Conserv Genet (2010) 11:1421–1433
DOI 10.1007/s10592-009-9969-y

RESEARCH ARTICLE

Genetic clustering methods reveal bull trout (*Salvelinus confluentus*) fine-scale population structure as a spatially nested hierarchy

Will G. Warnock · Joseph B. Rasmussen · Eric B. Taylor

Received: 4 February 2009 / Accepted: 27 July 2009 / Published online: 15 August 2009

Abstract Many conservation genetics studies in fishes define populations based on capture location. In salmonid fishes, this traditional a priori designation is made by spawning stream, with subsequent post hoc approaches used to define units of conservation. In this study of bull trout from southwestern Alberta, we provide evidence that a model-based Bayesian genetic clustering method may provide a more parsimonious alternative to designating population structure and units of conservation in comparison to traditional methods. The clustering method captured a hierarchical model of population structure, in which seven local populations were nested within three genetic archipelagos. This was in contrast to using simple F_{ST} based approaches between thirteen a priori designated populations, which found significant differences for nearly every pairwise comparison. In addition, assignment tests results from Bayesian clustering revealed that movement may be common between sampling locations. These clustering methods are easy to use, intuitive and provide substantial information on populations of fish; this study provides an example of their utility for local fisheries management and conservation.

Keywords Salmonid · Population structure · Genetic clustering · Units of conservation · Migration · Bull trout

Introduction

Stream-spawning salmonid fishes have long been known to display fine-scaled spatial population structure in stream networks, largely due to the strong homing tendencies of these organisms (Taylor 1991). In its most extreme manifestation, this population structure should lead to genetically distinguishable clusters of individuals around spawning locations (Wenburg et al. 1998). If such population structure existed, it would be entirely justified to define populations a priori, by site or stream-of-origin. Although populations of salmonid fishes are commonly defined in this way, such a paradigm hinges on two implicit assumptions: that each stream-of-origin represents a genetically distinguishable unit, and that each individual sampled at that site was born there and is not an immigrant from another site. If either of these assumptions is not met, it makes little sense to designate "genetic" populations according to where individuals were captured; moreover, it seems likely that both are likely to fail often in real stream systems.

An alternate approach to population identification that would not be affected by these issues would be to designate populations genetically, using model-based genetic clustering methods (Pritchard et al. 2000; Dawson and Belkhir 2001; Corander et al. 2004; Chen et al. 2007). Such methods may define populations statistically, on the basis of individual genotypes, regardless of capture location (Waples and Gaggiotti 2006). STRUCTURE (Pritchard et al. 2000) is the most common program used for genetic clustering. This program is based on Bayesian clustering methods,

Electronic supplementary material The online version of this article (doi:10.1007/s10592-009-9969-y) contains supplementary material, which is available to authorized users.

W. G. Warnock (✉) · J. B. Rasmussen
Department of Biological Sciences, University of Lethbridge, 4401 University Dr. W., Lethbridge, AB T1K3M4, Canada
e-mail: will.warnock@uleth.ca

E. B. Taylor
Department of Zoology, Biodiversity Research Centre and Native Fishes Research Group, University of British Columbia, 6270 University Blvd., Vancouver, BC V6T1Z4, Canada

probabilistically assigning individuals to K clusters (number of populations) in such a way as to minimize linkage disequilibrium (LD) and departures from Hardy–Weinberg equilibrium (HWE) at the n loci in each cluster identified. In this way, the program searches for the most probable structure in the dataset, assuming that all populations fit the equilibrium assumptions of the model. Multiple K values are tested, and log-likelihood values for each K value tested are returned, allowing the investigator to ascertain the most likely number of clusters within the dataset and therefore, population structure. This approach may yield a model of spatial population structure even if there is movement of individuals among sampling locations (Berry et al. 2004). Along these lines, assignment test results are provided for each individual's genotype in the dataset, and may be combined a posteriori with spatial data to provide information about movements among identified populations (Berry et al. 2004). Assignment tests based on this approach may therefore be powerful tools in answering a wide range of conservation and ecological questions (Manel et al. 2005).

An additional advantage of the clustering approach used in STRUCTURE is the ability to detect hierarchical population structure in accordance with a hierarchical island model of gene flow (Slatkin and Voelm 1991) that seems most appropriate for stream habitats. The analysis is sequential, with genetic "archipelagos" first differentiated from one another, followed by further population substructuring detected within each archipelago, and so forth (Evanno et al. 2005). Following this approach, population structure can be revealed at many levels within the study area, and may provide an intuitive guide for a meaningful conservation approach (Whiteley et al. 2006a).

The bull trout (*Salvelinus confluentus*) is a migratory inland salmonid that is a common model species in conservation genetics literature due to their fine-scale population structure and high conservation concern (Spruell et al. 1999). In theoretical and applied conservation genetics studies of this species, populations and units of conservation have always been defined by stream-of-origin, sometimes followed by post hoc distance-based clustering methods (Neraas and Spruell 2001; Costello et al. 2003; Spruell et al. 2003; Whiteley et al. 2006b; DeHaan and Arden 2007; Kassler and Mendel 2007); however, the results from these studies may have been influenced by flaws in an a priori designation based population structure. Firstly, the majority of representative samples used in these studies come from juvenile fish (0–3 years age), which are assumed to rear in the stream in which they were born. The assumption that juvenile fish remain in their stream-of-origin is consistent with a restricted movement paradigm (Gerking 1959; Rodriguez 2002); however, there is mounting evidence that has cast doubt on the ubiquity of this model, in both Pacific (Scrivener et al. 1994) and Atlantic (Erkinaro et al. 1997; Kennedy et al. 2002; Rasmussen et al. 2009) salmon and bull trout (Warnock 2008). Secondly, attempting to designate units of conservation based on pairwise-F_{ST} (index of population isolation) values is often problematic (Pearse and Crandall 2004), particularly for salmonids, which show a large range of F_{ST} values between sample locations due to variable intrapopulation heterozygosity (Hedrick 1999). In addition, where tributary populations are only composed of few breeders, family structure may also cause an overestimation of population divergence (Hansen et al. 1997). This is because putative populations may be the progeny of relatively few breeders, which may cause an upward bias in F_{ST} values, and go undetected by HWE departures. This phenomenon is known as the Allendorf–Phelps effect (Allendorf and Phelps 1981; Waples 1998), and may lead to misinterpretation of meaningful population structure for bull trout and other salmonids.

The objective of this study is to provide a hierarchical description of the population structure of bull trout in southwestern Alberta using genetic clustering methods, compared to a traditional stream-of-origin approach. Results from this objective may provide valuable information for a guided management strategy of bull trout and test the utility of the genetic clustering method as a tool in applied conservation genetics.

