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ARTICLE

Genetic analysis of Dolly Varden (*Salvelinus malma*) across its North American range: evidence for a contact zone in southcentral Alaska

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Abstract: Contact zones between divergent lineages of aquatic taxa have been described from the northeastern Pacific Ocean. We surveyed samples of Dolly Varden (*Salvelinus malma*) from their North American range for variation at 14 microsatellite DNA loci. After accounting for hybridization between Dolly Varden and co-occurring bull trout (*Salvelinus confluentus*) and Arctic char (*Salvelinus alpinus*), we found evidence for two genetic lineages of Dolly Varden consistent with the previously recognized subspecies, northern Dolly Varden (*S. m. malma*) and southern Dolly Varden (*S. m. lordii*). We documented a contact zone between the two subspecies from the eastern Alaska Peninsula to Cook Inlet, Alaska, where admixture values (i.e., the proportion of the genome estimated to be composed of northern Dolly Varden, Q_{NDV}) ranged between Q_{NDV} = 0.245 and 0.754 across about 700 ocean kilometres. Populations of Dolly Varden showing low admixture (i.e., less than 5%) were located a minimum of 346 km to the west to 1200 km to the southeast, respectively, from the contact zone. The two lineages of Dolly Varden probably stem from isolation and subsequent divergence in, and dispersal from, distinct northern and southern Pleistocene glacial refugia and substantiate the treatment of *S. malma* as two subspecies and as at least two designatable units under Canada's Species at Risk Act.

Résumé : Des zones de contact entre des lignées divergentes de taxons aquatiques ont été décrites dans le nord-est de l'océan Pacifique. Nous avons examiné des échantillons d'ombles Dolly Varden (*Salvelinus malma*) provenant de l'aire de répartition nord-américaine de l'espèce pour évaluer les variations dans 14 microsatellites d'ADN. Une fois les effets de l'hybridation entre les Dolly Varden et les ombles à tête plate (*Salvelinus confluentus*) et ombles chevaliers (*Salvelinus alpinus*) cooccurrents pris en compte, nous avons trouvé des indices de la présence de deux lignées génétiques de Dolly Varden coïncidant avec des sous-espèces déjà reconnues, les ombles Dolly Varden du Nord (*S. m. malma*) et du Sud (*S. m. lordii*). Nous avons documenté une zone de contact entre les deux sous-espèces allant de l'est de la péninsule de l'Alaska jusqu'à Cook Inlet (Alaska), où les valeurs de mélange (c.-à-d. la proportion estimée du génome provenant du Dolly Varden du Nord, Q_{NDV}) allaient de 0,245 à 0,754 sur environ 700 km d'océan. Les populations de Dolly Varden présentant un faible mélange (c.-à-d. moins de 5 %) étaient situées à au moins 346 km à l'ouest à 1200 km au sud-est, respectivement, de la zone de contact. Les deux lignées de Dolly Varden sont probablement issues de l'isolement et de la divergence subséquente dans des refuges glaciaires pléistocènes septentrional et méridional distincts, suivis de leur dispersion à partir de ces refuges. Les résultats soulignent la pertinence de traiter *S. malma* comme comprenant deux sous-espèces et au moins deux unités pouvant être désignées en vertu de la Loi sur les espèces en péril du Canada. [Traduit par la Rédaction]

Introduction

Fishes of the Holarctic genus *Salvelinus* (i.e., chars) have provided many opportunities to study geographic variation in morphology, genetics, and ecology, and their implications for understanding processes of evolutionary change and their relevance to taxonomy and conservation (e.g., Behnke 1972; Nordeng 1983; Reist et al. 2013). One of the more challenging groups within the genus comprises what was once known as the Arctic char (*Salvelinus alpinus*) "species complex" (Walters 1955; McPhail 1961). The Arctic char, first described by Linneaus in 1758, was at one time considered to comprise myriad forms that we now know constitute at least three species: Arctic char, found throughout the Holarctic usually above 55°N latitude, Dolly Varden (*Salvelinus malma*), found both in the northwestern and northeastern Pacific and western Arctic of Canada and the USA typically between 48°N and 65°N latitude, and the bull trout (*Salvelinus confluentus*), found in the eastern North Pacific and northwestern Canadian Arctic from 40°N to 65°N latitude (Scott and Crossman 1973; McPhail 2007). All three species may be anadromous or reside permanently in freshwater lakes and streams.

Notwithstanding the resolution of these three species within the former *S. alpinus* species complex, myriad subspecies, ecological forms, and evolutionary subgroups continue to be proposed within each taxon (Behnke 1972; Taylor et al. 1999; Klemetsen 2010). One of the longest studied aspects of intraspecific diversity in *Salvelinus* has been the suggestion that Dolly Varden consists of several subspecies based on geographic differences in various traits. One, a northern form designated *S. malma malma*, is found in Kamchatka and further north in the western Pacific and north of the Alaska Peninsula in the eastern Pacific and east to the Mackenzie River in Canada's western Arctic. At least three southern subspecies have been described: *S. m. miyabei* from Japan, *S. m. kraschenninikovi* from the western Pacific south of Kamchatka (Taranetz 1933), and *S. m. lordii* south of the Alaska Peninsula in the eastern Pacific (Günther 1866). Although he did not assign names to distinct forms

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of Dolly Varden, McPhail (1961) conducted a detailed morphological analysis among North American Dolly Varden and recognized two major groups: those found north of the Alaska Peninsula (with 65–71 vertebrae and 11–14 gill rakers on the lower limb of the first gill arch) and those south of the peninsula (57–67 vertebrae and 8–12 gill rakers on the lower limb of the first gill arch). Further subdivisions within geographic groupings of Dolly Varden have also been proposed (e.g., DeCicco and Reist 1999), but the most consistent distinction, whether accompanied by formal taxonomic recognition or not, has been between so-called "northern" and "southern" Dolly Varden in the eastern Pacific (reviewed by Kowalchuk et al. 2010). Phillips et al. (1999) reported variation in karyology and ribosomal ITS1 sequences that strongly supported distinctions between populations representing *S. m. malma* and *S. m. lordii*. Further, comparison of the molecular data in Dolly Varden with that in two subspecies of Arctic char, *S. a. alpinus* and *S. a. eurythrinus*, suggested that Dolly Varden may be paraphyletic and that the northern and southern groups have diverged independently from *S. a. alpinus* and *S. a. eurythrinus*, respectively (Phillips et al. 1999; see also Taylor et al. 2008).

Within the context of continuing uncertainty about the ultimate evolutionary origin of Dolly Varden lineages and their taxonomic status, the species remains an important aspect of subsistence and recreational fisheries, particularly in northern portions of its North American range (COSEWIC 2011). The vast majority of studies of population structure of North American Dolly Varden have occurred in Alaska, and there is virtually nothing known of the population structure of Dolly Varden south of the Alaska Panhandle despite the widespread occurrence of the species throughout coastal British Columbia and portions of western Washington State. In addition, areas from the Bering Sea south to the Alexander Archipelago in southeastern Alaska have been associated with contact zones in a variety of aquatic and terrestrial taxa (e.g., Seeb and Crane 1999; Cook et al. 2001; Hoffman et al. 2006). Given the evidence for two groups of Dolly Varden and the proposal that the Alaska Peninsula is a point of separation between the forms, more intensive sampling of Dolly Varden across its North American range may resolve a zone of contact between northern and southern Dolly Varden. If such a zone of contact exists, evaluation of the extent to which the two forms interbreed upon contact could be informative in evaluating the hypothesis that northern and southern Dolly Varden may represent distinct species rather than subspecies (Kowalchuk et al. 2010).

