

All dam-affected trout populations are not alike: fine scale geographic variability in resident rainbow trout in Icicle Creek, WA, USA

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Abstract Recognizing the genetic diversity within and among collections of allopatric rainbow trout is an important step in understanding and monitoring the dynamics of the metapopulation structure of a species like *Oncorhynchus mykiss* with resident and anadromous life history forms. Prior to the removal of a barrier and the recolonization of Icicle Creek with anadromous steelhead, we report the degree to which collections of above-barrier resident rainbow trout from 13 sites differ from downstream steelhead, and the pattern of genetic diversity and connectivity among resident collections using 14 microsatellite loci. Measures of genetic variability (H_e , A_R , and A/L) are low in the upper-most collections of residents and estimates of N_e change approximately 4-fold from the upper tributaries ($N_e \sim 90$) to the lowest main stem collections ($N_e \sim 360$) over 35 river kilometers (rkm). The overall comparison of resident rainbow trout versus below-barrier steelhead is $F_{ST} = 0.053$. A STRUCTURE analysis of all 1,730 fish indicated three populations within the above-barrier collections of resident fish. Notably, two sets of upstream collections of rainbow trout, separated at a minimum of 16.4 rkm, had a mean $F_{ST} = 0.128$. Natural passage barriers account for some of the observed stock

structure in Icicle Creek but the strongest differences are not associated with barriers by our analysis. No significant temporal variability was seen within four rainbow trout sites and one steelhead site; and no hatchery rainbow trout ancestry was detected in the watershed. In general these results highlight the need for conservation efforts to include fine-scale evaluations of population structure of riverine fishes above barriers to increase the accuracy of understanding and monitoring intra specific diversity and the biological effects of dams and dam removal.

Keywords Rainbow trout · Population structure · Conservation · Genetic divergence · Barriers

Introduction

A considerable number of studies in the Pacific Northwest of North America have described levels and patterns of genetic variability within and between populations of the two life history forms of *Oncorhynchus mykiss*, the freshwater form rainbow trout and the anadromous form steelhead (reviewed in Northcote 2010). Recent genetic work has shown that resident populations isolated from anadromous populations by natural or man-made barriers are genetically distinguishable from one another using microsatellite (mSAT) loci (Small et al. 2007; Narum et al. 2008; Winans et al. 2008; Van Doornik et al. 2010; Winans et al. 2010; Van Doornik et al. 2013). Although not as widely evaluated, phenotypic differences are also seen between ecotypes of *O. mykiss* (Keeley et al. 2005), and body shape and parr mark patterns differed between juvenile steelhead and above-barrier resident trout (Winans et al. 2010) as similarly reported in above-barrier collections of *O. clarkii* (Northcote and Hartman 1988). Missing in this general

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body of work are basic biological data concerning the extent of fine-scale genetic connectedness among collections of resident fish. Data from two other Pacific Northwest rivers, the Lewis and Elwha, indicate that significant differentiation may exist among resident sites (Winans et al. 2008; unpublished) at surprisingly small scales. Such data documenting fine-scale genetic differentiation among spatially proximate *O. mykiss* populations are critical for our understanding of the species as dams are bypassed or removed, putting recently allopatric resident and anadromous life history forms back into sympatry. To what extent will formerly isolated and differentiated resident trout genetically interact with steelhead, and how will 'introgression' affect the life history trajectories of these two life history forms and thus impact the broader metapopulation structure of the species?

Icicle Creek is a fifth-order watershed in the Wenatchee River Basin with a mean annual discharge 514 cubic feet per second. It enters the Wenatchee River at river kilometer (rkm) 41, which itself flows into the Columbia River at rkm 754. Icicle Creek contributes 21 % of the mean annual discharge of the Wenatchee River. Historically Icicle Creek supported anadromous steelhead, spring-run Chinook salmon *O. tshawytscha*, and bull trout *Salvelinus confluentus* (Mullan et al. 1992). Construction of the Leavenworth National Fish (LNF) Hatchery in 1939–1940 between Icicle Creek rkms 4.0–7.3 blocked returning migratory salmonids from more than 47 km of mainstem and tributary habitat. Currently, the US Fish and Wildlife Service plans to restore year-round upstream and downstream passage and thereby allow recolonization of the upper Icicle Creek basin by native anadromous salmonids.

The purpose of this paper is to genetically compare resident rainbow trout with steelhead, and evaluate the connectivity and patterns of variability among resident rainbow trout collections. The influence of hatchery trout in the collections is also evaluated. Our results provide not only a genetic baseline for monitoring Icicle Creek *O. mykiss* but also a framework for recognizing and monitoring potentially unique populations that may be identified throughout the species' range.

Methods

Study sites

Anthropogenic fish passage barriers associated with LNF Hatchery are located between Icicle Creek rkm 4.0 and 7.3 (Fig. 1). An irrigation diversion dam, passable by migratory salmonids at most flows, is located at rkm 9.2, just below US Geological Survey (USGS) gage station #12458000 (rkm 9.3). Our study took place upstream of the

gage station and we refer to the entire catchment upstream of rkm 9.3 as the Icicle Creek throughout this article, unless noted otherwise.

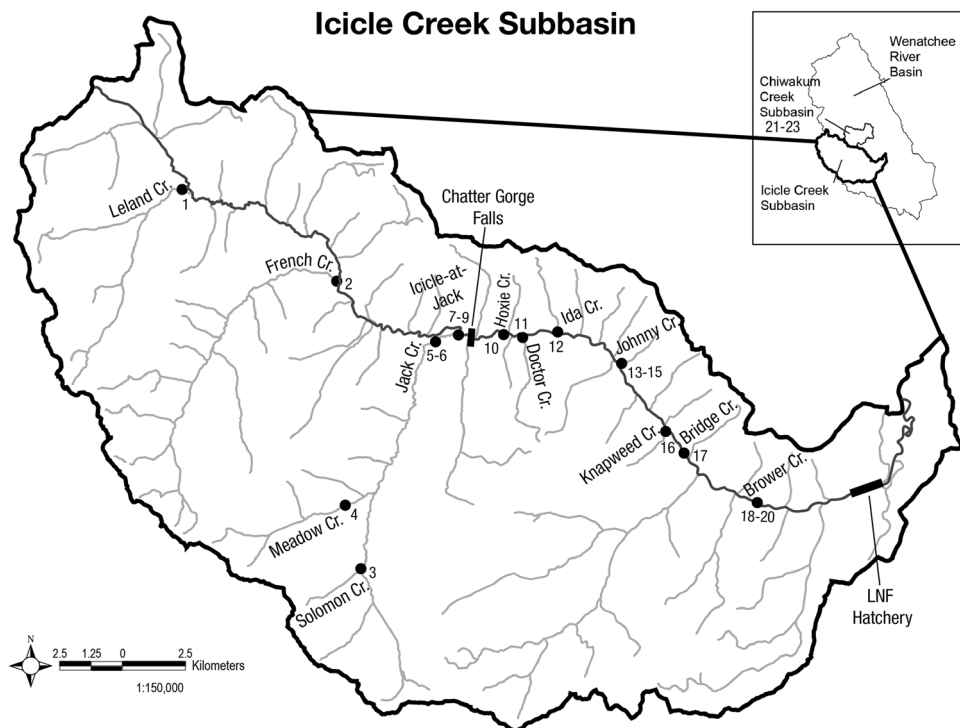
Resident rainbow trout in the upper Icicle Creek were sampled at eight mainstem sites between rkm 28 and 11, in the three largest tributaries (Leland, French, and Jack creeks) and in two tributaries to upper Jack Creek for a total of 13 resident sites (Fig. 1). Repeated sampling occurred in 2007, 2008, and 2009 at Icicle-at-Jack, Johnny, and Brower, and in 2007 and 2008 in lower Jack Creek for a total of 20 collections of resident trout from 13 sites. The geomorphology of the upper Icicle is strongly bedrock controlled resulting in cobble-dominated riffle/run habitats alternating with large pools dominated by bedrock and boulders. These habitat types are separated from one another at the scale of hundreds of meters by significant gradient breaks (drops) that create velocity and related hydraulic obstacles to fish migration, effectively preventing small-bodied resident salmonids from freely migrating upstream in most, if not all, flows (Webb 1976; Bjornn and Reiser 1991; Reiser et al. 2006). One of these hydraulic obstacles, Chatter Gorge Falls at rkm 26.3, is only passable by large-bodied migratory salmonids (adfluvial bull trout, steelhead, and spring-run Chinook salmon) (*ibid.*). We recognize seven more sites that are presumed potential barriers (Appendix 1).