Materials and methods

Study area and sample collection

The study area consisted of the core bull trout range of southwestern Alberta, in western Canada (Fig. 1). To sample all possible bull trout populations in the area, all spawning streams that could possibly support substantial bull trout populations were sampled by backpack electrofishing. Such streams were selected on the basis of accessibility for bull trout migrants, previously observed spawning activity (Gerrand and Watmough 1998), and suitable physical stream characteristics such as size and gradient (Dunham and Rieman 1999). On the basis of those criteria, 21 streams were sampled, with 13 yielding juvenile bull trout numbers large enough to presumably support a self-sustaining population (>3 juvenile fish per 100 m). The Castle River sub-basin contained six streams: the Carbondale River (Cb-CR), Gardiner Creek (Ga-CR), Lost Creek (Lo-CR), the West (Wca-CR) and South (Sca-CR) Castle Rivers, and Mill Creek (Mi-CR). The upper Oldman River sub-basin contained seven streams: South (Sra-OR) and North (Nra-OR) and the main-stem Racehorse (Ra-OR) Creeks, Dutch Creek (Du-OR), Hidden Creek (Hi-OR), and the Lower (Lli-OR) and Upper (Uli-OR) Livingstone Rivers (Fig. 1). The latter

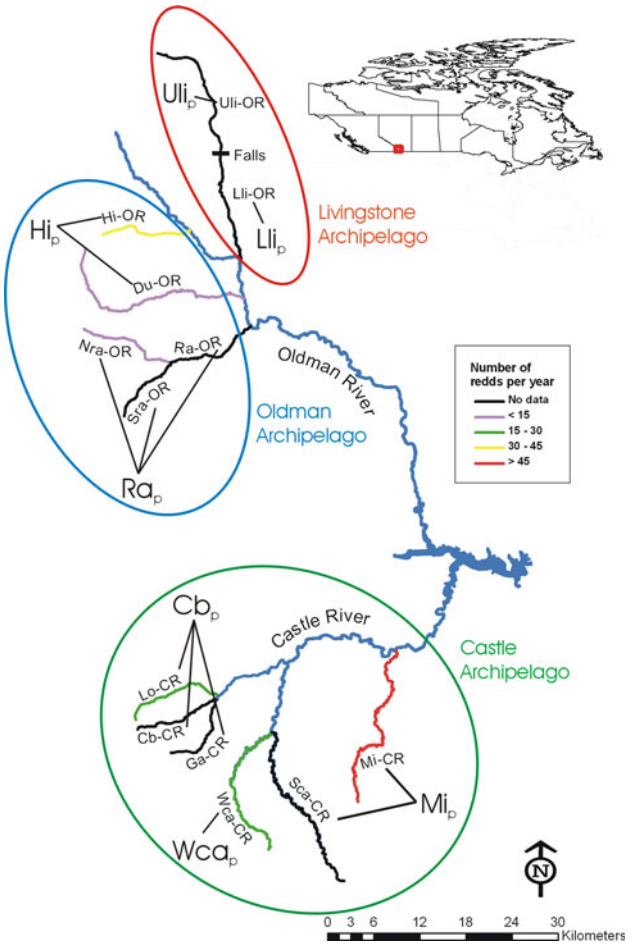

Fig. 1 Study area and 13 streams of origin for bull trout in southwest Alberta, Canada. Populations found by STRUCTURE are geographically represented by the streams from which the majority of the samples originate. *Ellipses* encompass the major genetic archipelagos found at the coarsest hierarchical level, as discerned by both STRUCTURE and individual-based PCA. Tributary stream shades represent different population densities, which are measured by the mean number of redds observed per year over a 3-year spawning survey (Gerrand and Watmough 1998)

two streams were separated on the basis of a seasonally passable set of falls, which may reduce gene flow between and lead to divergence of the two sites.

In 2006 and 2007, multiple sites within streams were sampled in the summer months until a target of 30 fish per stream was caught (364 individuals, Table 1). Each fish was weighed, measured and an adipose fin clip was taken and stored in 99% ethanol. In addition, each fish >20 mm FL had scales taken for aging to determine if it was an adult resident or migrant. These streams are used mainly by migrant populations of bull trout, are primarily used for spawning by adults and as nurseries for juveniles (McPhail and Baxter 1996), and sampling efforts did not coincide with spawning times; therefore, juvenile fish made up the bulk of the sample size for each collection.

DNA extraction, amplification and microsatellite genotyping

DNA was extracted from adipose fins using a QIAGEN DNeasy™ tissue kit following the recommended procedure in the manual.

All samples were screened for nine microsatellite loci, chosen based on clarity of resolution, degree of polymorphism and ability to distinguish bull X brook trout hybrids: *Sco102, Sco216, Smm22, Sfo18, Sco105, Sco106, Sco215, Sco220* and *Omm1128* (See Costello et al. 2003; DeHaan and Ardren 2005 for details). PCRs were conducted with fluorescently labeled primers (for PCR procedure, see Costello et al. 2003). PCR products were then assayed on a Beckman-Coulter CEQ 8000 automated genotyper. The program MICROCHECKER was used to screen for genotyping errors in the raw data (Van Oosterhout et al. 2004). Any individuals displaying missing data for more than four of the nine loci were discarded from analysis.

Genetic data analysis

The program STRUCTURE 2.2 (Pritchard et al. 2000) was used to infer population structure by genetic clustering methods. The model parameters of admixture and correlated allele frequencies were used. These account for recent gene flow between populations and some flexibility in linkage disequilibrium within populations. Such default settings are most flexible for dealing with real biological phenomena, although more parameters may be added to the analysis if there is more prior knowledge of the study system (Pritchard et al. 2007).

Recently, Evanno et al. (2005) assessed the ability of STRUCTURE to detect population structure according to a hierarchical model. The authors found that the program only captured the major structure in the data, at the archipelago level; however, subsequent STRUCTURE analysis may be performed on each of the identified clusters to find further population structure within each archipelago (e.g., Vähä et al. 2007).This study used the "hierarchical STRUCTURE analysis" approach outlined by Vähä et al. (2007) to account for varying levels of population structuring within the drainage. A first round of STRUCTURE was conducted with the entire pooled dataset to find the true value of K. Values of the natural logarithm of the probability of each K value (ln Pr ($X|K$)) were estimated for $K = 1$ to $K = 13$. Because STRUCTURE only captures the major population structure within the sample (Evanno et al. 2005), the true value of K was assumed to correspond to major archipelagos within the drainage. All fish assigning to each archipelago were then extracted from the dataset and separately analysed in a second, and where appropriate, a third round of STRUCTURE analysis until no further structure could be detected.