In this study, we made use of archival collections of Dolly Varden from across their entire North American range over a 25-year period to (*i*) assay microsatellite DNA variation across 14 loci to test for the existence of two or more geographically distinct genetic lineages, (*ii*) assess the correspondence between the geographic distribution of genetic lineages and the distribution of northern and southern Dolly Varden as currently understood, and (*iii*) explicitly test for the existence of areas of genetic admixture between genetic groups that might represent a contact zone between forms.

Materials and methods

Tissue collections

A total of 993 tissue samples (dried fin clips or fin clips stored in 95% ethanol) of Dolly Varden were analyzed from 58 localities, including 20 samples of *S. malma kraschenninikovi* from the Sorachi River in central Hokkaido, Japan (Table 1; Fig. 1). In addition, and because Dolly Varden have been shown to hybridize both with bull trout and Arctic char (Baxter et al. 1997; Redenbach and Taylor 2003; Taylor et al. 2008), we included 64 bull trout and 62 Arctic char from four and two localities, respectively, such that we could detect and account for any hybridization in our samples. Known samples of bull trout and Arctic char were diagnosed from each other and from Dolly Varden based on a combination of biogeography, morphological features, and nuclear DNA loci (see Haas and McPhail 1991; Redenbach and Taylor 2003; May-McNally 2014).

Many of the char samples were collected opportunistically and were associated with species-level diagnostic tests in environmental assessment studies by various agencies or private consultant companies. Still others were collected as part of targeted federal (US and Canada), territorial (Canada), provincial or state population assessments, or various graduate research projects (e.g., Redenbach 2000; May-McNally 2014) conducted since the 1990s. Consequently, precise locality data (i.e., specific reach of river, stream, or position in a lake) or methods of collection were not always available. In cases of samples missing specific locality information, geographic coordinates were estimated from physical site descriptions and Google Earth. In general, sampling included monitoring fences, electrofishing, minnow trapping, gill or seine-netting, and angling. Sample sizes varied from 1 to a maximum of 65 individuals and included age classes from newly emerged fry (30 mm) to adult fish (up to 1 m in length). The sampling is, therefore, highly variable in scope and methodology, and our study is restricted to examining the structure of Dolly Varden genetic variability across its North American range in the context of the distribution of putative northern and southern genetic groups or subspecies. Except in a small number of cases where sampling was extensive enough within a geographic area, the data were not examined in terms of interpopulation genetic structure, an area of investigation that requires further attention particularly in southern portions of the range of Dolly Varden in North America.

DNA extractions and microsatellite DNA analyses

We extracted genomic DNA from fin clips using either standard phenol–chloroform procedures (pre-2003 samples) or the DNeasy DNA blood and tissue extraction kit (Qiagen Inc., Valencia, California, USA) following kit protocols. Extracted DNA samples were stored at –20 °C for later use in multiplex polymerase chain reactions (PCR) using the Qiagen Multiplex Kit (Cat. No. 206145). The DNA was amplified in 10 μL PCR reactions at 95 °C for 15 min, 94 °C for 30 s, 35 cycles of 1.5 min at an annealing temperature of 55 °C followed by 72 °C for 1 min, and 60 °C for 30 min.

Microsatellite variation was assayed using primers end-labelled with infrared fluorophores and a 3730S 48-capillary DNA Analyzer with GS 500 LIZ or 600 LIZ internal size standards (Applied Biosystems, Carlsbad, California, USA). Alleles were manually scored using the program GeneMapper (GeneMapper version 3.7, Applied BioSystems). We genotyped fish using 14 microsatellite loci isolated from Atlantic salmon (*Salmo salar*; *SSOSL*456; Slettan et al. 1997), bull trout (*Sco*200, *Sco*202, *Sco*215, *Sco*216, *Sco*220; DeHaan and Ardren 2005), brook trout (*Salvelinus fontinalis*; *Sfo*18; Angers et al. 1995) Chinook salmon (*Oncorhynchus tshawytscha*; *Ots*G83b, *Ost*G253b; Williamson et al. 2002), Dolly Varden (*Smm*-17, *Smm*-21, *Smm*-22, *Smm*-24; Crane et al. 2004), and rainbow trout (*Oncorhynchus mykiss*; *OMM*1105; Rexroad et al. 2002).

To detect any possible genotype scoring errors we used MICRO-CHECKER software (van Oosterhout et al. 2004) to identify instances where alleles failed to amplify owing to the presence of null alleles or to large allele dropout. We defined all "population" samples as those localities with a sample size of least 15 and all other samples as "localities". Genetic polymorphism was summarized for population samples by determining the number of alleles per locus (A), allelic richness (A_R), and observed (Ho) and expected (H_E) heterozygosity using FSTAT version 2.9.3 (Goudet 2001). Tests for departures from Hardy–Weinberg equilibrium (HWE) in populations were performed for each locus–population combination using an exact test in which probability values were determined using a Markov chain method using GENEPOP (version 4.2, Raymond and Rousset 1995). Tests for genotypic linkage disequilibrium for all combinations of locus pairs within populations were made using a Markov chain method also with GENEPOP default values. Corrections for multiple simultaneous testing were applied following the sequential Bonferroni procedure (Rice 1989).

Table 1. List of localities of Dolly Varden (*Salvelinus malma*, except where noted) sampled, their north latitude (Lat.) and west longitude (Long.), sample size (*N*), year of sampling, and mean admixture score across individuals sampled from each locality and assayed at 13 microsatellite DNA loci expressed as Q_{NDV} (standard deviation (SD) in parentheses), the proportion of the genome estimated to be composed of northern Dolly Varden.