Field collections

Fish were collected by angling from riffle-run habitats (sites) over a 3 years period (2007–2009) from July to September when river flow permitted collection opportunities. Sampling began in the second or third week of July when discharge at the USGS gage declined to 400 cfs and continued through September when base flow discharge ranged from 130 to 200 cfs. The majority of the fish sampled ranged from 100 to 300 mm fork length and one to four years of age (based on analysis of scale patterns of more than 400 sampled individuals). In 2009 young of the year were sampled at night by dip netting. All main stem sites ranged from 100 to 150 m in length and 18–23 m in width. At each site, collections were obtained from typical rearing habitats that ranged in depth from 15 to 150 cm. Collections from Leland, French, and Jack creeks represent the only tributaries year-round flow capable of supporting independent populations of resident rainbow trout.

Steelhead collections were made over three years at Chiwaukum Creek, a tributary in a subbasin of the Wenatchee River located 8 rkm upstream of the Icicle Creek confluence and from a smolt trap 12 rkm downstream from the Icicle-Wenatchee confluence in Peshastin Creek (at Peshastin rkm 10.2). Data were also collected from two widely-used strains of hatchery rainbow trout

Fig. 1 Location of 20 collections of resident rainbow trout made in the Icicle Creek subbasin, Wenatchee River watershed, and three collections of steelhead made in the Chiwaukum Creek subbasin, Wenatchee River watershed, and three collections of steelhead made in the Chiwaukum Creek subbasin (*inset*). A collection of steelhead from Peshastin Creek downstream of Icicle Creek on the Wenatchee River and two collections of out-of-basin hatchery rainbow trout are not included here. The river flows left to right and enters the Wenatchee River at rkm 41 which exits the Wenatchee River Basin in the Columbia River at rkm 754



outplanted by the Washington Department of Fish and Wildlife. Fin clips were non lethally collected and stored on chromatography paper.

Genetics

Genomic DNA was extracted and purified from caudal fin clips using the Qiagen DNeasy 96 Blood and Tissue kit (Qiagen, Valencia, California, USA). We collected genotypic data for fourteen mSAT loci (Appendix 2) using previously described procedures (Winans et al. 2008, 2010). LIZ 500 was used as an internal size standard for each sample and fragment size was determined using Genescan 3.7 (Applied Biosystems, Foster City, California, USA). Genotyping and tabling of the data for further analysis were performed using Genotyper 3.7 (Applied Biosystems, Foster City, California, USA). A control specimen was included in each run to standardize allele scoring. To screen out pure or F1 hybrid cutthroat trout (*O. clarki*), we established allele protocols at *Ocl1*, *Ots3*, and *Ots100* using known animals as determined by intron markers (Baker et al. 2002).

Descriptive population statistics were calculated with GENEPOP version 3.3 (Raymond and Rousset 1995) and Genetix 4.05 (Belkhir et al. 1996). Effective population size N_e was estimated with the linkage disequilibrium method in the computer program LDNE (Waples and Do 2008) based on the lowest allele frequency of 0.02 and confidence intervals estimated with the parametric method

(which were highly similar to the jackknife method). An analysis of molecular variance AMOVA was conducted to evaluate temporal variability in allele frequencies within collection sites in comparison to between-site variability using ARLEQUIN 3.5 (Excoffier et al. 2005). F statistics and allele richness were calculated after Weir and Cockerham (1984) with FSTAT version 2.9 (Goudet 2001); significance testing was determined with permutation over alleles by 10,000 bootstraps. Differences among collections were illustrated in a dendrogram using Cavalli-Sforza and Edwards (CSE) chord metric (Cavalli-Sforza and Edwards 1967) calculated with POPULATIONS (Langella 2001). Precision of branching patterns was evaluated by bootstrapping over loci 1,000 times (Belkhir et al. 1996). To further evaluate the number of gene pools, we implemented STRUCTURE 2.2 (burn-in of 50,000 iterations and a run of 500,000 iterations; Pritchard et al. 2000) using the admixture model with correlated allele frequencies and examined the mean and variance of the likelihood value over 10 iterations of K between 1 and 10 where K is the hypothetical number of populations. Isolation by distance (IBD) was explored in a plot of adjusted F_{ST} values (Rousset 1997) and river kilometers.

Results

One F1 cutthroat hybrid was identified in the Chiwaukum 2009 collection and dropped from the analysis. Individual

fish with greater than two missing loci were excluded; 4.6 % of the fish were excluded leaving 1,730 fish. A total of 270 alleles was revealed over 14 loci in the 1,730 fish (Appendix 2). The number of alleles per locus ranged from 8 at *Ssa289* to 30 at *Omy100*. The lowest average expected heterozygosity per locus was 0.528 (*One14*), whereas 7 loci had observed heterozygosity between 0.808 and 0.876. Mean observed heterozygosity was 0.743. Estimates of F_{ST} among collections ranged from 0.048 (*Ocl1* and *Ots100*) to 0.146 (*One14*), and averaged 0.068 over all loci (Appendix 2).

Significant departures from Hardy–Weinberg equilibrium were detected at Icicle-at-Jack 2007, and Brower 2009; pooled temporal data at Icicle-at-Jack and Brower were also significant. F_{IS} was significantly positive in each case (indicating heterozygote deficiency; Table 1). There was no systematic pattern to loci deviating from Hardy–Weinberg equilibrium. Similarly, there was no pattern to the significant pair-wise linkage disequilibrium comparisons across collections (Table 1). Seven of the 20 resident collections had from 1 to 6 significant comparisons and the 4 steelhead collections had 2 (Peshastin) to 17 (Chiwaukum 2007) significant comparisons in the 91 pair-wise tests per collection.

