columbia River chains	–0.81	0.002	–0.81	0.001	–0.73	0.01
Thompson River lake chains	–0.45	0.06	–0.45	0.07	–0.4	0.1

Note: The effects of elevation were conducted within each lake and stream chain of the three major watersheds (upper Fraser, upper Columbia River, and Thompson River).

colonization and perhaps more disruptions in gene flow between populations relative to genetic drift and contribute to lack of equilibrium in these more distant areas of the Fraser River (Hutchison and Templeton 1999).

The northeastern corner of Graham Island (Queen Charlotte Islands) is considered to be one of two areas in British Columbia that were ice-free during the last glaciation; the other area often cited is northwestern Vancouver Island (e.g., Moodie and Reimchen 1976; Warner et al. 1982; Byun et al. 1997). In rainbow trout, high mitochondrial DNA diversity was found among Queen Charlotte Islands populations compared with other populations in British Columbia consistent with this area as a glacial refuge (McCusker et al. 2000). Our results provide some limited support for the idea that the Queen Charlotte Islands, or at least that a more general coastal area, acted as a glacial refuge. A coastal group was evident from the NJ tree and the PCA of allele frequencies. Furthermore, some coastal populations that were more than 450 km from the Queen Charlotte Islands grouped with the other coastal populations despite the fact that they were geographically more proximate to some interior populations located east across the Coast Mountains (e.g., Ealue and Moosevale creeks).

By contrast, a genetic signature of postglacial colonization was not clearly evident within the coastal group of *O. mykiss*, and no significant correlations were found between straight-line geographic distance from the putative refugium and measures of genetic variation. Two of the coastal populations that had very high levels of genetic variation and low levels of genetic differentiation from other coastal populations and that were farthest away from the presumed heart of the coastal refuge (Queen Charlotte Islands) were the sea-run (anadromous) steelhead from the Gold and Nimpkish rivers. The greater potential for dispersal among populations of sea-run trout in the coastal group may explain the lack of relationship between measures of genetic variation and latitude, longitude, and geographic distance. Fish species limited to dispersal by river and streams often demonstrate high genetic population subdivision compared with marine species where greater connectivity resulting in the exchange of individuals (gene flow) could promote reduced levels of genetic differentiation and population subdivision but also maintain relatively higher levels of intrapopulation variation (Ward et al. 1994; DeWoody and Avise 2000). Microsatellite-based assays of anadromous rainbow trout show F_{ST} values that are consistently low, ranging from 0.01 to 0.07, and may reflect the greater connectivity of steelhead promoted by their anadromous behaviour (Reisenbichler and Phelps 1989; see also Wenburg et al. 1998 for coastal cutthroat trout (*Oncorhynchus clarkii*) and Bouza et al. 1999 for anadromous brown trout (*Salmo trutta*)). The potential for ongoing gene flow between the Queen Charlotte Islands populations and other nearby populations including those on Vancouver Island may, therefore, make detection of historical processes like postglacial colonization difficult. Alternatively, it is possible that our samples of coastal fish, especially those on Vancouver Island, were influenced by postglacial dispersal from another coastal refuge located south of the ice sheet in Washington or Oregon. Beacham et al. (2004), for instance, noted closer affinities between Vancouver Island steelhead and fish from coastal Washington and the lower Columbia River than compared with steelhead from central and north coastal British Columbia.

Contemporary influences on genetic diversity within populations

Notwithstanding the existence of broad genetic groups of populations that tend to cluster geographically, the majority of genetic variation at microsatellite loci is typically found within salmonid populations (75%: Nielsen and Fountain 1999; 96%: Beacham et al. 2000; 96%: Heath et al. 2001). Consequently, it is at the within-population level that great potential exists to investigate how geomorphology influences genetic variation. Theory and some evidence in mammals and fish suggest that a positive relationship exists between population size and genetic variation in a variety of species (Frankham 1996; Bouza et al. 1999; Heath et al. 2001). It is often difficult to assess true population size, particularly for aquatic species such as fish; however, habitat size can act as an approximate proxy for relative population size (e.g., Angers et al. 1999; Castric et al. 2001; Heath et al. 2001). For instance, in brown trout (Jorde and Ryman 1996) and brook trout (Angers et al. 1999; Castric et al. 2001), lake size was unrelated to the level of genetic variation among populations. Heath et al. (2001), however, found that habitat area used by fish less than 1 year of age was related to the level of genetic variation in steelhead. Our data provide some support for a relationship between habitat size and genetic variation because the average number of alleles, allelic richness, and expected heterozygosity were all found to be positively related to lake size, although only the effect of lake size on allelic richness was statistically significant. These modest relationships may, however, reflect the idea that population size may not be strongly related to overall habitat size, such as we measured, but rather to habitat area during specific life history

Table 4. Correlations between genetic differentiation (θ) and fluvial distance, elevation, and presence of stream nodes among population chain sample sites in rainbow trout (*Oncorhynchus mykiss*) in the major drainages sampled (upper Fraser River, Thompson River, and upper Columbia River).