Locality	Lat., Long.	*N*	Year	Q_{NDV}
1. Quinault River, WA	47.27.28, 123.53.23	22	2002	0.003 (0.001)
2. Soleduck River, WA	47.57.10, 124.33.11	18	2002	0.007 (0.004)
3. Dungeness River, WA	48.08.19, 123.08.04	14	2002	0.007 (0.002)
4. Nooksack River, WA	48.55.20, 122.04.27	7	2009	0.007 (0.007)
5. Garnet Creek, BC	49.21.31, 121.36.09	8	2002	0.003 (0.001)
6. Ruby Creek, BC	49.21.00, 121.37.08	8	2002	0.003 (0.001)
7. Indian River, BC	49.35.56, 122.58.18	16	2014	0.008 (0.002)
8. Mamquam River, BC	49.43.19, 123.05.45	32	2002	0.008 (0.010)
9. Zeballos Lake, BC	50.04.33, 126.46.03	20	2002	0.029 (0.079)
10. Eve River, BC	50.28.04, 126.15.27	5	2002	0.003 (0.002)
11. Tsitika River, BC	50.28.41, 126.35.19	8	2002	0.015 (0.019)
12. Homathko River, BC[a]	50.58.04, 124.54.30	10	1999	NA
13. Klinaklini River, BC[a]	51.13.17, 123.38.39	10	1999	NA
14. Dallery Creek, BC	51.49.20, 127.02.33	13	2002	0.011 (0.010)
15. Cascade Creek, BC	56.02.45, 130.02.17	10	2002	0.009 (0.008)
16. Chown Creek, BC	54.01.29, 132.00.20	18	2004	0.006 (0.001)
17. Demon Creek, BC	53.23.58, 132.19.12	14	2004	0.091 (0.198)
18. Ain River, BC	53.45.15, 132.28.36	23	2004	0.011 (0.010)
19. Honna River, BC	53.14.52, 132.08.00	20	2004	0.023 (0.043)
20. Unnamed Creek-1, BC	53.19.44, 132.24.51	26	2004	0.007 (0.004)
21. Unnamed Creek-2, BC	53.24.40, 132.31.34	5	2004	0.005 (0.004)
22. Cove Creek, BC	53.51.40, 133.05.25	8	2004	0.006 (0.005)
23. Unnamed Creek-3, BC (Skeena River)	54.35.36, 128.25.08	8	2010	0.003 (0.001)
24. Sedan Creek, BC	55.05.15, 128.11.36	14	2010	0.010 (0.012)
25. Kutcho Creek, BC[a]	58.28.22, 128.38.34	30	2007	NA
26. Nass River, BC	55.57.47, 129.03.45	19	2011	0.013 (0.015)
27. Teigan Creek, BC	56.44.00, 129.51.14	16	2009	0.019 (0.041)
28. Serratus Creek, BC	56.56.55, 126.28.59	14	2010	0.005 (0.003)
29. Duncan Lake, BC	57.03.29, 126.48.19	15	2006	0.004 (0.002)
30. Ingenika River, BC	56.50.25, 126.05.31	15	2006	0.005 (0.002)
31. Sustut River, BC	56.20.53, 127.23.54	14	2006	0.004 (0.001)
32. Forrest-Kerr Cr., BC	56.44.40, 130.36.11	1	2009	0.003 (0.001)
33. Kitsault R., BC	55.28.53, 129.20.00	15	2009	0.009 (0.005)
34. More Creek, BC	56.46.06, 131.25.26	31	2010	0.023 (0.041)
35. Harris Creek, AK	55.27.40, 132.41.40	3	2013	0.008 (0.001)
36. Logjam Creek, AK	55.55.52, 132.59.04	5	2013	0.006 (0.001)
37. Stanley Creek, AK	56.00.42, 132.39.36	1	2013	0.011 (NA)
38. Admiralty Island, AK	57.49.48, 134.26.56	8	2007	0.043 (0.079)
39. Tulsequah River, BC[a]	58.40.43, 133.36.48	9	2000	NA
40. Hobbit Creek, BC	57.30.40, 129.21.20	22	2002	0.004 (0.001)
41. Little Rabbit Creek, AK	61.04.44, 149.48.02	16	2013	0.375 (0.206)
42. Chakachatna River, AK	61.05.37, 151.31.2	15	2013	0.245 (0.208)
43. Karluk Lake, AK	57.22.34, 154.03.02	15	2013	0.263 (0.199)
44. Chignik Lake, AK	56.15.49, 158.58.14	65	2009	0.754 (0.182)
45. Russell Creek, AK	55.09.50, 162.42.40	20	2013	0.992 (0.034)
46. Frosty Creek, AK	55.10.06, 162.48.37	21	2013	0.996 (0.050)
47. Bear River, AK	56.06.13, 160.18.55	20	2013	0.988 (0.034)
48. Egegik River, AK	**58.13.42, 157.30.25**	**29**	**2013**	**0.992 (0.007)**
49. Iliamna River, AK	59.44.11, 153.58.23	30	2009	0.996 (0.002)
50. Pedro Ponds, AK	59.46.27, 154.02.41	24	2009	0.996 (0.002)
51. Lake Aleknagik-1, AK	**59.20.16, 158.49.56**	**50**	**2012–2013**	**0.982 (0.071)**
51. Lake Aleknagik-2, AK[b]	59.20.16, 158.49.56	32	2012–2013	NA
52. Lake Nerka-1, AK	**59.34.34, 158.48.23**	**22**	**2012–2013**	**0.996 (0.002)**
52. Lake Nerka-2, AK[b]	59.34.34, 158.48.23	30	2012–2013	NA
53. Wulik River, AK	68.01.13, 163.19.48	14	2005	0.992 (0.005)
54. Avan River, AK	68.29.33, 161.40.43	22	2005	0.993 (0.004)
55. Ekokpuk River, AK	68.17.55, 151.57.17	10	2005	0.994 (0.002)
56. Graylime Creek, AK	68.06.05, 151.03.09	12	2005	0.993 (0.002)
57. Anaktuvuk River-1, AK	68.52.52, 158.08.50	11	2005	0.958 (0.086)
58. Anaktuvuk River-2, AK	68.07.02, 151.06.59	20	2005	0.978 (0.038)
59. Anaktuvuk River-3, AK	68.06.07, 151.03.16	15	2005	0.994 (0.002)
60. Big Fish River, YT	68.17.59, 136.21.17	10	2010	0.991 (0.005)
61. Babbage River, YT	68.36.03, 138.44.07	10	2010	0.992 (0.007)

Table 1. (*concluded*).

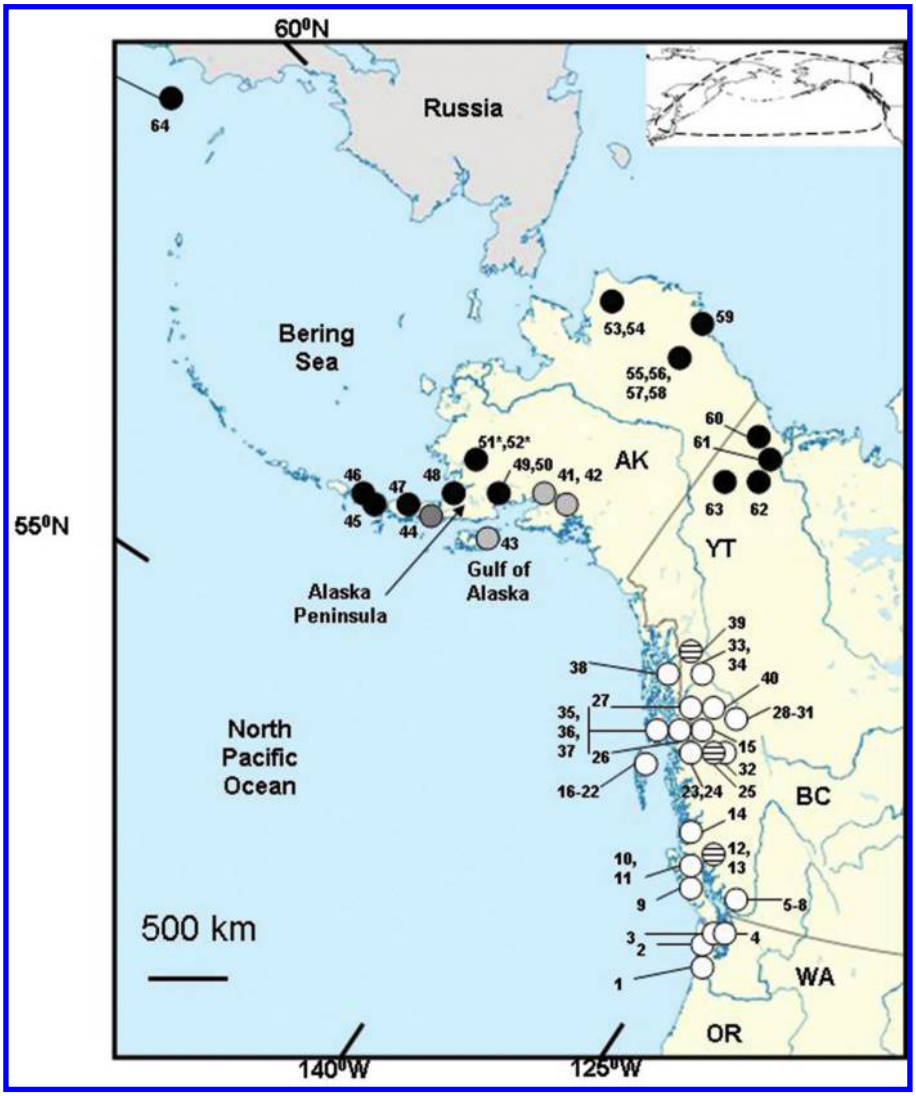
Locality	Lat., Long.	*N*	Year	Q_{NDV}
62. Bonnet Plume River, YT	65.19.20, 134.28.18	10	2008	0.989 (0.014)
63. Blackstone River, YT	65.45.08, 137.25.11	11	2008	0.996 (0.001)
64. Sorachi River, JP	43.18.13, 142.28.26	20	2013	0.988 (0.015)