Eighteen private alleles were detected in the resident trout collections and were distributed throughout the watershed without any general pattern, i.e., from 1 to 4 per collection in half of the collections. There were 23 private alleles in the steelhead; eleven private alleles were detected in the Chiwaukum 2008 collection. Measures of genetic diversity (allele richness A_R , and observed and expected heterozygosity H_O and H_E) for the resident trout collections were generally lower than the steelhead collections: e.g., A_R : 6.76 versus 7.73 ($P = 0.084$), H_O : 0.727 versus 0.776 ($P = 0.024$), and H_E : 0.726 versus 0.778 ($P = 0.030$) for resident rainbow trout versus steelhead respectively. The average number of alleles per locus varied in the resident collections from 4.36 to 12.57 and generally decreased in the upstream sites (Table 1). For example, Brower had 11.5–12.5 alleles per locus while the two Jack Creek tributaries had 4.36–5.0 alleles per locus. Estimates of A_R varied significantly with upstream river distance ($r^2 = 0.676$; $P = 0.000$) and elevation ($r^2 = 0.846$; $P = 0.000$, Fig. 2). Similar patterns were seen for observed heterozygosity and gene diversity (not shown).

N_e estimates ranged about 4 fold for the resident trout from an estimate of 89 (CI 65–136) at French Creek to a mean of 367 (mean CI 244–770) at the Brower collections (Table 2). The mean N_e estimate for steelhead sampled at Chiwaukum was 77 (mean CI 67–88), a value similar to that seen at Peshastin (88, CI 68–122). Note that even if the point estimate is infinity, the data provide a plausible lower limit of N_e (Waples and Do 2010).

In the global AMOVA, a significant portion of the variance was identified among sites (1.91 %, d.f. 273, $P < 0.001$), and temporal variability of collections within a site was not a statistically significant source of variation (0.09 %, d.f. = 42, $P = 0.055$). In lieu of pooling the temporal collections in subsequent analyses, they remain separate to demonstrate this minor source of variability. Approximately 81 % of the among-collection values of F_{ST} were significantly greater than zero (Appendix 3). The overall F_{ST} value was 0.136. Average F_{ST} between the resident trout and steelhead was 0.053, and the average F_{ST} between the resident trout and hatchery fish was 0.202. Sixty eight percent of the among-resident comparisons were significant (mean $F_{ST} = 0.027$). Mean F_{ST} between steelhead and the hatchery strains of trout was 0.165. The IBD relationship among the resident trout collections suggested two distributions: any comparisons involving collections from Jack Creek (upper distribution in Fig. 3: $r^2 = 0.55$; slope = 0.004, $P = 0.00$) and all other comparisons involving only the main stem collection sites (lower distribution in Fig. 3: $r^2 = 0.80$; slope = 0.001, $P = 0.00$).

In a neighbor joining dendrogram the steelhead collections and the resident trout collections from the Jack Creek watershed (Solomon and Meadow creeks) were at extreme ends of the tree with moderate to strong boot strap support (Fig. 4). Collections from the lower river at Brower and Bridge were distinctive and most similar to the steelhead groups. The branching patterns in the middle of the tree were not well resolved including the upstream collections from Leland and French creeks. Temporal collections at Brower, Johnny, and Chiwaukum clustered closely to their respective group. The hatchery rainbow trout strains (Spokane and Goldendale) formed a distinctive branch.

Five populations were indicated in the STRUCTURE analysis (Fig. 5) where mean $-\ln$ probability (K) for $K = 5$ (−87535) was significantly different from $K = 4$ (−88391; $P = 0.00$) but not K of 6 (−86303; $P = 0.26$). The five groups were the two upper-most collections (Leland and French creeks), the Jack Creek watershed collections, Brower collections, the steelhead collections, and the hatchery rainbow trout strains (Fig. 5). No fish in the watershed had any ancestry of the hatchery trout strains. We note that steelhead-like genotypes appear in Leland and French creeks, and downstream in Brower; similarly, Brower-like genotypes are identified in Chiwaukum Creek (Fig. 5, 6). Although it is possible that these identities are correct, we feel it is as likely that they represent individuals that by chance have a combination of alleles that resemble steelhead or Brower genotypes, respectfully.

We also used the assignment values from STRUCTURE ($K = 5$) to evaluate the composition of collections with respect to fish with ancestry ≥ 80 % for any of four

Table 1 Summary of site information and basic genetic analyses per collection, for expected and observed heterozygosity H_e and H_o , Wright's inbreeding coefficient F_{IS} (P over 325,000 permutations; significant values are bolded), alleles per locus (A/L), allele richness

(A_R), the number of private alleles, and the number of pair-wise locus comparisons out of 91 comparisons in locus disequilibrium (DisEqu). River km for the resident trout refers to Icicle Creek

No./Site	Year	No. of fish	River km	Elevation (m)	H_e	H_o	F_{IS}	P	A/L	A_R	Priv.alleles	DisEqu.
Resident trout												
1 Leland Cr.	2008	22	46.8	987	0.713	0.701	0.041	0.08	7.07	6.09	1	
2 French Cr.	2008	40	35.8	875	0.740	0.743	0.009	0.34	8.86	6.65		
3 Solomon Cr.	2008	18	39.7	1107	0.572	0.582	0.013	0.39	5.00	4.59		
4 Meadow Cr.	2008	16	36.4	1071	0.551	0.554	0.028	0.24	4.36	4.09		
5 Jack Cr.	2007	65	28.9	812	0.684	0.697	-0.012	0.733	7.57	5.90		1
6 Jack Cr.	2008	82	28.9	812	0.678	0.681	0.0012	0.46	8.64	5.79	1	4
							-0.006 ^a	0.67	8.11			4 ^a
7 Icicle-at-Jack	2007	219	27.9	810	0.746	0.723	0.034	0	12.00	5.85	4	
8 Icicle-at-Jack	2008	109	27.9	810	0.737	0.717	0.031	0.007	9.93	6.76	1	
9 Icicle-at-Jack	2009	31	27.9	810	0.729	0.715	0.036	0.067	11.00	6.62		
							0.034^a	0	10.98			11 ^a
10 Hoxie	2007	53	25.3	774	0.743	0.729	0.028	0.059	10.29	6.75		
11 Doctor	2007	24	24.1	766	0.725	0.702	0.055	0.0247	8.07	6.71	1	3
12 Ida	2007	23	23.0	756	0.751	0.759	0.01200	0.27	8.64	6.85		
13 Johnny	2007	52	19.8	704	0.763	0.770	0.011	0.48	10.21	6.82	1	
14 Johnny	2008	79	19.8	704	0.767	0.763	0.009	0.24	11.21	7.25		1
15 Johnny	2009	70	19.8	704	0.763	0.770	-0.001	0.529	9.36	7.27	1	2
							-0.0004 ^a	0.32	10.26			6 ^a
16 Knapweed	2007	19	17.3	633	0.743	0.757	0.010	0.39	8.57	7.39		
17 Bridge	2007	30	16.5	626	0.762	0.765	0.013	0.27	9.64	7.35		1
18 Brower	2007	163	11.5	492	0.782	0.770	0.019	0.019	14.50	7.34	4	6
19 Brower	2008	52	11.5	492	0.783	0.770	0.026	0.68	11.50	7.50	1	1
20 Brower	2009	70	11.5	492	0.788	0.749	0.057	0	12.57	7.51	3	2
							0.029^a	0	12.86			12 ^a
Steel head												
21 Chiwaukum	2007	84	-		0.787	0.787	0.006	0.3184	11.57	8.04	4	17
22 Chiwaukum	2008	123	-		0.787	0.770	0.026	0.007	13.36	7.94	11	6
23 Chiwaukum	2009	53	-		0.758	0.777	-0.015	0.8	11.00	7.99	4	8
							0.015 ^a	0.0208 ^a		7.99 ^a		21 ^a
24 Peshastin Cr.	2005	45	-		0.781	0.771	0.025	0.09	10.21	7.82	4	2
Hatchery trout strain												
25 Goldendale	2006	92	-		0.704	0.715	-0.014	0.8	6.14	8.01	1	5
26 Spokane	2005	96	-		0.739	0.742	0.002	0.46	7.29	7.56	2	3