Table 1 Summary of pertinent tests for stream-of-origin defined populations

Population	Cb-CR	Ga-CR	Lo-CR	Wca-CR	Sca-CR	Mi-CR	Ra-OR	Sra-OR	Nra-OR	Du-OR	Hi-OR	Lli-OR	Uli-OR	Global
N	29	23	29	27	30	32	25	18	27	27	31	29	37	364
pw F_{ST}, Cb		*	*	*	*	*	*	*	*	*	*	*	*	NA
pw F_{ST}, Ga	0.0505		*	*	*	*	*	*	*	*	*	*	*	NA
pw F_{ST}, LO	0.0497	0.0742		*	*	*	*	*	*	*	*	*	*	NA
pw F_{ST}, Wca	0.114	0.0787	0.1051		*	*	*	*	*	*	*	*	*	NA
pw F_{ST}, Sca	0.0613	0.0484	0.0452	0.0672		*	*	*	*	*	*	*	*	NA
pw F_{ST}, Mi	0.1455	0.1327	0.0688	0.1686	0.0622		*	*	*	*	*	*	*	NA
pw F_{ST}, Ra	0.1962	0.1873	0.1627	0.2878	0.1646	0.1907		NS	NS	*	*	*	*	NA
pw F_{ST}, Sra	0.1869	0.1891	0.1645	0.2827	0.1612	0.193	−0.008		*	*	*	*	*	NA
pw F_{ST}, Nra	0.2398	0.2359	0.1883	0.3057	0.1907	0.2109	0.0559	0.0601		*	*	*	*	NA
pw F_{ST}, Du	0.0998	0.1357	0.0561	0.1729	0.1003	0.1349	0.1159	0.1146	0.1502		*	*	*	NA
pw F_{ST}, Hi	0.1232	0.1576	0.0842	0.2181	0.1304	0.1612	0.0909	0.1018	0.1527	0.0344		*	*	NA
pw F_{ST}, Lli	0.1674	0.1698	0.1145	0.2053	0.1685	0.2284	0.2625	0.275	0.3174	0.159	0.1547		NS	NA
pw F_{ST}, Uli	0.1775	0.1992	0.1142	0.232	0.1898	0.2396	0.2863	0.2968	0.3129	0.1577	0.1483	0.0419		NA
Mean allelic richness (A)	4.916	4.862	4.558	4.420	5.117	4.394	3.616	3.522	3.076	4.150	3.931	2.818	2.555	5.558
Total private alleles	1	1	1	2	3		1			3			1	12
H_e	0.563	0.524	0.560	0.470	0.593	0.547	0.455	0.454	0.411	0.517	0.483	0.456	0.409	0.496
F_{ST}														0.158
F_{IS}	0.012	−0.021	−0.056	0.089	0.038	0.037	0.045	−0.142	−0.039	0.011	0.031	0.082	0.066	
No. of loci	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Out of HWE?	No	No	No	1 locus	No	No	No	No	No	1 locus	No	No	No	Yes
Heterozygote deficiency?	No	No	No	1 locus	No	No	No	No	No	1 locus	No	1 locus	No	NA
Heterozygote excess?	No	No	No	No	No	No	No	No	No	No	No	No	No	NA
Linkage Disequilibrium?	No	No	No	No	No	No	No	No	No	No	No	No	1 pair	NA
Bottleneck? alpha <0.05	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	NA
Bottleneck? alpha <0.01	No	No	No	No	Yes	No	Yes	Yes	Yes	Yes	No	No	No	NA

* Indicates statistical significance at $P < 0.05$

Values of K were estimated by the ΔK method of Evanno et al. (2005). This method is an ad hoc statistic which aids the researcher in finding the optimal value of K across multiple iterations. Where ΔK failed to reveal a signal, the highest mean value of (ln Pr ($X|K$)) was used (Pritchard et al. 2007; Vähä et al. 2007).

A burn-in length of 100,000 with 100,000 MCMC repeats was sufficient to capture major structure in the data, but for subsequent rounds a burn-in length of 300,000–500,000 with 300,000–500,000 MCMC repeats was used due to higher success in estimating ΔK. All analyses were conducted with 10 iterations per K estimate. To visualize data, the run of highest value for (ln Pr ($X|K$)) was selected for the true K found.

Values of membership (q) were assessed for all individuals within each genetic cluster found by the program. Individuals were assigned to a population based on their highest q-value. To avoid assigning individuals to multiple populations, any individual found in the second round to show membership ($q > 0.75$) to a particular group that had displayed $q < 0.5$ in the previous round was discarded from analysis (Vähä et al. 2007). Proportion of individuals mis-assigned were determined by the numbers of fish assigned to a population by STRUCTURE that were caught in a geographical area distal to the streams of their respective population of origin, relative to the sample size of the population.

We ran a principle coordinate analysis (PCA) in GenAlEx6 (Peakall and Smouse 2006) on streams-of-origin to see if we could visualize the same basic population structure as identified by hierarchical STRUCTURE analysis. The first PCA was created from pairwise genetic differences among individuals found at all streams (Peakall et al. 1995), while the second was created from the matrix of pairwise F_{ST} values between streams.

Departures from HWE across loci and identified clusters and streams-of-origin were tested in the program GENEPOP 4.0 (Raymond and Rousset 1995) using Markov chain parameters of 10,000 dememorizations, 100 batches and 5,000 iterations per batch (Guo and Thompson 1992). P-values of tests were adjusted using sequential Bonferroni adjustment (Rice 1989) to test for significance. Where significance was encountered, GENEPOP was used to identify whether departures were due to heterozygote excess or deficiency.

Basic statistical data on allele frequencies, observed (H_o) and expected (H_e) heterozygosity were calculated in GenAlEx 6 (Peakall and Smouse 2006) across loci and clusters or streams of origin. Mean allelic richness (A) per cluster and stream was calculated in FSTAT v 2.9.3 (Goudet 1995) because the program corrects for differences in sample size between given populations. F_{IS} and $F_{ST}(\theta)$ were also calculated in FSTAT, using the methods outlined in Weir and Cockerham (1984). Additionally, FSTAT was used to test for genotypic (linkage) disequilibrium at all locus pairs within and across clusters and streams-of-origin. To test for significance, P-values were adjusted using the sequential Bonferroni technique (Rice 1989).

Family structure was determined a posteriori on STRUCTURE identified clusters. Individual fullsibs were identified in the program KINSHIP v1.3.1 (Goodnight and Queller 1999) using 1,000 pairwise simulations and assuming that fullsibs have a relatedness value of 0.5. A half-matrix of significant pairwise values ($\alpha = 0.05$) was compiled to visualize the family structure in each cluster.

Finally, population bottlenecks were evaluated by the program BOTTLENECK (Piry et al. 1999). Although microsatellites largely evolve via a step-wise mutation model (SMM), occasional mutations result in multi-repeat differences that violate the stepwise model (Balloux and Lugon-Moulin 2002). This may be the case for some loci screened, such as *Sfo18*, *Sco106* and *Sco220*, which show multi-repeat differences in allelic patterns for bull trout in the study area. To account for this, the program allows for a two-phase mutation model (TPM), which allows the user to specify the proportion of stepwise and multi-step mutations within the model. The recommended value of 90% SMM within the TPM was chosen, which is realistic for microsatellite data (Luikart et al. 1998). To test for significance, the 1-way Wilcoxon sign-rank test was used, which is the most powerful in detecting significance with limited sample sizes and/or loci (Piry et al. 1999).

Other considerations

Mill Creek (Mi) is the only stream in the drainage that contains sympatric bull and the introduced exotic, brook trout. A single F1 bull X brook trout hybrid from Mill Creek was identified by being heterozygous for bull and brook trout specific alleles at all 5 hybrid-detecting loci. This individual was the only hybrid detected in the drainage, and was excluded from all analyses.

The migratory tendencies of bull trout in the Oldman River complex are poorly understood. In order to determine whether streams had adult resident or migrant populations, all potential adult and subadult (>20 mm FL) bull trout caught were aged by scales and weighed. Size-at-age for these fish was used to determine if the population consisted of migrant, resident or both life histories. This stems from the commonly documented "dwarfing" phenomenon that occurs in non-migratory salmonid fish (Northcote 1992), where a resident will be smaller than a migrant of the same species of equivalent age. Migratory bull trout were considered as such if they were young and large (<6 years; >350 mm). Residents were determined as old, small bull trout (>5 years; <350 mm). These threshold

values were based on size-at age values described in previous bull trout literature for residents and migrants (McPhail and Baxter 1996; Mogen and Kaeding 2005). In addition, we used secondary sex characteristics to support life history conclusions, since resident fish will display phenotypic signs of maturity (kyped jaws, vibrant coloring etc.) at a smaller size than will migrant life histories (McPhail and Baxter 1996).