Note: WA, Washington State; BC, British Columbia; AK, Alaska; YT, Yukon Territory; JP, Japan. Localities in boldface contained hybrids between Dolly Varden and Arctic char (i.e., at least 5% admixture between the species), and those fish were removed before calculating Q_{NDV}. Numbers accompanying locality names refer to those in Fig. 1. Latitude and longitude and expressed in degrees.minutes.seconds north and west, respectively, except for the Sorachi River, where it is east longitude.

[a]Bull trout, *Salvelinus confluentus*.

[b]Arctic char, *Salvelinus alpinus*.

Fig. 1. Map of localities of Dolly Varden (*Salvelinus malma*, except where noted) sampled in North America and assayed at 14 microsatellite DNA loci. Number codes are defined in Table 1. Open symbols depict Dolly Varden populations with Q_{NDV} (the proportion of the genome estimated to be composed of northern Dolly Varden) of less than 0.05 (putative *S. m. lordii*), black symbols are populations with Q_{NDV} greater than 0.95 (putative *S. m. malma*), dark grey symbols are populations with Q_{NDV} between 0.70 and 0.95, and light grey symbols are populations with Q_{NDV} less than 0.70 but greater than 0.05 (putative admixed populations of *S. m. malma* and *S. m. lordii*). Localities accompanied by asterisks (*) were also sampled for Arctic char (*Salvelinus alpinus*), and those represented by circles filled with horizontal bars are bull trout (*Salvelinus confluentus*). The black symbol in the extreme northwestern Pacific (No. 64) represents a sample of *S. m. kascheninnikovi* from the Sorachi River, Hokkaido, Japan. OR = Oregon, WA = Washington State, BC = British Columbia, AK = Alaska, YT = Yukon Territory. Inset shows the approximate global distribution of *S. malma*. Note that localities 46–52 drain to the Bering Sea, while localities 44–45 drain to the Gulf of Alaska.



Large-scale genetic structure

We used factorial correspondence analysis (FCA) as a multivariate measure of differentiation to obtain and visualize samples in "allelic space" with GENETIX (Belkhir et al. 2004). Ordination along three FCA axes was visualized using bubble plots as implemented in PAST (version 3.04, Hammer et al. 2001). Next, the genetic clustering program STRUCTURE version 2.3 (Falush et al. 2003) was used to determine the number of distinct genetic clus-

ters (*K*) among the Dolly Varden sampling locations. STRUCTURE uses a Bayesian clustering method to assign individuals to genetic clusters based on their genotypes. An individual may be assigned to more than one cluster if its genotype indicates admixture of two or more genetic groups. A Markov chain Monte Carlo method (MCMC) is used to estimate posterior probability distributions for each possible number of clusters. In an initial analysis, we used the known bull trout and Arctic char samples to identify any Dolly Varden with admixture coefficients (i.e., Q_{DV}) of $Q_{DV} < 0.95$ owing to admixture with either Arctic char or bull trout and eliminated them from further analysis. Subsequently, our simulations in STRUCTURE were performed using values of *K* between 1 and 10. Although we had samples from 58 Dolly Varden localities, we limited *K* to a maximum of 10 genetic groups because our focus was on testing for major genetic lineages within Dolly Varden, not to resolving smaller geographic scale population structure. Our sampling of Dolly Varden was not well-suited to examining fine geographic scale genetic structure owing to the high variation in sample sizes and year of sampling. Each analysis used parameters that allowed admixture, correlated allele frequencies, and did not include a location prior. Each run consisted of a 500 000 step burn-in followed by an additional 1 000 000 steps. Ten iterations were run for each value of *K*. We then examined the log-likelihood for each proposed *K*, and when the highest likelihood involved a *K* greater than 1, we used the method of Evanno et al. (2005) to calculate the second-order rate of change in the log probability of successive *K* values (ΔK) and evaluate different solutions for *K*. When admixture was observed between groups of Dolly Varden, we used NEWHYBRIDS (version 1.1, Anderson and Thompson 2002) to assess whether any fish considered to be admixtures of northern and southern Dolly Varden might represent first-generation hybrids. NEWHYBRIDS uses multilocus genotypes and MCMC simulation to estimate the joint posterior probability of membership of any individual in one of six genotypes: parental 1 (northern Dolly Varden), parental 2 (southern Dolly Varden), F_1 hybrid, F_2 hybrid, and backcrosses to either northern or southern Dolly Varden. We used the uniform prior option (use of the Jeffreys-like prior produced similar result) and ran NEWHYBRIDS for at least 100 000 steps or sweeps of the Markov chain replicated ten times.

Finally, we quantified the extent of variation in allele frequencies in Dolly Varden samples that was attributable to geographic group (north or south of the Alaska Peninsula) and location within geographic group using the hierarchical approach in an analysis of molecular variance (AMOVA) of allele frequencies using ARLEQUIN (version 3.5, Excoffier and Lischer 2010).

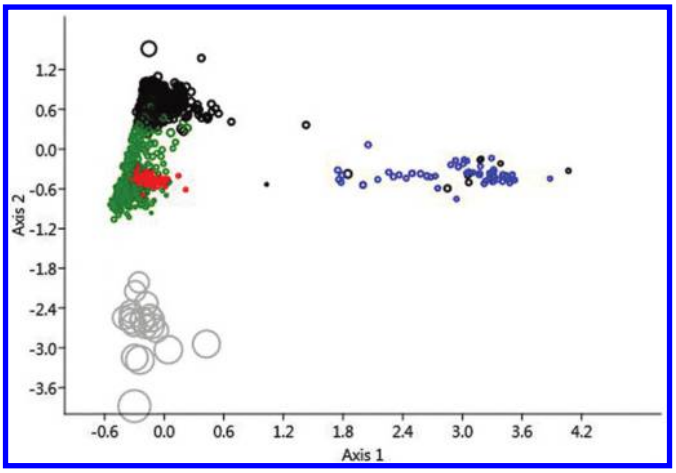
Results

Microsatellite amplification and variation within samples

Given the range of species and geographic locations examined as well as the age of some samples (some dated back to 1999), variation in amplification success at some loci was expected. On average, there were missing data at 1.8 of the 14 loci assayed per individual (SD = 11.6, range = 0–8). Any individual with missing data at eight or more loci was removed from subsequent analyses (*N* = 10 individuals), and more than 95% of all fish were assayed at a minimum of eight loci and 85% at a minimum of 12 loci.