^a Calculated over all temporal collections of fish from this site

populations (hatchery rainbow trout were excluded). The pattern to the distribution of these “non-introgressed” fish in the water shed may indicate patterns of movements among the populations within the Icicle Creek drainage. Upper Icicle Creek fish were the primary population downstream to the Hoxie-Doctor-Ida sites (Fig. 6); 33 % of the non-introgressed fish at Johnny

Creek were upper Icicle Creek fish (Fig. 6). In comparison, Jack Creek fish constituted 26 % of the non-introgressed fish at Icicle-at-Jack but were not common in collections downstream of this site. Brower fish were frequent upstream to Johnny (63 %), varied from 13 to 27 % of the collections at the Hoxie-Doctor-Ida sites, and were absent at Icicle-at-Jack.

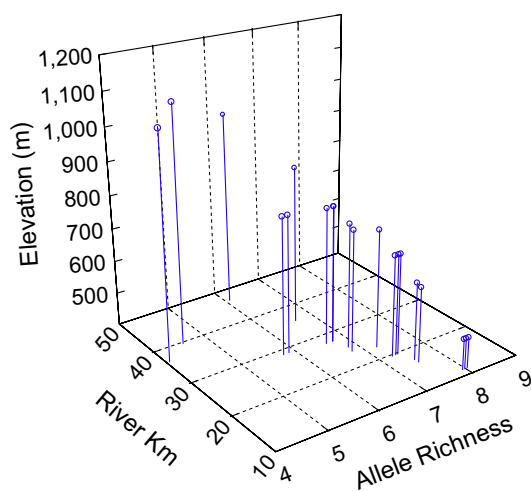


Fig. 2 Scatterplot of allele richness A_R by elevation (m) and river kilometer

We also singled out the assignment values from STRUCTURE ($K = 5$) for 149 young of the year caught in three locations, Icicle-at-Jack, Johnny, and Brower. Origin of these fish with $\geq 80\%$ ancestry matched that seen in Fig. 6, i.e., the local ancestral group dominated at a site. For example, of the 13 $\geq 80\%$ ancestry fish at Icicle-at-Jack, 8 (62%) were upper Icicle Creek origin, and 5 (38%) were Jack Creek origin (in other words, no Brower young of the year were detected). Of the 28 $\geq 80\%$ ancestry fish at Johnny, 20 (72%) were Brower origin, 6 (21%) were upper Icicle Creek, and 2 (7%) were Jack Creek origin. 28 young of the year with $\geq 80\%$ ancestry assignments were seen at Brower: they were 96% Brower with only one upper Icicle Creek young of the year seen.

Discussion

We report significant genetic differences between above-barrier trout and downstream steelhead collections, and significant and systematic differences in measures of genetic diversity among the resident trout.

Genetic diversity within gene pools

We report a decrease in genetic variability in upstream collections of rainbow trout. Although the resident trout collections in the lowest part of upper Icicle Creek have levels of genetic richness comparable to the steelhead collections sampled in neighboring streams, measures of genetic variability (H_e , A_R , and A/L) drop off in the upper populations, decreasing significantly upstream over 35 river km and an elevation gain of 615 m (Fig. 2). Resident trout in the upstream tributaries of Jack Creek, for example, had 5 alleles per locus in comparison to lower mainstem

Table 2 Estimates of N_e from LDNE (Waples and Do 2008) using the lowest allele frequency of $P_{crit} = 0.02$ except when collection size N was <40 , then $P_{crit} = 1/2 N$; using fish with no missing data only

	Harmonic collection Mean	Estimate of N_e	Min	Max
Resident trout				
Leland	21	∞	89	∞
French	39	89	65	136
Solomon	16	32	16	135
Meadow	16	∞	58	∞
Jack 2007	58	126	91	195
Jack 2008	82	122	95	166
Icicle-at-Jack 2007	209	490	373	694
Icicle-at-Jack 2008	107	330	229	553
Icicle-at-Jack 2009	27	130	65	314
Hoxie	50	382	188	924
Doctor	22	∞	93	∞
Ida	22	86	45	655
Johnny 2007	45	∞	636	∞
Johnny 2008	77	639	312	343
Johnny 2009	66	456	245	944
Knapweed	17	∞	66	∞
Bridge	29	∞	538	∞
Brower 2007	158	472	351	703
Brower 2008	52	244	152	560
Brower 2009	65	384	229	1048
Steelhead				
Chiwaukum 2007	80	36	32	40
Chiwaukum 2008	121	109	96	123
Chiwaukum 2009	66	85	72	103
Peshastin	42	88	68	122
Hatchery trout				
Spokane trout	95	125	98	168
Goldendale trout	89	62	52	89

collections that have ~ 13 alleles per locus (Table 1). This is congruent with recent work of *O. mykiss* that has reported decreasing diversity with increasing elevation and isolation (Deiner et al. 2007; Narum et al. 2008; Kozfkay et al. 2011). Paralleling these changes in estimates of genetic richness, the estimates of the effective number of spawners N_e changed approximately 4-fold from the upper tributaries where $N_e \sim 90$ to the lower mainstem collections where $N_e \sim 360$ (Table 2).

Genetic differentiation among gene pools

We report several genetic differences of interest for future monitoring of steelhead recolonization of Icicle Creek.

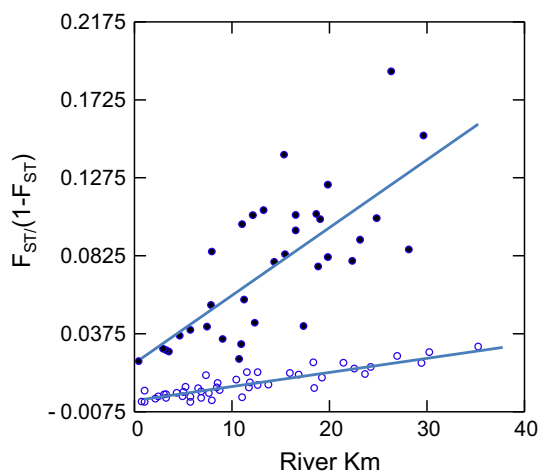


Fig. 3 Plot of all pair-wise comparisons among resident rainbow trout collections of genetic distance estimated as $F_{ST} (1-F_{ST})$ and geographic distance (river kilometer). Comparisons with Jack Creek collections are filled circles ($y = 0.004x + 0.02$) and comparisons among main stem collections are open circles ($y = 0.001x - 0.002$)

First, the resident rainbow trout are genetically distinctive from nearest-neighbor collections of steelhead. These two allopatric groups of *O. mykiss*, distinguished in the branching patterns of a CSE tree and in a STRUCTURE analysis, differ at an F_{ST} value of 0.053. Note that residents and steelhead differ in the frequency of occurrence of shared alleles and not in the occurrence of fixed allele differences.