Results

Hierarchical population structure

STRUCTURE returned $\Delta K = 3$ in the first round of the hierarchical analysis, indicating three primary genetic archipelagos within the drainage (Castle, Upper Oldman and Livingstone archipelagos). A second round of analysis returned $\Delta K = 2$, indicating two secondary clusters within each of these archipelagos, a grouping which reflected geographical proximity. Populations detected at this level were the West and Mill (Mi_p) group within the Castle area, the Racehorse (Ra_p) and Hidden (Hi_p) groups within the Upper Oldman area, and the Lower (Lli_p) and Upper (Uli_p) Livingstone groups within the Livingstone area. A third round of analysis only found further structuring within the West Castle group, where $\Delta K = 2$ was found, representing the Carbondale (Cb_p) and West Castle (Wca_p) groups. The finest population structure detected by STRUCTURE therefore consisted of seven populations, nested hierarchically within three archipelagos (Figs. 1, 2).

PCA based on pairwise genetic differentiation of individuals seemed to reveal the same three major genetic archipelagos as the genetic clustering method (Fig. 3a). The PCA based on pairwise genetic differentiation of streams-of-origin was more difficult to interpret for clustering individual streams from the Castle River sub-basin, and did not reveal the same genetic clusters as found by STRUCTURE in the Castle River sub-basin and in the Livingstone system (Fig. 3b); however, at the coarser level of population structure, the same three archipelagos could roughly be approximated by the fact that all streams of each archipelago appeared in a different quadrant of the plot (Fig. 3b).

Coarsest level of structure: the three archipelagos

Within-archipelago variation ranged from $A = 3.604$ and $H_e = 0.437$ in the Livingstone to $A = 7.185$ and $H_e = 0.597$ in the Castle (Table 2). All three pairwise examinations of F_{ST} between archipelagos were significant (Table 2), with values ranging from 0.1212 to 0.2177. Global F_{ST} over all populations was found to be 0.146.

As expected in subdivided populations (Castric et al. 2002), tests for deviations from HWE revealed all archipelagos were out of HWE (Table 2), with four loci in the Castle and one locus in the Livingstone showing significant heterozygote deficiency (supplementary online Appendix 2).

Evidence for LD was found in all archipelagos, but only in 1–2 locus pairs in each (Table 2). Evidence for a population bottleneck was only found in the Oldman archipelago at both the 0.05 and 0.01 significance levels (Table 2).

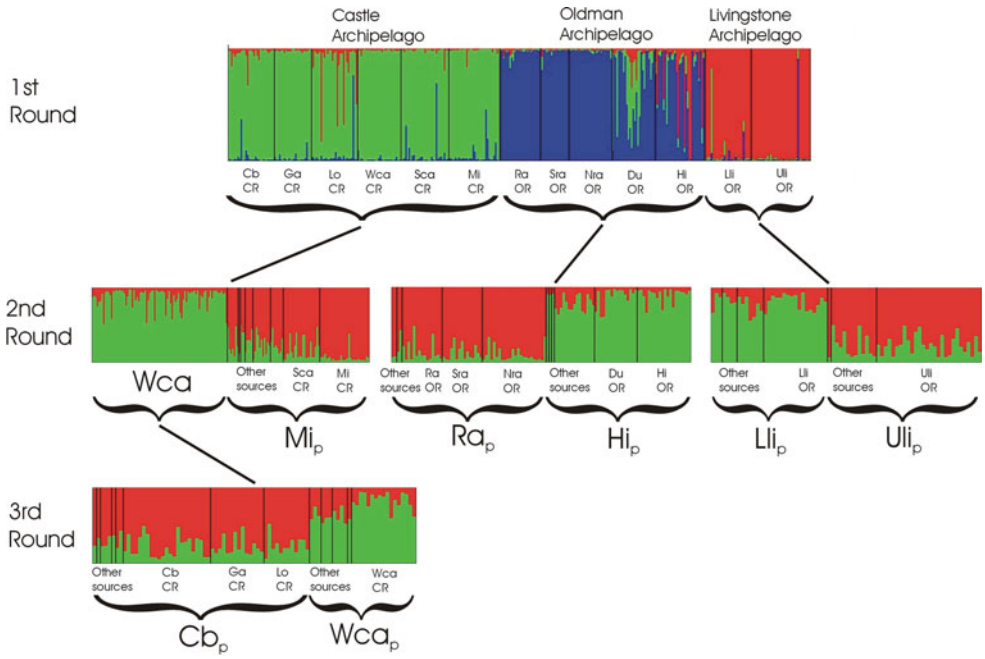

Fig. 2 Flow chart of hierarchical STRUCTURE analysis. *Each chart* represents the output from an analysis, with thin *vertical columns* each representing individual fish on the *x*-axis. On the *y*-axis is the likelihood of assignment to any given cluster *K*, which is represented by *different shades* in the chart. Sampling locations are separated by *black lines*

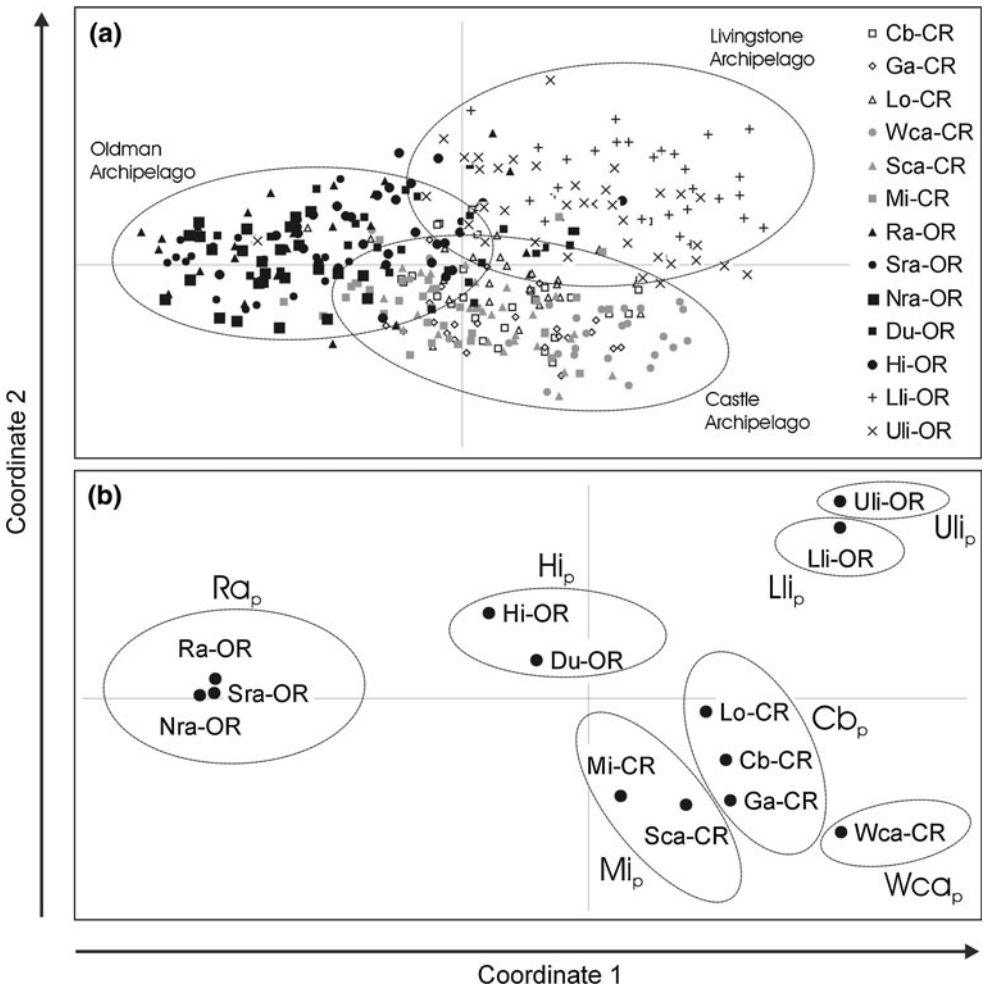

Fig. 3 PCA analysis from **a** genetic distance between all individuals (47% of total variation in two axes), and **b** from pairwise-F_{ST} values of all 13 streams-of-origin (48% of total variation in two axes). *Broken ellipses* represent the clusters found by hierarchical STRUCTURE analysis for comparison