There were 34 localities for Dolly Varden (23 south of the Alaska Peninsula or on the peninsula, but draining to the Gulf of Alaska, and 11 north of the peninsula or on it, but draining to the Bering Sea or Arctic Ocean) and two localities each for bull trout and Arctic char, with sufficient samples sizes (i.e., ≥15) to evaluate HWE, linkage disequilibrium, and levels of genetic variation. Most such population samples (27/34) had at least one locus that was out of HWE, but the only locus that was consistently out of HWE was *Sco*216 (23/28 samples where HWE could be tested), and MICRO-CHECKER suggested the existence of one or more null alleles at this locus. Consequently, *Sco*216 was removed from subsequent analysis. Further, many of the deviations from HWE occurred in samples with relatively few fish (i.e., <30 fish), sometimes of mixed age classes, and (or) in samples subsequently found to contain admixtures of genetically distinct groups within samples (see below), all of which are phenomena that may generate such deviations (see Waples 2015). Consequently, and given that our focus was on resolving major lineages of Dolly Varden across a wide geographic area and not on details of population structure within areas, we did not consider that deviations from HWE at other loci to be consequential to our analyses. Out of 1558 tests for departures from linkage equilibrium, 83 were significant, only marginally higher than expected by chance (78). Because only four locus pairs were found to deviate from linkage equilibrium in as many as three samples, we retained all loci (other than *Sco*216) for analyses.

Fig. 2. Factorial correspondence analysis projection along three axes via a bubble plot of individual Dolly Varden, bull trout, and Arctic char based on variation at 13 microsatellite DNA loci. Relative ordination along the third dimension is represented by the size of each bubble. Blue symbols are bull trout, black symbols are southern Dolly Varden, green symbols are northern Dolly Varden, red symbols are Arctic char, and grey symbols are Japanese Dolly Varden.

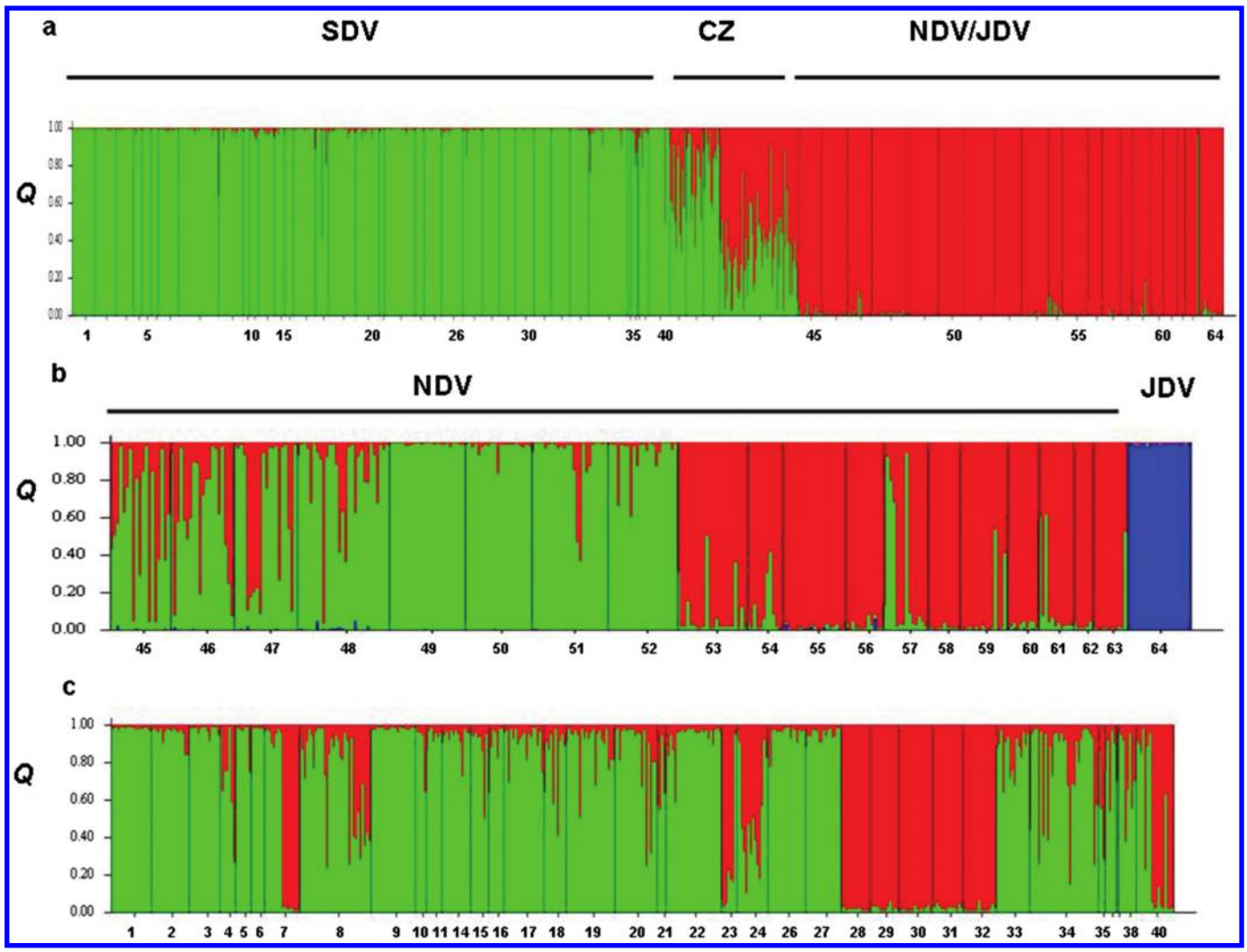


Large-scale population structure

The FCA segregated all samples into four broad groupings: bull trout, Dolly Varden south of the Alaska Peninsula, Dolly Varden north of the Alaska Peninsula and Arctic char, and the Japanese sample of Dolly Varden (Fig. 2). The samples from Yukon Territory clustered with the northern Dolly Varden. There were several fish that clustered in positions intermediate between the main bull trout and southern Dolly Varden groups and between the southern and northern groups of Dolly Varden (Fig. 2).

Bayesian analysis of population structure using STRUCTURE and setting *K* = 3 (Dolly Varden, bull trout, and Arctic char) identified 98 presumptive Dolly Varden with >5% admixture with bull trout (*N* = 88) or Arctic char (*N* = 10). All the individuals admixed with bull trout occurred in British Columbia or Washington State, and all of the individuals admixed with Arctic char occurred in southcentral or southwestern Alaska. The Japanese sample contained no admixed individuals. When admixed individuals were removed and the data subjected to another round of analysis (*N* = 923), a *K* = 2 was the most supported model, with one group predominating south and southeast of the Alaska Peninsula and another on or north of the Alaska Peninsula (including the Japanese sample; Fig. 3*a*; Table 2). Considerable admixture between northern and southern Dolly Varden, expressed as the proportion of the genome from northern Dolly Varden (Q_{NDV}), was found in an area from Chignik Lake (on the Alaska Peninsula, but draining to the Gulf of Alaska, not the Bering Sea) to about 300 km southeast to Karluk Lake (Kodiak Island) and further east some 733 km to Little

Fig. 3. Results of STRUCTURE analysis based on variation in allele frequencies at 13 microsatellite loci in Dolly Varden and Arctic char. Each fish is represented by a thin vertical line, which depicts the proportional representation (*Q*) of distinct genetic groups (*K*) represented by different colours. (*a*) Analysis of all Dolly Varden. The green group is dominated by southern Dolly Varden (SDV) and the red group by northern Dolly Varden (NDV) and Japanese Dolly Varden (JDV). Samples with CZ above represent the putative contact zone between SDV and NDV where Q_{NDV} (representing the proportion of the red genetic group) was between 0.06 and 0.94. (*b*) Analysis of NDV and JDV. The green and red groups are NDV, and the blue group is JDV. (*c*) Analysis of SDV. The red group is dominated by central coast and interior British Columbia SDV and the green group by all other samples. Sample codes along the horizontal axis are defined in Table 1, but do not include numbers 12, 13, 25, 39, which were composed of bull trout, or any Arctic char from localities 51 and 52.