Distinguishing above-barrier *O. mykiss* from anadromous *O. mykiss* has been reported with allozyme markers (e.g., Parkinson et al. 1984, Currens et al. 1990) and more recently with mSAT markers in British Columbia (Heath et al. 2001), Washington (Small et al. 2007, Kozfkay et al. 2011), and in coastal California (Deiner et al. 2007; Clemento et al. 2008; Pearse et al. 2009). Levels of mSAT differentiation has ranged from minimal levels ($F_{ST} = 0.030$) in the Green River, Puget Sound basin (Winans et al. 2008) to higher values in northern California, ($F_{ST} = 0.158$; 7 locales in the Russian River, (Deiner et al. 2007). Dampened values may be the result of upstream transplants and mixing of trout (i.e., Winans et al. 2010) whereas elevated values may be the result of the greater isolation time and, in some cases, the involvement of small fragmented populations inhabiting the southern extreme of the species distribution and experiencing periodic bottlenecks (Deiner et al. 2007). Here we found a moderate level of differentiation between resident rainbow trout versus downstream steelhead at $F_{ST} = 0.053$.

Secondly, we identify separate genetic groups of resident fish above the anthropogenic barrier in Icicle Creek. Over the ~35 rkm of mainstem corridor, the lowest elevation collections (at Brower and Bridge) and the highest elevation collections (Leland and French) are significantly different at $F_{ST} = 0.024$. Moreover, collections from the upper Jack Creek are 3.7 times more differentiated from the lower river collections at $F_{ST} = 0.089$, (separated by

Fig. 4 Neighbour joining dendrogram of Cavalli-Sforza and Edwards chord values. Number at nodes indicates the percentage (when >60 %) of 1,000 dendrograms in which collections beyond the nodes grouped together. Ellipses enclose in some cases temporal collections from 2007 to 2009 (labeled 07, 08, and 09)

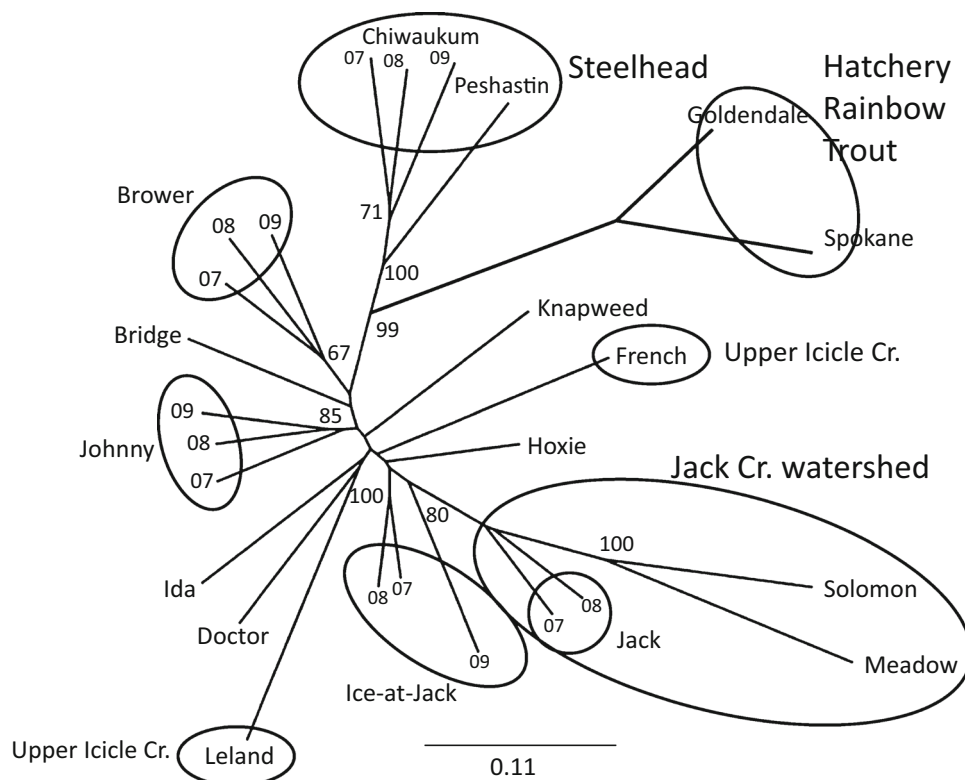
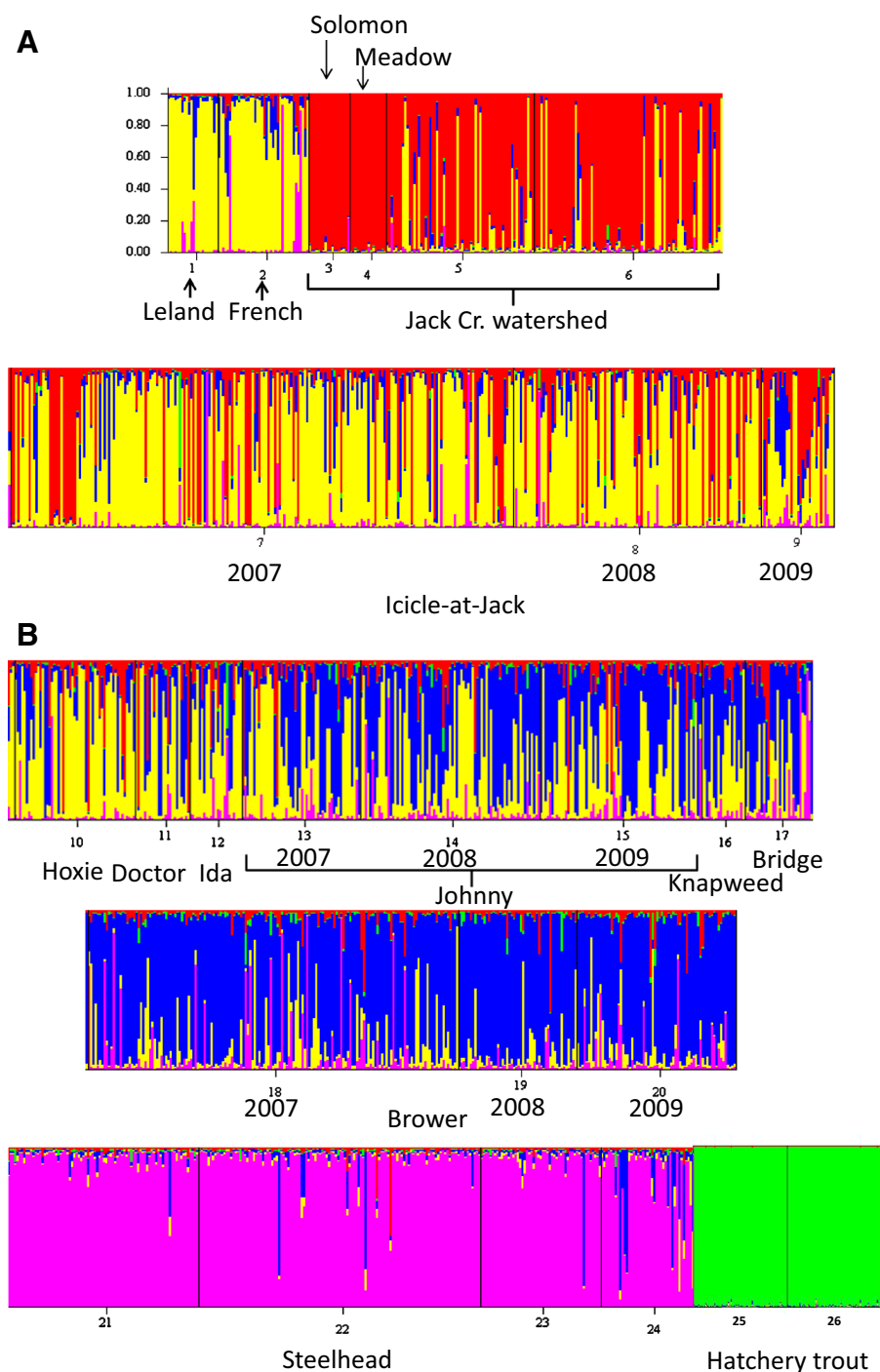