	Gen vs. Geo cont Elev		Gen vs. Nodes cont Elev		Gen vs. Elev cont Geo		Gen vs. Nodes cont Geo		Gen vs. Elev cont Nodes		Gen vs. Geo cont Nodes	
	Mantel *r*	*p*	Mantel *r*	*p*	Mantel *r*	*p*	Mantel *r*	*p*	Mantel *r*	*p*	Mantel *r*	*p*
Fraser River chains	0.200	0.100	0.370	0.003	0.290	0.004	0.370	0.002	0.225	0.013	0.096	0.240
Columbia River chains	0.650	0.020	0.030	0.450	0.350	0.046	–0.130	0.270	0.480	0.030	0.710	0.020
Columbia River chains without barrier populations	0.876	0.002	0.380	0.036	–0.030	0.560	–0.007	0.480	0.870	0.060	0.920	0.007
Thompson River chains	–0.46	0.078	–0.19	0.24	0.67	0.21	0	0.5	0.58	0.019	–0.2	0.3

Note: Correlation analyses were conducted between genetic differentiation (Gen) and elevation (Elev), fluvial distance (Geo), and presence of nodes (Nodes) before and after controlling (cont) for the effects of Geo, Nodes, and Elev, respectively.

stages such as spawning or fry feeding habitats (Jorde and Ryman 1996; Heath et al. 2001). Further, the level of intrapopulation genetic variation observed is likely related to the level of connectivity between populations because we found a stronger relationship between all measures of genetic variation and stream order, a measure of connectivity. Shaw et al. (1991, 1994) found that the levels of polymorphism and heterozygosity were positively correlated with stream order in Trinidadian guppies (*Poecilia*). The greater genetic variation within lowland populations inhabiting larger-order streams may be explained by the greater amount of potential gene flow into them because lowland streams can receive immigrants from upstream reaches and tributaries (of lower order) as well as from other lowland streams. Likewise, contiguous populations often display lower levels of population subdivision, especially when they are interconnected by large river systems (Beacham et al. 2000; Hendry et al. 2002). The results of our assignment tests are consistent with these ideas because larger-order streams that we assayed contained a higher number of misassigned individuals compared with smaller-order and upstream localities. Elevation, however, is consistently associated with genetic variation, as observed in our study, although its effects are likely not independent from the effects of stream order and connectivity. Similar results have been observed in an alpine snail (*Arianta arbustorum*) (Arter 1990), Trinidadian guppies (Shaw et al. 1991), and rainbow trout within single drainages (Narum et al. 2008).

Contemporary influences on genetic differentiation among populations

The strong correlation between genetic distance and geographical distance among the Columbia River localities suggests that population differentiation may, in some cases, be largely governed by IBD. Perhaps, the lack of IBD in the upper Fraser River chain and Thompson River system results from strong gene flow across the relatively small spatial scale studied and presence of migration barriers as well as their more recent population founding (e.g., Slatkin 1993; Hansen and Mensberg 1998; Crispo and Hendry 2005). Populations above migration barriers are, of course, physically isolated from downstream populations and they may experience greater genetic drift from smaller effective population sizes. Migration barriers typically are more frequent in lower-order streams associated with the high-elevation and smaller habitat area of narrow river valleys that are the sources of streams especially in montane areas. These smaller habitat areas would be expected to support lower population abundances (and presumably lower effective population sizes). By contrast, populations below migration barriers may show reduced levels of differentiation owing to greater connectivity and gene flow with neighbouring populations (Slatkin 1985; Hughes et al. 1996; Riginos and Nachman 2001), including downstream movement of fish over barriers (Shaw et al. 1991, 1994; Marshall et al. 1992). The position of a migration barrier within a watershed is an important factor in influencing both levels of variation and divergence within systems (Guy et al. 2008). For instance, not all rainbow trout populations located above migration barriers exhibited low genetic variation and high genetic differentiation from each other. Within the upper Fraser River drain-

age, the Blanchet Lake chain is located above a migration barrier (E. Parkinson, unpublished data), but the lakes within the chain are interconnected among themselves. Costello et al. (2003) found that while most above-barrier bull trout populations show reduced genetic variation and were often monomorphic at several loci, those above-barrier populations in larger watersheds retained higher levels of genetic variation. Similarly, mainstream populations of rainbow trout populations from watersheds in portions of the US range exhibit high levels of genetic variation, whereas populations located above migration barriers but interconnected above the barrier with other populations exhibit intermediate levels of genetic variation and isolated headwater populations show the lowest levels of variation (Knudsen et al. 2002; Narum et al. 2008). Our AMOVA results also supported the influence of migration barriers in structuring genetic differentiation and this appears to be a general phenomenon in stream fishes (e.g., Shaw et al. 1991; Carlsson and Nilsson 2001; Costello et al. 2003).