Table 2 Summary of data and pertinent tests for each archipelago found by hierarchical STRUCTURE analysis

Population	Ca	Omr	Li	Global
N	174	113	72	359
pw F_{ST}, Ca		*	*	NA
pw F_{ST}, OMR	0.1212		*	NA
pw F_{ST}, Li	0.1335	0.2177		NA
Mean allelic richness (*A*)	7.185	4.884	3.604	7.433
Total private alleles	28	4	2	34
H_e	0.597	0.496	0.437	0.510
F_{ST}				0.146
F_{IS}	0.091	0.031	0.112	
#loci	9	9	9	9
Out of HWE?	Yes, 4 loci	Yes, 1 locus	Yes, 1 locus	Yes
Heterozygote deficiency?	Yes, 4 loci	No	Yes, 1 locus	NA
Heterozygote excess?	No	No	No	NA
Linkage Disequilibrium?	Yes, 2 pairs	Yes, 2 pairs	Yes, 1 pair	NA
Bottleneck? alpha <0.05	No	Yes	No	NA
Bottleneck? alpha <0.01	No	Yes	No	NA
Proportion mis-assigned	0.02	0.12	0.03	0.06

* Indicates statistical significance at $P < 0.05$

Table 3 Summary of data and pertinent tests for each population found by hierarchical STRUCTURE analysis

Population	Cb_p	Wca_p	Mi_p	Ra_p	Hi_p	Lli_p	Uli_p	Global
N	57	28	89	58	54	31	41	358
pw F_{ST}, Cb_p		*	*	*	*	*	*	NA
pw F_{ST}, Wca_p	0.0988		*	*	*	*	*	NA
pw F_{ST}, Mi_p	0.0782	0.1218		*	*	*	*	NA
pw F_{ST}, Ra_p	0.2206	0.3355	0.1787		*	*	*	NA
pw F_{ST}, Hi_p	0.1168	0.2198	0.1015	0.1125		*	*	NA
pw F_{ST}, Lli_p	0.1708	0.2644	0.1648	0.3036	0.1569		*	NA
pw F_{ST}, Uli_p	0.1776	0.2734	0.191	0.3555	0.2269	0.1485		NA
Mean allelic richness (*A*)	5.433	4.207	5.952	3.339	4.454	3.462	2.292	6.133
Total private alleles	1	0	8	1	2	1	0	13
H_e	0.558	0.448	0.592	0.432	0.499	0.437	0.372	0.477
F_{ST}								0.178
F_{IS}	0.004	0.001	0.059	−0.069	0.005	0.087	−0.011	
#loci	9	9	9	9	9	9	9	9
Out of HWE?	No	No	Yes	No	No	No	No	Yes
Heterozygote deficiency?	No	No	Yes, 3 loci	No	No	No	No	NA
Heterozygote excess?	No	No	No	No	No	No	No	NA
Linkage disequilibrium?	No	No	Yes, 1 pair	No	No	No	Yes, 1 pair	NA
Bottleneck? alpha <0.05	No	No	No	Yes	Yes	No	Yes	NA
Bottleneck? alpha <0.01	No	No	No	Yes	Yes	No	No	NA
Proportion mis-assigned	0.14	0.39	0.39	0.07	0.33	0.45	0.32	0.29

* Indicates statistical significance at $P < 0.05$

Finest level of structure: the seven populations

Intra-population variation ranged from $A = 2.292$ and $H_e = 0.372$ in Uli_p to $A = 5.952$ and $H_e = 0.592$ in Mi_p (Table 3). All 21 pairwise F_{ST} values were significant, with between-population variation ranging from 0.0782 to 0.3555 (Table 3). Global F_{ST} over all populations was found to be 0.178.

Only a single population (Mi_p) deviated from HWE (Table 3) with three loci exhibiting heterozygote deficiency (Table 3). The population also displayed high F_{IS} values.

Evidence for LD was found in a single locus pair in Uli_p and Mi_p (Table 3). Family structure was very strong in populations showing low genetic diversity, and weaker, but potentially "patchy" in populations showing higher genetic diversity (Fig. 4). Evidence for population bottlenecks were found in Ra_p, Hi_p and Uli_p at a significance level of 0.05, but only Ra_p and Hi_p at a significance level of 0.01 (Table 3).

Within and between-stream variation

Within-stream variation ranged from $A = 2.555$ and $H_e = 0.409$ in the Upper Livingstone River (Uli) to $A = 5.117$ and $H_e = 0.593$ in the South Castle River (Sca; Table 1). For between-stream variation, out of 78 pairwise estimates of F_{ST}, all but three were significant (Table 1). Absolute F_{ST} values ranged from −0.0077 to 0.3174. Global F_{ST} over all streams was 0.158.

Two streams deviated significantly from HWE across individual or pooled loci (Table 1). This was due to heterozygote deficiency found at these streams (Table 1).

Evidence for LD was found in a single locus pair in the Upper Livingstone River (Table 1) and evidence for population bottlenecks was found in nine out of the 13 streams at the 0.05 significance level and 5 at the 0.01 significance level (Table 1).

Mis-assignment within the hierarchically structured drainage and life history of populations

Mis-assignment levels (proportion of fish in a population that may be emigrants) were low for archipelagos, but at the finer population level, were variable, and in certain cases, very high (Tables 2, 3). These fish were presumably migrants that were sampled in streams distant from the home streams of their genetic population of origin.

No evidence of sympatric resident and migrant bull trout were found anywhere in the drainage. The Upper Livingstone River (Uli-OR) had no fish >320 mm FL, and many of the fish found were 5 years of age or older (data not shown). In addition, many of the fish between 250 and 320 mm FL had secondary sex characteristics typical of

sexually mature trout (vibrant coloring, kyped jaws etc.); on the basis of these characteristics, we interpret the Upper Livingstone area to contain exclusively resident and all other areas migrant life-histories.

Discussion

Hierarchical population structure

Genetic clustering methods revealed population structure in a hierarchical manner to a relatively fine spatial scale in our population complex of bull trout. At the coarsest level of population structure, the archipelago level, divergence was relatively high, as shown in the range of pairwise F_{ST} values (Table 2). The Oldman and Livingstone archipelagos were the most differentiated, which would not have been expected on the basis of their relative close geographical proximity. All three archipelagos showed significant HWE departures due to heterozygote deficit, which was expected due to further population structuring (Wahlund effect). This was confirmed by subsequent STRUCTURE analyses, which successfully detected population structure within each archipelago.