Rabbit Creek near Anchorage in Cook Inlet, Alaska (Fig. 1; Table 1; Q_{NDV} between 0.25 and 0.75). Dolly Varden from localities west of Chignik Lake along the Alaska Peninsula to the Egegik River area on the northeastern edge of the Alaska Peninsula (about 230 km northeast of Chignik Lake, but about 1100 km by sea route) showed only between 0.4% and 1.2% admixture with southern Dolly Varden (Q_{NDV} = 0.988 to 0.996, but also showed some admixture with Arctic char — see below; Table 1). Similarly, the next southern location closest to Little Rabbit Creek (in southcentral Alaska with Q_{NDV} = 0.37) was Admiralty Island in southeast Alaska (about 930 km straight line distance and about 1300 km by sea route), which showed less than 5% admixture with northern Dolly Varden (Q_{NDV} = 0.043; Table 1). A *K* = 3 was the next most supported model and consisted of Dolly Varden south of the Alaska Peninsula, Dolly Varden north of the Alaska Peninsula (with admixture between these two groups), and the Japanese sample of Dolly Varden, and Arctic char (Table 2).

Across the four localities showing high admixture between northern and southern Dolly Varden (i.e., Q_{NDV} between 0.25 and 0.75), no fish were identified as probable F_1 hybrids by NEWHYBRIDS; the posterior probability of being an F_1 hybrid was consistent across the 10 runs of NEWHYBRIDS and within any one run averaged less than 0.005 (SD = 0.01, range 0.0000 to 0.067, *N* = 103). The highest mean posterior probability was for fish in the putative contact zone to be one of either parental lineage (0.132, SD = 0.293, to 0.350, SD = 0.432, for southern and northern Dolly Varden, respectively) or at least a second generation hybrid (0.478, SD = 0.411).

The samples from north of the Alaska Peninsula and those south of this point were subject to separate STRUCTURE analyses and with any fish showing more than 5% admixture between these groups removed (*N* = 341). For all samples north of Alaska Peninsula (or on the peninsula, but draining to the Bering Sea), a *K* = 3 was the most supported model; there were two groups of fish resolved within the northern Dolly Varden, and the Japanese sample of Dolly Varden (Fig. 3*b*). Dolly Varden from the Alaska Peninsula and Bristol Bay predominated in one genetic group (localities 45–52), while Dolly Varden from north and east of that area (northwestern Alaska and Yukon) predominated in the second

Table 2. Results of STRUCTURE analysis on samples of Dolly Varden (DV), all southern Dolly Varden (SDV), and all northern Dolly Varden (NDV) and Japanese Dolly Varden (JDV) based on variation at 13 microsatellite DNA loci.

Data set	*K*	ln*P*(*K*)	Δ*K*
All DV samples	1	–55 076.2 (1.2)	NA
	2	**–49 334.4 (11.0)**	**427.5**
	3	–48 287.1 (51.9)	3.8
NDV and JDV	1	–21 327.7 (1.7)	NA
	2	–22 964.8 (569.1)	8.4
	3	**–19 800.9 (4.9)**	**616.3**
SDV	1	–21 043.3 (1.2)	NA
	2	**–20 215.3 (17.5)**	**12.8**
	3	–19 611.4 (5.0)	6.2

Note: Shown for the top three models of population structure (*K*) are the mean (standard deviation (SD) in parentheses) natural logarithm of the probability of *K* (ln*P*(*K*)) and the second-order rate of change of the mean ln*P*(*K*) divided by the standard deviation (Δ*K*). The best model according to the Δ*K* criterion is in boldface; NA, not applicable.

group (localities 53–63), with some admixture between these two genetic groups particularly within the western-most samples on the Alaska Peninsula (Figs. 1 and 3). A $K = 2$ was the second most supported model and separated all northern Dolly Varden from the Japanese sample, with considerable admixture of the latter within many of the northern Dolly Varden. For the STRUCTURE analysis run on Dolly Varden south of the Alaska Peninsula ($N = 479$), K values of 2 and 3 were about equally supported; in both scenarios, Dolly Varden from the upper Skeena River (Pacific drainage, localities 23–24) and adjacent watersheds of the upper Finlay River (Mackenzie River drainage, localities 28–31) tended to form their own cluster distinct from other Dolly Varden within the southern lineage (Fig. 3c; Table 2).

Finally, when all Dolly Varden samples were partitioned into two groups (those north of or on the Alaska Peninsula, but draining to the Bering Sea or Arctic Ocean ($N = 18$ localities) and those south of or on the peninsula, but draining to the Gulf of Alaska ($N = 40$ localities)), 15.3% of the variation in allele frequencies was explained by this partition, 16.7% by site within each such group, and 68.0% by variation within site (all $p < 0.001$). When three groups were composed by treating the samples with ≥5% admixture between northern and southern Dolly Varden as a separate group, 13.0% was explained by among-group differences, 16.8% by differences among sites within groups, and 70.2% by variation within sites (all $p < 0.001$). Northern Dolly Varden had private alleles that were present at a frequency of at least 5% at *Sco*215, *Sco*200, and *Omm*1105 (one allele each; see online supplementary data, Table S1[1]). Southern Dolly Varden had one private allele at *Sfo*18, while the sample of Dolly Varden from Japan had private alleles at *Sfo*18 and *Ots*g83b (four alleles each), *Smm*22, *Sco*215, and *Ssos*1456 (one allele each; Table S1[1]).

Discussion

Our study represents the most extensive examination of broad-scale population structure in North American populations of Dolly Varden, a relatively understudied salmonid in this regard. We found strong evidence for two regional groupings of Dolly Varden, an assemblage concentrated north of the Alaska Peninsula and east to the Mackenzie River and an assemblage concentrated south of the Alaska Peninsula, as well as a zone of admixture between the two groups in and around the Gulf of Alaska. The two regional groupings of Dolly Varden resolved with microsatellite DNA data correspond almost exactly to the distribution of two recognized North American subspecies: the northern Dolly Varden (*S. m. malma*) and the southern Dolly Varden (*S. m. lordii*) that have been proposed based on morphology, life history, and biochemical and other molecular traits (reviewed by Kowalchuk et al. 2010). Our data, therefore, substantiate the current differentiation of *S. malma* into two North American genetic lineages that would indeed appear to fit the definition of subspecies: two or more largely allopatric and diagnosable groups that meet and interbreed at a zone of contact (Bailey et al. 1954; Mallet 2007). Our work is the first to provide evidence that a zone of intergradation occurs between these subspecies and provides hints as to its location and geographical extent.

Yamamoto et al. (2014) presented mitochondrial DNA data for Dolly Varden from throughout the Pacific Rim (but with no samples from the extensive Canadian range) and documented the existence of three major mtDNA lineages: western Pacific, central Pacific, and eastern Pacific lineages. By contrast with our data, the mtDNA data showed extensive areas of overlap among the western and central lineages and between the central and eastern lineages (e.g., the latter two overlapped from the Aleutian Islands to Washington State). The concentration of mtDNA haplotypes from each lineage in distinct geographic areas of the North Pacific is also consistent with the recognition of three North Pacific subspecies of *S. malma*. By contrast, our microsatellite DNA data resolved a much narrower zone of overlap between *S. m. malma* and *S. m. lordii* and suggest that perhaps both historical hybridization and incomplete mtDNA lineage sorting characterize the history of each lineage (cf. Yamamoto et al. 2014).