Fig. 5 Results of STRUCTURE analysis of 1738 *O. mykiss*; for $K = 5$. Individual estimates of percent ancestry to the hypothetical populations (Y axis) versus collection site numbers (X axis) as provided in Table 1. Hypothetical populations are yellow (*upper Icicle*), red (*Jack Creek*), blue (*Brower*), purple (steelhead), and green (hatchery rainbow trout). Single analysis illustrated in **A** and **B**



~ 28 rkm) and highly differentiated from the upper Icicle Creek collections at $F_{ST} = 0.128$ (separated on average 16.4 rkm). We propose the recognition of three populations of resident rainbow trout in Icicle Creek. Although there is reproductive exchange between these three groups (Fig. 5), the data suggest that the three populations remain distinctive over at least a three year period.

Fine-scale geographic differentiation among collections of *O. mykiss* has been alluded to in several studies, but none

of these works has provided precise geographic information for comparison (Clemento et al. 2008; Small et al. 2007; Winans et al. 2008). Fine-scale patterns of differentiation have been also reported in other salmonids, including Brook trout *Salvelinus fontinalis* (Kanno et al. 2011), brown trout *Salmo trutta* (Stelkens et al. 2012), and Atlantic salmon *S. salar* (Dionne et al. 2009; Gudmundsson et al. 2013) where collections separated from 4 to 140 rkm had reported F_{ST} values as great as 0.085 (i.e., Stelkens et al. 2012).

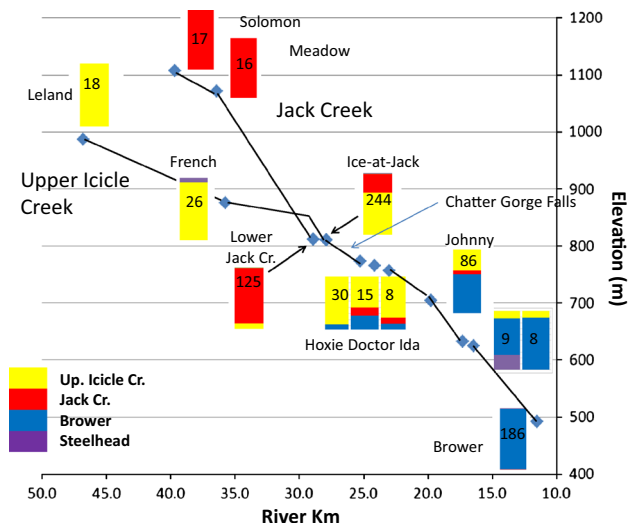


Fig. 6 Percent composition of collections for fish with $\geq 80\%$ ancestry to a single population where the populations are yellow (upper Icicle), red (Jack Creek), blue (Brower), and purple (steelhead) (hatchery rainbow trout are excluded). Number of fish in a group is indicated; lines connect contiguous sites for visual aid only

We assume that the majority of the mSAT variability is driven by random genetic drift and gene flow. Given that the upriver populations have estimates of N_e of 136 or less, it is likely that drift will be affecting the genetic variability in these collections. Likewise we assume that, as salmonids these fish exhibit strong philopatry and habitat selection (Taylor 1991; Quinn 2005). These behaviors have been recognized as important premating isolating mechanisms (sensu Mayr 1963) that can lead to isolation and differentiation through reduced gene flow (although see Fraser et al. 2011). We present data that show, for the life history stages of fish studied here, there is apparently limited dispersal among the resident populations. Upstream fish from Jack Creek and upper Icicle Creek appear downstream but in diminishing numbers past Chatter Gorge Falls (traveling 20–30 rkm), whereas Brower fish do not appear above the Falls (Fig. 6). The pattern of yearlings in the creek generally confirms that upstream populations are found downstream in diminishing numbers and Brower yearlings are seen in Johnny but not upstream above Chatter Gorge Falls at Icicle-at-Jack.

Genetic connectivity may also be affected by environmental features. For example, IBD is seen among collections along the main stem of the river and is stronger when comparisons with the Jack Creek collections are involved. Whereas instream cascades and local gradient breaks routinely contribute to population structure in stream fishes (Frank et al. 2011), the largest F_{ST} values were observed here between the two pairs of headwater collections (Leland/French and Solomon/Meadow) between which there

are no migration barriers. In fact, less differentiation was seen between either of these two groups and the third population downstream at Brower from which they were both separated by the maximum number of barriers in the watershed. Although passage barriers may account for some of the observed stock structure in the Icicle Creek by restricting upstream movement, the strongest differences are not associated with passage barriers in our analysis. Separating these two pair of head water collections is the Jack Creek/Icicle Creek confluence, a branching point or node—a feature reported to affect within-river connectivity (Tamkee et al. 2010; Kanno et al. 2011). A fish traveling between these two locations must change direction at the node, and swim upstream, presumably an energetically expensive behavior. This node may also be inhibiting gene flow among the two groups. This is the only tributary node in Icicle Creek and we suggest that the effect of nodes on fine-scale variability be examined in more complex watersheds. Finally, it is also possible that the observed differentiation is associated with genetic changes due to local adaptation. The upstream populations are subjected to harsh winter conditions and short growing season. We estimate that the average water temperatures in these streams is $<2\text{ }^\circ\text{C}$ for 6 months (Gayeski, unpublished data). Temperature-related adaption in physiological compensation and early life history traits may be expected (Jensen et al. 2008). Although the gene pools mix to some degree in parts of the watershed, they may remain genetically distinctive due, in part, also to some degree of local adaptation to their specific environments. Further exploration into the causes of these patterns of differentiation may include common garden experiments, (e.g., Doctor et al. 2014, Doctor et al. *submitted*).

Thirdly, there is no indication of any genetic influence from the two hatchery trout stocks in the *O. mykiss* collections studied here. Icicle Creek was periodically stocked with one or both of the hatchery strains up to the mid-1990s when Washington State ceased the practice of planting hatchery rainbow trout in rivers and streams. Headwater lakes are still stocked and it is physically possible for some of these fish to migrate to small tributaries into the basin via lake outlets. Nevertheless, the mSAT loci provide a strong genetic signature, hatchery trout were correctly assigned 100% of the time in the structure analysis, and there is no indication of hatchery influence in the resident trout collections. Although the two strains we tested are the primary hatchery strains planted in eastern Washington, other unknown hatchery transplants may have occurred in the Icicle Creek watershed from other hatchery sources and thus are undetected here, although this seems unlikely.