Within the Oldman and Livingstone archipelagos, two populations were found. In the Castle archipelago, three populations were revealed by an additional round of STRUCTURE analysis. All populations conformed to HWE with the exception of Mi_p. This may indicate the existence of further sub-structuring which was not detectable by the analysis, or by the presence of patchy family structure creating a family Wahlund effect (Castric et al. 2002) in this population (Fig. 4). The groups appear different in $K = 2$ output from STRUCTURE, though neither ΔK or the highest mean value of (ln Pr ($X|K$)) suggested that this was the most likely solution (data not shown). STRUCTURE may have failed to subdivide this group because of insufficient genetic differentiation between them (Waples and Gaggiotti 2006). It may be warranted in future studies to use multiple clustering programs (e.g., Vähä et al. 2007; Fedy et al. 2008) to explore multiple avenues of solutions for correctly determining population structure.

The previous case reflects an example where a false negative may have been encountered using STRUCTURE. False positives may also be generated by STRUCTURE if family groupings are partitioned by the program, rather than true populations (e.g., Vähä et al. 2007). In our study, such a case may have occurred in the Livingstone system because we observed strong family structure in both clusters identified by kinship analysis (Fig. 4); however, given the unique life history and geomorphology of the system (Fitch 1997), it seems more likely that the two clusters truly are separate populations. The strongest reason for this is that they are separated by a set of falls that are not easily passed in the upstream direction, which should drastically reduce gene flow. This is supported by the fact that the majority of observed movement between the two areas is in a downstream direction, and these were exclusively juveniles (Table 3, Fig. 2). The second reason is that the two populations show different life history strategies as adults, which implies divergent selection between the two areas and has even greater implications for managing evolutionarily relevant genetic diversity (Taylor 1991).

Stream-of-origin, compared to clustering technique, and management implications

Since most streams show significant genetic divergence (measured by F_{ST}) at neutral microsatellite markers (Moritz 1994), each of these streams-of-origin might strictly speaking be viewed as different management units, or stocks (Dehaan and Arden 2007; Kassler and Mendel 2007; Whiteley et al. 2006b). It is unlikely, however, that all these streams contain truly distinct populations, since pairwise F_{ST} values may be inflated by an effect of family structure between streams (Hansen et al. 1997) or possibly low intra-stream heterozygosity (Hedrick 1999). In comparison to this a priori population designation, genetic clustering methods are less likely to generate such spurious groupings, giving the manager a more realistic picture of the true spatial population structure in the drainage. For example, pairwise F_{ST} values between all three streams in the Carbondale River area (Carbondale, Lost and Gardiner Creeks) range between 0.05 and 0.07, and are statistically significant (Table 1). By the stream-of-origin approach, these could be interpreted as three separate populations for management purposes; however, the genetic clustering approach groups these three areas into one population (Cb_p, Fig. 1). Additionally, this general overestimate of population structure when using a stream-of-origin approach will yield larger tables of statistical results and more pairwise comparisons of genetic divergence between populations. The standard statistical method of evaluating tables of statistical results in population genetics is the sequential Bonferroni correction procedure (Rice 1989), which becomes increasingly prone to type 2 error with an increasing number of comparisons, exponentially escalating difficulty in interpreting the data (Moran 2003).

Using a PCA from genetic distances calculated between individuals, we were able to discern the same three genetic archipelagos defined by STRUCTURE (Fig. 3a). Like the methods STRUCTURE uses, this first analysis is not based on any a priori spatial assumptions, and clusters groups based on individual genetic pairwise comparisons; therefore, it may be useful as an initial exploratory analysis for defining major groupings in the dataset.

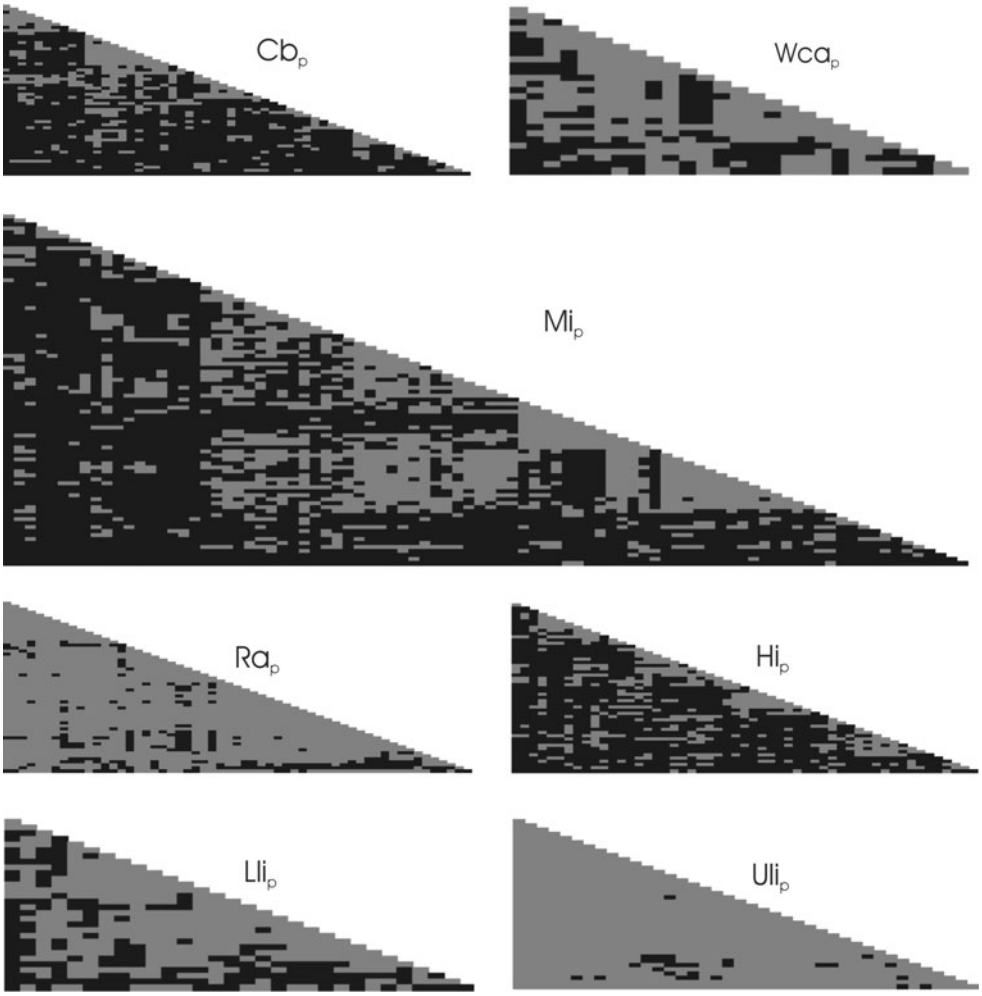

Fig. 4 Family structure in each population found by hierarchical STRUCTURE analysis. Each half-matrix contains *blocks* indicating significance or non-significance for fullsib relatedness between individuals in a population. *Gray* indicates significant values at $\alpha = 0.05$. These individuals are assumed to be fullsibs. *Black* indicates non-significance between individuals for fullsib occurrence

The second PCA constructed from pairwise F_{ST} values between streams-of-origin is difficult to interpret. While the same archipelagos may roughly appear and hierarchical structure may be ascertained in this case, fine-scale populations identified by STUCTURE do not clearly stand out in the matrix (Fig. 3b). This method, in essence, attempts to redefine spatial population structure in a post hoc manner from analyses that had already assumed a certain geographic population structure. This circular logic lacks the parsimony of genetic clustering methods and is more open to interpretation when designating meaningful units of conservation.