Admixture zone in the North Pacific

Our data suggest that populations of Dolly Varden from areas of the Alaska Peninsula that drain to the Gulf of Alaska east to at least Cook Inlet constitute admixtures of northern and southern Dolly Varden. Across the four populations within the putative contact zone between northern and southern Dolly Varden, admixture values relative to the northern lineage averaged 0.33 (SD = 0.23). This is in marked contrast with areas north and south of the putative contact zone where admixture between the two groups in these areas averaged only 0.012 (SD = 0.022) and 0.008 (SD = 0.011), respectively. Interestingly, only about 300 km of ocean distance separates two localities that have strongly divergent proportions of the two lineages; Chignik Lake has a mean admixture proportion expressed in terms of the proportion of the northern lineage (Q_{NDV}) equal to 0.754 compared with a value of 0.263 (or $Q_{SDV} = 0.737$) in Karluk Lake. Similarly, only about 340 km separates Chignik Lake from Russell Creek to the west where Q_{NDV} rises to 0.992. There are, however, large distances between these populations (and others with high levels of admixture between the two subspecies) and genetically nonadmixed populations within the northern and southern subspecies (about 1000 ocean kilometres each). Further, the two genetically nonadmixed populations of the two subspecies closest to the admixture zone in our survey (Russell Creek, Alaska Peninsula, and Admiralty Island, southeast Alaska) are separated by about 1800 km of ocean. Detailed sampling will be necessary to better estimate the width of the contact zone between the two subspecies, particularly in the eastern Gulf of Alaska, and whether this contact zone represents recurrent, contemporary interbreeding between the subspecies, or is a result of historical interactions. For instance, although long-distance migrations exceeding 1500 km by Dolly Varden have been recorded between northwestern Alaska and Russia (DeCicco 1992), it is unknown if fish move regularly between the Bering Sea and the Gulf of Alaska through the Aleutian Archipelago. Our analysis using NEWHYBRIDS, however, suggests that the admixture zone probably represents historical rather than contemporary contact between these lineages because none

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2015-0003.

of the fish within the contact zone were identified as probable F_1 hybrids.

Interestingly, the morphological data of McPhail (1961) that strongly suggested the existence of the two subspecies also suggest that they may form a contact zone in the Gulf of Alaska – Alaska Peninsula area. His so-called northern form of Dolly Varden were distributed north of the peninsula and were characterized by mean total gill raker counts on the first arch of 21–22 and mean total vertebrae of 65–71, while the values for the southern form were 16–17 and 57–67, respectively. Fish collected from areas ranging from Kodiak Island to Cook Inlet had generally intermediate values of 15–20 and 64–65, respectively (McPhail 1961).

The area of contact between *S. m. malma* and *S. m. lordii* is remarkably similar in general distribution to contact zones for several other taxa. For instance, Seeb and Crane (1999) studied allozyme variation in chum salmon (*Oncorhynchus keta*) and reported a contact zone between Beringian and Cascadian lineages across a 150 km wide stretch of the northwestern portion of the Alaska Peninsula. Chignik Lake falls within this zone on the southern portion of the Alaska Peninsula. Seeb and Crane (1999) also noted that this general area is thought to be a contact zone between two human aboriginal language groups: Aleut and Yu'pik (Muller-Beck 1967). O'Corry-Crowe et al. (1997) and Westlake and O'Corry-Crowe (2002) used mtDNA to resolve major distinctions between populations within both beluga whales (*Delphinapterus leucas*) and harbor seals (*Phoca vitulina*) sampled north and south of the Alaska Peninsula and suggested that the peninsula may represent a major biogeographical boundary. Further, and at least for the harbor seal, samples from the Gulf of Alaska (Kodiak Island) were intermediate genetically to those north of the Alaska Peninsula and those in southeast Alaska. Finally, Hoffman et al. (2006) studied populations of Steller's sea lion (*Eumetopias jubatus*) across the North Pacific using microsatellite DNA and also noted a strong shift in composition of "western" (Asian and North American animals east to Prince William Sound, Alaska) and "eastern" (from southeast Alaska to northern California) lineages, with the greatest level of admixture occurring in the Prince William Sound area. The general concordance in pattern among these taxa is striking and indeed suggests that the Gulf of Alaska – Alaska Peninsula area is a contact zone between intraspecific lineages that ultimately originated in distinct glacial refugia north (or west) and south of this point. Interestingly, if one assumes that the northern Dolly Varden survived the Pleistocene glaciations in North America as far south as the ice-free area around Bristol Bay in Beringia, and southern Dolly Varden persisted in either or both of the Chehalis refuge or in the Pacific refuge in and around the lower Columbia River (McPhail and Lindsey 1986), then the subspecies have dispersed from ~2700 km (for the southern Dolly Varden) to over 3000 km (for the northern Dolly Varden) to reach the current northern limits of their respective ranges (the eastern Gulf of Alaska and at least 350 km up the Mackenzie River in the western Canadian Arctic, respectively). Yet, the area of current overlap appears to be relatively small, and the Alaska Peninsula – Aleutian Island chain appears to be an effective dispersal barrier.

Interestingly, our data, although limited to a single population from Japan, suggest that these Dolly Varden from the western Pacific are more similar to northern Dolly Varden than they are to southern Dolly Varden from the eastern Pacific. This is consistent with the idea that southern Dolly Varden across the North Pacific consist of at least two subspecies (*S. m. lordii* in the eastern Pacific and *S. m. krascheninnikovi* in the western Pacific). Curiously, recent mtDNA phylogenetic analysis suggests that among these taxa and Arctic char, *S. m. krascheninnikovi* is the most basal lineage and that the origin of the complex may have originated in the western Pacific (cf. Oleinik et al. 2007).

Conservation implications

Our analysis of the differentiation between the putative subspecies of *S. malma* is the most geographically comprehensive to date and strongly supports the recognition of these two lineages as two subspecies in the classical sense of the term: *S. m. malma* and *S. m. lordii* (see also McPhail 1961; Behnke 1980; Phillips et al. 1999; Kowalchuk et al. 2010), which reinforces their distinct conservation treatment. Furthermore, contact zones between distinct lineages are considered high priorities for conservation (e.g., Moritz and Faith 1998; Moritz et al. 2009). Certainly, more extensive sampling is required to better understand the spatial distribution of each subspecies and their area of admixture. In particular, the characterization (as southern Dolly Varden or admixed northern and southern Dolly Varden) across a 1000 km stretch of coastline from the Kenai Peninsula to Admiralty Island in southeast Alaska should be a high priority. Also, areas along the western portion of the Alaska Peninsula need to be examined to pinpoint exactly where the zone of admixture (e.g., in populations such as Chignik Lake) switches to one that is almost exclusively northern Dolly Varden (i.e., is there a population closer to Chignik Lake than Russell Creek that is predominantly northern Dolly Varden? cf. Seeb and Crane 1999). In addition, physical tagging perhaps accompanied by detailed genetic analyses of locality of origin of fish would be very useful to better understand if the area of admixture is generated by contemporary or historical interbreeding between subspecies.