In summary, two pair of head water collections exhibit decreased levels of genetic richness (see also Deiner et al. 2007; Narum et al. 2008) and correspondingly small N_e

values. With limited or zero upstream gene flow and small population sizes, differences among these populations may be driven in part by genetic drift. On the other hand, larger N_e values in the downstream gene pool at Brower make genetic drift less likely in this portion of the water shed. We further hypothesize that the population structure of resident trout in Icicle Creek may be attributed in part to strong environmental differences (principally temperature and length of growing season) along the mainstem and in the upper head water locations.

Conservation issues

Little is known about the diversity and biological characteristics of resident rainbow trout, although it is an important life history form of a species that is threatened or endangered throughout most of its distribution (Good et al. 2005, Ford 2011). Throughout the species' distribution, there are a large number of recognized barriers. For example, there are 798 dams in the State of Washington (CorpsMap National Inventory of Dams www.geo.usace.army.mil/pgis) with over 400 in the Puget Sound Basin alone (Schlenger et al. 2011). If even only a fraction of these barriers harbor isolated resident fish, a considerable number of distinctive genetic populations are yet to be recognized. We provide the laboratory protocol and an incipient mSAT baseline that can be applied to recognize population diversity and help monitor metapopulation dynamics through time in *O. mykiss*. A thorough characterization of resident fish conducted on a fine scale is needed to begin to understand the extent of their variability and their potential role in the population structure of the species.

When barriers are breached or removed, it will be particularly important to observe how resident rainbow trout will respond to an opportunity to smolt and exit to the ocean. In one case (Sashin Creek, Alaska), a small percentage of resident fish (founded from a steelhead gene pool) isolated from anadromy for 80+ years still smolted and exited to seawater under experimental conditions—but exhibited an exceedingly low adult return rate (Thrower et al. 2004). How successful will smolts from resident × resident trout families recruit as steelhead, what will be the frequency of resident × steelhead matings, and how will the recruit rates of smolts from either of these two mating types compare to the recruiting success of steelhead × steelhead matings? In sympatry, the return rate of resident × steelhead smolts has been measured even when the

genetic differences are minimal or nil because of substantial gene flow between the two life history forms (Christie et al. 2011; Courter et al. 2013; Van Doornik et al. 2013). When multiple resident populations are recognized, each resident type, by nature of its habitat and genetics, may respond differently to opportunities to smolt and/or genetically interact with steelhead. Given the various trajectories of adaptation to upstream conditions including growth, movement behavior, body shape, and coloration (Morita et al. 2000; Morita and Yamamoto 2001; Winans et al. 2010; Stelkens et al. 2012) and plasticity of these and other life history characters (summarized in Garcia de Leaniz et al. 2007), any predictions about the effect of introgression of resident and steelhead are conjectural at best.

On a broader scale, many riverine fish are affected by dams and barriers. Habitat fragmentation and disruption of genetic connectivity among populations are conservation concerns not only in other salmonids, e.g., brook trout *S. fontinalis* (Kelson et al. 2014), bull trout *S. confluentus* (DeHaan et al. 2011), and Lahontan cutthroat trout *O. clarkii henshawi* (Neville et al. 2006), but in other freshwater species, e.g., bullhead *Cottus gobio* (Junker et al. 2012); tallapoosa shiner *Cyprinella gibbsi*, and tallapoosa darter *Etheostoma tallapoosae* (Fluker et al. 2014); Yazoo darter *E. raneyi* (Sterling et al. 2012); and creek chub *Semotilus atromaculatus* (Hudman and Gido 2013). Our work highlights the need to conduct, when possible, fine scale sampling above the barrier to evaluate for the presence of multiple resident/affected stocks. Not all dam-affected fish may be alike. And in general, small isolated populations can play critical roles in maintaining gene diversity in a species (Allendorf et al. 2008). Conservation efforts that include fine-scale evaluations of population structure above barriers will increase the accuracy of understanding and monitoring the genetic and biological effects of dams and dam removal.

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Appendix 1

Barriers to upstream movement of resident-sized rainbow trout in Icicle Creek. Known barriers are **; all others are potential barriers. Relevant collection sites are included for perspective

Barrier name	rkm	Elevation drop (m)	Distance (m)	Gradient (%)
Jack Creek site	28.9			
Jack/Icicle confluence	28.7	1.1	150	0.7
Icicle-at-Jack site	27.9			
Chatter Gorge Falls**	26.3	4.5	100	4.5
Hoxie	25.3			
Doctor	24.1			
Ida	23.0			
Johnny site	19.8			
Icicle-at-Johnny	19.7	2	150	1.3
Cascade Reach 1	19.4	8.7	140	6.2
Cascade Reach 2	18.2	28.1	710	4.0
Knapweed	17.3			
Bridge	16.5			
Cascade Reach 3**	16	14.4	200	7.2
Cascade Reach 4	13.2	24.1	370	6.5
Icicle-Brower	11.6	3.5	190	1.8
Brower	11.5			
Cascade Reach 5	9.8	32.4	610	5.3

Appendix 2

Summary data for 14 microsatellite loci where H_o and H_e are observed and expected heterozygosity, and F_{ST} is the index of differentiation (S.E. standard error) after Weir and Cockerham (1984). Size range of observed alleles is provided

Locus	Number of alleles	Observed alleles	H_o	H_e	F_{ST}	(S.E.)
<i>Ocl1</i>	23	150–230	0.815	0.830	0.048	(0.017)
<i>Ogo4</i>	13	115–151	0.708	0.715	0.071	(0.023)
<i>Oke4</i>	22	234–272	0.671	0.680	0.091	(0.040)
<i>Oki23</i>	23	116–204	0.757	0.767	0.063	(0.024)
<i>Omy07</i>	21	234–304	0.812	0.808	0.060	(0.005)
<i>Omy1001</i>	30	172–246	0.819	0.834	0.058	(0.018)
<i>Omy1011</i>	21	118–214	0.820	0.834	0.073	(0.022)
<i>One14</i>	11	146–192	0.482	0.528	0.146	(0.081)
<i>Ots100</i>	27	158–226	0.827	0.876	0.048	(0.017)
<i>Ots3</i>	12	68–94	0.600	0.607	0.094	(0.043)
<i>Ots4</i>	10	105–129	0.712	0.694	0.054	(0.023)
<i>Ssa289</i>	8	105–119	0.551	0.581	0.115	(0.059)
<i>Ssa407</i>	26	159–263	0.836	0.822	0.052	(0.012)
<i>Ssa408</i>	23	165–265	0.819	0.825	0.075	(0.028)
Overall	270		0.731	0.743	0.068	(0.006)

Appendix 3

Below diagonal are pairwise F_{ST} values among collections. Bolded values were significantly different from zero ($P < 0.0004$, adjusted 0.05 level for multiple comparisons). Above diagonal is the number of loci out of 14 that are significantly different at $P = 0.05$ level