Assignment tests from the clustering approach revealed significant mis-assignment levels in the dataset (Tables 2, 3). Because mis-assignment potentially reflects true real-time dispersal (Berry et al. 2004), this introduces a further problem of including immigrant individuals into a population sample when using a stream-of-origin approach. Throughout the drainage, F_{ST} values among STRUCTURE generated clusters were higher than between-stream F_{ST} values (Global F_{ST}: Tables 1, 3), likely owing to the genetically homogenizing effect of movement among natal areas of separate populations (Hendry et al. 2000).

The differences found using these two methods have many management implications. Fundamental measurements in fisheries biology are census size estimates of populations. If populations are inappropriately designated, or there is movement between home ranges of populations, census size estimates may be very inaccurate (Palstra et al. 2007). For example, many fish that were assigned to the Mi_p population were found in the Carbondale River system (home range of the Cb_p; Figs. 1, 2; Table 3), which would result in an underestimation of the Mi_p population size and overestimation of the Cb_p population size. This may also confound estimates of effective population size (N_e) if census size estimates are used to derive this value (Harris and Allendorf 1989). Estimates of historic gene flow (number of effective migrants—N_em) are traditionally derived from F-statistics. F_{ST} values between STRUCTURE generated clusters differ greatly from among-stream values (Tables 1, 3), and thus would greatly affect estimates of N_em, which are sensitive to small changes in F_{ST} (Allendorf and Luikart 2007).

These examples illustrate the types of artefacts that can be generated from the stream-of-origin approach, when the

population structure is not congruent with the drainage network. The genetic clustering approach may therefore provide a more objective approach to the assessment of stocks or conservation units than traditional methods, even when combined with post hoc approaches to interpret the data, such as PCA plots. It should be noted, however, that clustering may work especially well in bull trout and some other salmonine fish because of the finely dissected population structure they show (Whiteley et al. 2006a; Vähä et al. 2007), and the technique may underestimate population structure in other systems or taxa where gene flow is moderate to high (Waples and Gaggiotti 2006). Alternatively, overestimates of population structure may also occur when there is strong family structure (Vähä et al. 2007). In such systems, traditional methods may work just as well or better than clustering methods, or the two may be used in concert to describe population structure (Fedy et al. 2008).

Genetic bottlenecks

Genetic bottlenecks were found only in the Hi_p and Ra_p populations, using a threshold of $\alpha = 0.01$. This is consistent with the strong trends of bottleneck detection seen in these areas at both the stream specific and archipelago levels of structure. There are two possible explanations for such a bottleneck. First, angling pressure may have historically reduced the numbers of migratory bull trout far more in the upper Oldman area than in the Castle area (Fitch 1997). Because anglers would likely have selectively targeted the larger bodied migrants over the smaller residents for harvest, this explains why no bottleneck is seen in the Livingstone archipelago, which is found in the same area but contains primarily resident fish. Secondly, there is a seasonal migration barrier for fish in the main-stem of the Oldman River located downstream of all spawning areas of the archipelago. Because bull trout migrate in late summer when stream flows are reduced (McPhail and Baxter 1996), this barrier may, in at least the driest summers, limit the numbers of returning spawners to these migrant populations. Such seasonal barriers are likely common aspects of mountain stream geomorphology, but their influences on shaping genetic structure of populations are rarely discussed. Note that these two explanations may not be mutually exclusive, and may have interacted in the past century to create the observed bottleneck.

Conservation implications

Hierarchical STRUCTURE analysis revealed levels of population structure within the drainage system that differed from traditional approaches. It is, however, still uncertain at exactly what level to draw the line when describing populations as units of conservation. Major clusters defined by the first round of genetic clustering analysis and PCA are likely a good starting point, as it appears that gene flow is likely very low between the three major archipelagos (Table 2); however, significant genetic subdivision within each of these archipelagos is present and may reflect adaptive differences (Taylor 1991). Therefore, it could be argued that all levels of hierarchical population structure detectable by this analysis reveal potentially important information for determining conservation strategies (Pearse and Crandall 2004; Whiteley et al. 2006a). While the scale of this study was rather small, larger scale studies are likely to reveal more levels of hierarchical structure, therefore defining units of conservation for the species as a whole may require an integrated management plan that reflects this multi-layered structure.

Some of the populations identified (e.g., the Uli_p) were highly divergent, and displayed low diversity, but neutral genetic divergence measured for this study may mirror a high degree of adaptive divergence within these "genetically meager," sometimes resident populations, which may face different ecological and genetic challenges than robust populations (Hedrick and Kalinowski 2000; Amos and Balmford 2001). It should be noted that populations of lower effective size or genetic diversity should not necessarily be treated with lower "conservation priority," as smaller populations are important in maintaining genetic diversity to an overall system (Rieman and Allendorf 2001; Allendorf et al. 2008); furthermore, population sizes may vary over evolutionary timescales (Palstra et al. 2007), thus determining conservation priorities based on contemporary genetic structure and population size seems short-sighted. Management should focus on maintaining overall genetic diversity, and conserving all populations and habitats, rather than selectively conserving only specific populations.

In conclusion, our results appear generally consistent with findings of other authors that distal headwater salmonid populations (Vähä et al. 2008) and populations isolated above barriers (Costello et al. 2003; Whiteley et al. 2006b) display reduced intrapopulation diversity and increased interpopulation divergence. There is cause for added complexity to the latter, however, by interpreting most barriers as not all-or-none migration impediments, but as semi-permeable genetic filters which may operate in a temporally variable manner to shape population structure and evolution (Haugen et al. 2008).

The population structure revealed by genetic clustering in this study is hierarchical, in which movement may be common between streams. Population structure of bull trout revealed by this method may be more objective and parsimonious than when using traditional stream-of-origin methods, which may be biased by unrealistic a priori assumptions of geographic population structure. As results are largely interpretable and intuitive given a posteriori

geographic and ecological information, it appears that the genetic clustering approach has merit in efficiently identifying true hierarchical population structure and proper units of conservation at fine spatial scales for salmonid fish.

Acknowledgments We thank F. Allendorf, C. Goater, A. Hurley, R. MacDonald and T. Burg for their comments, discussion and technical support. This project was funded by the National Science and Engineering Research Council of Canada, an Alberta Conservation Association Biodiversity Grant and Trout Unlimited Canada's Coldwater Conservation Fund.