In addition to the presence of migration barriers, the number of stream nodes and elevation both explained significant amounts of variation in differentiation among populations within the upper Fraser River drainage. By contrast, within the Thompson River watershed, elevation was the only variable that was found to help shape genetic structure. Johnson and Black (1995) found significant IBD in regions where habitat was continuous and no IBD in regions that were highly fragmented. These observations suggest the importance of landscape features instead of, or in addition to, geographic distance in explaining spatial patterns of genetic divergence among populations.

More generally, our data suggest that the structure of drainage networks influences interpopulation connectivity and, hence, patterns of diversity within populations and divergence among populations (Meffe and Vrijenhoek 1988). Ultimately, the extent of genetic variation and divergence in neutral traits is influenced by effective population size and interlocality dispersal. The more dispersal that occurs, the more likely it is that individuals from nearby populations contribute genetic material to the pool of diversity. By contrast, environmental variables that help promote isolation (nodes, barriers, fluvial distance, and elevation) are those that are likely to reduce effective population size and, consequently, result in the reduction of intrapopulation variation while promoting interpopulation differentiation. The common thread through these ideas is the influence of environmental variables on connectivity, and the level of genetic variation and divergence is strongly influenced by the level of both historical and contemporary connectivity among localities (Poissant et al. 2005; Segelbacher et al. 2003; Turgeon and Bernatchez 2001).

Synthesis and implications

Our study showed that genetic traces of postglacial colonization from refugia were resolved using microsatellite data, particularly in the interior lineage of *O. mykiss*. At the largest spatial (across British Columbia) and temporal scale (Pleistocene glaciations), our results illustrate the importance of considering the role of history in explaining patterns of genetic variation and differentiation in contemporary populations, which, in some cases, can persist as the dominant factor influencing these genetic parameters (e.g., Poissant et al. 2005). Layered on top of this template of population history is the influence of contemporary hydrology; at smaller physical and shorter time scales, our results illustrate the potential for contemporary hydrological features to modulate historical patterns of population diversity and subdivision (cf. Hurwood and Hughes 1998; Hébert et al. 2000; Guy et al. 2008). Consequently, it is important to recognize that historical and contemporary processes are not strictly independent. The histories of a lineage and its habitats are intertwined and their interactions result in patterns of genetic variation and differentiation that will be influenced by subsequent and contemporary changes in habitat structure, connectivity, and population demography. For instance, the glacial history imposed on a lineage might result in genetic divergence of that lineage and its founding of populations above migration barriers. Such historical effects will be subject to contemporary influences on population demography (population size changes, migration, and environmental fluctuation) that together can influence levels of variation within, and differentiation between, populations (e.g., Johnson and Stinchcombe 2007).

Our results have important implications for conservation of rainbow trout and, more generally, for freshwater fishes living within hierarchically arranged stream and lake habitats. Genetic diversity in *O. mykiss* is nested: major differences exist among regions and among drainages within regions and differences within drainages are variable and often depend on the localized geographic matrix of topography across which populations are located. Biodiversity managers should appreciate this hierarchical nature of genetic diversity within rainbow trout in conservation plans for the species. At the most local scale, our results highlight the often highly interconnected nature of apparently discrete habitats (i.e., adjacent lakes interconnected via streams) and that single genetic populations of fish may use multiple habitats. Consequently, fisheries habitat managers should account for such "dispersal corridors" in much the same way as wildlife managers do for terrestrial vertebrates (Rosenberg et al. 1997; Hale et al. 2001). Notwithstanding these implications for stream salmonid conservation, microsatellite variation is only one aspect of biodiversity. Phenotypic variation, much of it genetically based and likely the result of local selective environments, is abundant in rainbow trout (Keeley et al. 2005, 2007) and distinctiveness in these aspects of trout biodiversity can be integrated with molecular biodiversity for a comprehensive biodiversity plan for *O. mykiss* and for species with similar histories and contemporary biology (Taylor et al. 2010).

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