	Lela	Fren	Solo	Mead	Jack7	Jack7	Jack7	Jack7	Icelk7	Icelk7	Icelk8	Icelk9	Hox	Doc	Ida	Joh7	Joh8	Joh9	Knap
Leland	0.000	5	9	11	11	10	10	4	3	6	2	2	3	2	2	2	3	3	2
French	0.030	0.000	11	10	9	12	7	7	6	3	3	3	3	3	3	4	7	7	0
Solomon	0.132	0.096	0.000	2	1	2	8	8	5	4	5	4	8	5	7	4	8	8	4
Meadow	0.159	0.123	0.027	0.000	5	4	8	8	8	6	7	8	9	7	8	9	8	10	7
Jack07	0.067	0.047	0.022	0.040	0.000	0	7	7	8	2	3	7	4	3	7	7	9	9	2
Jack08	0.072	0.052	0.023	0.040	-0.001	0.000	9	9	10	4	4	8	6	4	8	10	10	11	5
Icelk07	0.020	0.013	0.055	0.078	0.018	0.023	0.000	0.000	0	2	0	1	0	0	1	4	8	8	0
Icelk08	0.019	0.013	0.057	0.083	0.020	0.026	0.000	0.000	1	1	0	0	1	0	0	1	4	7	0
Icelk09	0.038	0.022	0.058	0.081	0.016	0.016	0.010	0.011	0.000	1	0	1	1	0	1	1	1	1	0
Hoxie	0.020	0.011	0.073	0.091	0.023	0.027	0.003	0.001	0.012	0.000	1	0	0.000	1	0	0	1	1	0
Doctor	0.017	0.015	0.077	0.096	0.032	0.036	0.005	0.003	0.006	0.004	0.000	0.006	0.004	0.000	0	0	0	1	0
Ida	0.014	0.015	0.088	0.098	0.036	0.038	0.007	0.007	0.012	0.000	-0.002	0.012	0.000	-0.002	0.000	0	0	0	0
John07	0.021	0.011	0.073	0.092	0.028	0.033	0.006	0.006	0.005	0.012	0.000	0.012	0.000	0.002	-0.001	0.000	0	0	0

	Lela	Fren	Solo	Mead	Jack7	Jack8	Jack8	Bro9	Ch7	Ch7	Ch8	Ch9	Doc	Ida	Joh7	Joh8	Joh9	Knap
Leland	4	8	8	8	7	7	6	6	13	13	12	10	10	10	14	14	14	14
French	5	9	9	10	10	10	8	8	13	13	13	11	11	11	13	14	14	14
Solomon	9	10	10	7	7	9	9	9	10	10	12	11	11	11	12	14	14	14
Meadow	9	10	10	9	9	10	10	10	12	12	12	12	12	12	11	14	14	14
Jack07	7	11	11	9	9	9	9	9	13	13	13	13	13	13	12	14	14	14
Jack08	8	12	12	10	10	11	11	11	13	13	13	13	13	13	12	14	14	14
Icelk07	4	13	13	11	11	12	12	12	13	13	13	13	13	13	12	14	14	14
Icelk08	4	12	12	10	10	11	11	11	13	13	13	12	12	11	14	14	14	14
Icelk09	2	5	5	5	5	1	1	1	10	10	12	10	10	9	14	14	14	14
Hoxie	0	4	4	4	4	4	4	4	11	11	12	11	11	11	14	14	14	14
Doctor	1	1	1	2	2	0	0	0	11	11	11	10	10	9	14	14	14	14
Ida	0	1	1	1	1	1	1	1	9	9	7	7	7	8	14	14	14	14
John07	0	3	3	3	3	3	3	3	11	11	11	9	9	10	14	14	14	14

	Lela	Fren	Solo	Mead	Jack7	Jack8	Jack8	Icelk7	Icelk8	Icelk9	Hox	Doc	Ida	Joh7	Joh8	Joh9	Knap
John08	0.025	0.014	0.080	0.101	0.030	0.035	0.009	0.010	0.010	0.009	0.004	0.003	0.003	-0.001	0.000	0	0
John09	0.026	0.018	0.079	0.100	0.033	0.036	0.012	0.013	0.013	0.010	0.007	0.004	0.004	0.001	0.000	0.000	0
Knap	0.020	0.006	0.073	0.094	0.024	0.028	0.001	0.000	0.005	0.005	-0.001	0.000	-0.002	-0.003	0.000	0.005	0.000
Bridge	0.026	0.012	0.084	0.110	0.038	0.042	0.010	0.009	0.013	0.013	0.005	0.003	0.006	0.000	0.001	0.000	-0.002

continued

	Lela	Fren	Solo	Mead	Jack7	Jack8	IccJk7	IccJk8	IccJk9	Hox	Doc	Ida	Joh7	Joh8	Joh9	Knap
Brow07	0.028	0.017	0.080	0.097	0.037	0.041	0.013	0.014	0.019	0.008	0.007	0.007	0.005	0.006	0.007	0.001
Brow08	0.031	0.021	0.080	0.095	0.039	0.045	0.013	0.015	0.022	0.010	0.008	0.006	0.006	0.006	0.006	0.001
Brow09	0.031	0.019	0.084	0.097	0.037	0.042	0.015	0.015	0.015	0.007	0.009	0.006	0.005	0.007	0.008	0.001
Chiw07	0.054	0.058	0.133	0.149	0.081	0.082	0.053	0.054	0.046	0.046	0.051	0.036	0.039	0.038	0.038	0.040
Chiw08	0.050	0.057	0.131	0.151	0.082	0.084	0.051	0.050	0.046	0.045	0.048	0.039	0.040	0.039	0.041	0.038
Chiw09	0.052	0.062	0.143	0.172	0.088	0.090	0.054	0.055	0.051	0.051	0.055	0.044	0.042	0.041	0.043	0.040
Peshastin	0.053	0.052	0.130	0.142	0.079	0.080	0.050	0.049	0.042	0.043	0.047	0.036	0.034	0.035	0.033	0.034
Goldendale	0.194	0.197	0.288	0.297	0.230	0.236	0.194	0.199	0.205	0.195	0.208	0.186	0.183	0.173	0.177	0.186
Spokane	0.205	0.203	0.275	0.272	0.235	0.242	0.200	0.201	0.210	0.197	0.205	0.183	0.184	0.182	0.184	0.18
		Brid	Bro7	Bro8	Bro9	Chi7	Chi8	Chi9	Pesh	Gold	Spok					
John08	0	5	3	3	3	12	14	12	11	14	14	11	11	14	14	14
John09	0	5	4	4	4	14	14	11	10	14	14	10	10	14	14	14
Knap	0	0	0	0	0	7	7	6	10	7	6	10	10	14	14	14
Bridge	0.000	1	0	0	1	8	8	6	5	8	6	5	5	14	14	14
Brower07	0.000	0.000	0	0	1	13	14	12	10	14	12	10	14	14	14	14
Brower09	0.003	0.001	0.000	0.000	0.000	13	13	9	11	13	9	11	11	14	14	14
Chiwaukm07	0.029	0.035	0.036	0.036	0.032	0.000	2	1	5	2	1	5	5	14	14	14
Chiwaukm08	0.028	0.034	0.034	0.034	0.031	0.004	0.000	1	5	0.000	1	5	5	14	14	14
Chiwaukm09	0.032	0.036	0.036	0.039	0.034	0.008	0.008	0.000	3	0.005	0.000	3	3	14	14	14
Peshastin	0.026	0.031	0.031	0.030	0.027	0.008	0.008	0.013	0.000	0.010	0.013	0.000	0.000	14	14	14
Goldendale	0.188	0.169	0.165	0.167	0.165	0.171	0.170	0.179	0.167	0.170	0.179	0.167	0.167	0.000	0.000	14
Spokane	0.182	0.173	0.168	0.169	0.168	0.153	0.156	0.171	0.151	0.156	0.171	0.151	0.151	0.141	0.141	0.000

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