References

Allendorf FW, Luikart G (2007) Conservation and the genetics of populations. Blackwell Publishing, Malden

Allendorf FW, Phelps SR (1981) Use of allelic frequencies to describe population structure. Can J Fish Aquat Sci 38:1507–1514

Allendorf FW, England PR, Luikart G, Ritchie PA, Ryman N (2008) Genetic effects of harvest on wild animal populations. Trends Ecol Evol 23:327–337

Amos W, Balmford A (2001) When does conservation genetics matter? Heredity 87:257–265

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. Mol Ecol 11: 155–165

Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? Mol Ecol 13:551–561

Castric V, Bernatchez L, Belkhir K, Bonhomme F (2002) Heterozygote deficiencies in small lacustrine populations of brook charr *Salvelinus Fontinalis* Mitchill (Pisces, Salmonidae): a test of alternative hypotheses. Heredity 89:27–35

Chen C, Durand E, Forbes F, Francois O (2007) Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. Mol Ecol Notes 7:747–756

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 Int J org Evolution 57:328–344

Dawson KJ, Belkhir K (2001) A Bayesian approach to the identification of panmictic populations and the assignment of individuals. Genet Res 78:59–77

DeHaan P, Arden W (2007) Genetic analysis of bull trout in the Jarbidge River watershed, Nevada/Idaho. U.S. Fish and Wildlife Service, Reno

DeHaan PW, Ardren WR (2005) Characterization of 20 highly variable tetranucleotide microsatellite loci for bull trout (*Salvelinus confluentus*) and cross-amplification in other *Salvelinus* species. Mol Ecol Notes 5:582–585

Dunham JB, Rieman BE (1999) Metapopulation structure of bull trout: influences of physical, biotic, and geometrical landscape characteristics. Ecol Appl 9:642–655

Erkinaro J, Dempson JB, Julkunen M, Niemela E (1997) Importance of ontogenetic habitat shifts to juvenile output and life history of Atlantic salmon in a large subarctic river: an approach based on analysis of scale characteristics. J Fish Biol 51:1174–1185

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Fedy BC, Martin K, Ritland C, Young J (2008) Genetic and ecological data provide incongruent interpretations of population structure and dispersal in naturally subdivided populations of white-tailed ptarmigan (*Lagopus leucura*). Mol Ecol 17:1905–1917

Fitch L (1997) Bull trout in Southwestern Alberta: notes on historical and current distribution. In: Mackay W, Brewin M, Monita M (eds) Friends of the bull trout. Trout Unlimited Canada, Calgary, pp 147–160

Gerking SD (1959) The restricted movement of fish populations. Biol Rev Cambridge Philosophic Soc 34:221–242

Gerrand M, Watmough M (1998) 1998 Spawning survey of bull trout in the Oldman River drainage. Alberta Conservation Association, Blairmore

Goodnight KF, Queller DC (1999) Computer software for performing likelihood tests of pedigree relationship using genetic markers. Mol Ecol 8:1231–1234

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate *F*-statistics. J Hered 86:485–486

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48:361–372

Hansen MM, Nielsen EE, Mensberg KLD (1997) The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. Mol Ecol 6:469–474

Harris RB, Allendorf FW (1989) Genetically effective population size of large mammals: an assessment of estimators. Conserv Biol 3:181–191

Haugen TO, Aass P, Stenseth NC, Vøllestad LA (2008) Changes in selection and evolutionary responses in migratory brown trout following the construction of a fish ladder. Evol Appl 1:319–335

Hedrick PW (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution Int J org Evolution 53:313–318

Hedrick PW, Kalinowski ST (2000) Inbreeding depression in conservation biology. Annu Rev Ecol Syst 31:139–162

Hendry AP, Wenburg JK, Bentzen P, Volk EC, Quinn TP (2000) Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science 290:516–518

Kassler T, Mendel G (2007) Genetic characterization of bull trout from the Walla Walla River basin. Washington Department of Fish and Wildlife, Olympia

Kennedy BP, Klaue A, Blum JD, Folt CL, Nislow KH (2002) Reconstructing the lives of fish using Sr isotopes in otoliths. Can J Fish Aquat Sci 59:925–929

Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. J Hered 89:238–247

Manel S, Gaggiotti OE, Waples RS (2005) Assignment methods: matching biological questions techniques with appropriate. Trends Ecol Evol 20:136–142

McPhail JD, Baxter JS (1996) A review of bull trout (*Salvelinus confluentus*) life-history and habitat usi in relation to compensation and improvement opportunities. Fisheries management report 104. University of British Columbia, Vancouver

Mogen JT, Kaeding LR (2005) Identification and characterization of migratory and nonmigratory bull trout populations in the St. Mary River drainage, Montana. Trans Am Fish Soc 134:841–852

Moran MD (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100:403–405

Moritz C (1994) Defining evolutionarily-significant-units for conservation. Trends Ecol Evol 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. Mol Ecol 10:1153–1164

Northcote TG (1992) Migration and residency in stream salmonids. Some ecological considerations and evolutionary consequences. Nord J Freshw Res 67:5–17

Palstra FP, O'Connell MF, Ruzzante DE (2007) Population structure and gene flow reversals in Atlantic salmon (*Salmo salar*) over contemporary and long-term temporal scales: effects of population size and life history. Mol Ecol 16:4504–4522

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Peakall R, Smouse PE, Huff DR (1995) Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalograss *Buchloe dactyloides*. Mol Ecol 4:135–147

Pearse DE, Crandall KA (2004) Beyond F-ST: analysis of population genetic data for conservation. Conserv Genet 5:585–602

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered 90:502–503

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pritchard JK, Wen X, Falush D (2007) Documentation for STRUCTURE software: version 2.2. Available from http://pritch.bsd.uchicago.edu/software

Rasmussen JB, Trudeau V, Morinville GR (2009) Estimating the scale of fish feeding movements in rivers using $\delta^{13}C$ signature gradients. J Anim Ecol 78:674–685

Raymond M, Rousset F (1995) GENEPOP (version-1.2)—population-genetics software for exact tests and ecumenicism. J Hered 86:248–249

Rice WR (1989) Analyzing tables of statistical tests. Evolution Int J org Evolution 43:223–225

Rieman BE, Allendorf FW (2001) Effective population size and genetic conservation criteria for bull trout. North Am J Fish Manage 21:756–764

Rodriguez MA (2002) Restricted movement in stream fish: the paradigm is incomplete, not lost. Ecology 83:1–13

Scrivener JC, Brown TG, Andersen BC (1994) Juvenile chinook salmon (*Oncorhynchus tshawytscha*) utilization of Hawks Creek, a small and nonnatal tributary of the upper Fraser River. Can J Fish Aquat Sci 51:1139–1146

Slatkin M, Voelm L (1991) F-st in a hierarchcial island model. Genetics 127:627–629

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

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

Taylor EB (1991) A review of local adaptation in salmonidae, with particular reference to pacific and atlantic salmon. Aquaculture 98:185–207

Vähä JP, Erkinaro J, Niemela E, Primmer CR (2007) Life-history and habitat features influence the within-river genetic structure of Atlantic salmon. Mol Ecol 16:2638–2654

Vähä JP, Erkinaro J, Niemela E, Primmer CR (2008) Temporally stable genetic structure and low migration in an Atlantic salmon population complex: implications for conservation and management. Evol Appl 1:137–154

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J Hered 89:438–450

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Mol Ecol 15:1419–1439

Warnock WG (2008) Molecular tools reveal hierarchical stucture and patterns of migration and gene flow in bull trout (*Salvelinus confluentus*) populations of South-Western Alberta. M.Sc. Thesis, University of Lethbridge, Lethbridge, p 174

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution Int J org 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*). Mol Ecol 7:733–749

Whiteley AR, Spruell P, Allendorf FW (2006a) Can common species provide valuable information for conservation? Mol Ecol 15:2767–2786

Whiteley AR, Spruell P, Rieman BE, Allendorf FW (2006b) Fine-scale genetic structure of bull trout at the southern limit of their distribution. Trans Am Fish Soc 135:1238–1253