The distinction between subspecies of Dolly Varden is already recognized for conservation purposes in the Canadian Species at Risk Act (COSEWIC 2011). In Canada, the northern Dolly Varden has been assessed as a wildlife species of Special Concern, largely owing to the relatively small number of populations and the small area (estimated at less than 1 km^2) of known and critical overwintering habitat in Canada's western Arctic (COSEWIC 2011). By contrast, the conservation status of the southern Dolly Varden, at least in Canada, is essentially unknown. The British Columbia Conservation Data Centre (http://www.env.gov.bc.ca/cdc/) recognizes two "lineages" (but not subspecies) of Dolly Varden and, based on the mtDNA data of Taylor et al. (2001), considers that both exist in British Columbia. The northern mtDNA lineage extends as far south as Vancouver Island, while the southern mtDNA lineage was found only as far north as the northern edge of Vancouver Island (Taylor et al. 2001). Interestingly, Dolly Varden mtDNA is paraphyletic with respect to bull trout mtDNA, and Taylor et al. (2001) suggested that this resulted from historical and contemporary hybridization between the species, which has been widely reported (e.g., see Baxter et al. 1997; Redenbach and Taylor 2003; this study). In our study, hybridization with bull trout was assessed and admixed fish were removed before characterizing Dolly Varden further. Based on our results, while two mtDNA lineages of Dolly Varden exist in British Columbia, only one subspecies appears to be found in British Columbia, and it is *S. m. lordii*. Even without the added complication of mtDNA introgression, contact zones between lineages defined using mtDNA may be well displaced geographically from those defined using biparentally inherited loci for a variety of reasons (e.g., Boissinot and Boursot 1997; reviewed by Toews and Brelsford 2012; cf. Yamamoto et al. 2014).

In British Columbia, the mtDNA lineages of *S. m. lordii* have conservation statuses of S4S5 ("apparently secure to secure"; northern lineage) and S4 ("apparently secure"; southern lineage) according to the NatureServe qualitative ranking system (http://www.natureserve.org/). In general, the apparently different conservation statuses of *S. m. malma* and *S. m. lordii* in Canada are consistent with differences in their geographic range sizes, especially in terms of the number of populations and extent of critical habitat, and the generally more benign environment occupied by *S. m. lordii* in Canada. On the other hand, at least in southern portions of its range in British Columbia, *S. m. lordii* probably faces a greater range and intensity of anthropogenic threats from human develop-

ment as shown by declines of many salmonids across that area (e.g., Slaney et al. 1996; Dunham et al. 2008).

Finally, our data suggest that there is population structure within the broadly defined southern and northern Dolly Varden, but sample sizes were not sufficient for population-level analyses. For instance, we found a general distinction between northern Dolly Varden from the Alaska Peninsula and Bristol Bay and those from areas north and west of these areas. Our survey, however, includes a gap between these areas of over 1000 km where we had no samples, which could explain the apparent genetic discontinuity. Certainly, population subdivision has been suggested to occur based on mtDNA and nDNA analyses of regional groups of Dolly Varden (e.g., Krueger et al. 1999; Omelchenko et al. 2002; Gordeeva et al. 2010), but much remains to be resolved especially within the southern North American range of *S. malma lordii*, where no analyses outside of southeastern Alaska have been conducted.

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References

Anderson, E.C., and Thompson, E.A. 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics, **160**: 1217–1229. PMID:11901135.

Angers, B., Bernatchez, L., Angers, A., and Desgroseillers, L. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. J. Fish Biol. **47**(Suppl. A): 177–185. doi:10.1111/j.1095-8649.1995.tb06054.x.

Bailey, R.M., Winn, H.E., and Smith, C.L. 1954. Fishes from the Escambia River, Alabama and Florida, with ecological and taxonomic notes. Proc. Acad. Nat. Sci. Phila. **106**: 109–164.

Baxter, J.S., Taylor, E.B., Devlin, R.H., Hagen, J., and McPhail, J.D. 1997. Evidence for natural hybridization between Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in a northcentral British Columbia watershed. Can. J. Fish. Aquat. Sci. **54**(2): 421–429. doi:10.1139/f96-289.

Behnke, R.J. 1972. The systematics of salmonid fishes of recently glaciated lakes. J. Fish. Res. Bd. Can. **29**(6): 639–671. doi:10.1139/f72-112.

Behnke, R.J. 1980. A systematic review of the genus *Salvelinus*. *In* Charrs: salmonid fishes of the genus *Salvelinus*. *Edited by* E.K. Balon. Dr. W. Junk, The Hague, the Netherlands. pp. 441–481.

Belkhir, K., Borsa, P., Chikhi, N., Raufaste, N., and Bonhomme, F. 2004. GENETIX 4.05.2, Logiciel Sous Windows TM Pour la Genetique des Populations. Laboratoire Genome, Populations, Interactions, CNRS UMR 5000, Universite de Montpellier II, Montpellier, France.

Boissinot, S., and Boursot, P. 1997. Discordant phylogeographic patterns between the Y chromosome and mitochondrial DNA in the house mouse: selection on the Y chromosome? Genetics, **146**: 1019–1034. PMID:9215905.

Cook, J.A., Bidlack, A.L., Conroy, C.J., Demboski, J.R., Fleming, M.A., Runck, A.M., Stone, K.D., and MacDonald, S.O. 2001. A phylogeographic perspective on endemism in the Alexander Archipelago of southeast Alaska. Biol. Conserv. **97**: 215–227. doi:10.1016/S0006-3207(00)00114-2.

COSEWIC 2011. COSEWIC assessment and status report on the Dolly Varden *Salvelinus malma malma* (Western Arctic populations) in Canada. Committee on the Status of Endangered wildlife in Canada. Canadian Wildlife Service, Ottawa, Ont.

Crane, P.A., Lewis, C.J., Kretschmer, E.J., Miller, S.J., Spearman, W.J., DeCicco, A.L., Lisac, M.J., and Wenburg, J.K. 2004. Characterization and inheritance of seven microsatellite loci from Dolly Varden, *Salvelinus malma*, and cross-species amplification in Arctic char, *S. alpinus*. Conserv. Genet. **5**: 737–741. doi:10.1007/s10592-004-1853-1.

DeCicco, A. 1992. Long distance movements of anadromous Dolly Varden between Alaska and the U.S.S.R. Arctic, **45**: 120–123. doi:10.14430/arctic1382.

DeCicco, F., and Reist, J.D. 1999. Distribution of Dolly Varden in northern Bering and Chukchi Sea drainages, and provisional organization. *In* International Society of Arctic Char Fanatics Information Series 1996. *Edited by* F.W. Kircheis. Institute of Freshwater Research, Drottingholm, Sweden. pp. 13–18.

DeHaan, P.W., and Ardren, W.R. 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. doi:10.1111/j.1471-8286.2005.00997.x.

Dunham, J., Baxter, C., Fausch, K., Fredenberg, W., Kitano, S., Koizumi, I., Morita, K., Nakamura, T., Rieman, B., Savvaitova, K., Stanford, J., Taylor, E., and Yamamoto, S. 2008. Evolution, ecology, and conservation of Dolly Varden, white spotted char, and bull trout. Fisheries, **33**: 537–550. doi:10.1577/1548-8446-33.11.537.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. **14**: 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x. PMID:15969739.

Excoffier, L., and Lischer, H.E. 2010. Arlequin suite version 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. **10**: 564–567. doi:10.1111/j.1755-0998.2010.02847.x.

Falush, D., Stephens, M., and Pritchard, J.K. 2003. Inference of population structure: Extensions to linked loci and correlated allele frequencies. Genetics, **164**: 1567–1587. PMID:12930761.

Gordeeva, N.V., Chukova, E.I., and Oleinik, A.G. 2010. Microsatellite genetic variation of Asian populations of Dolly Varden char. Hydrobiologia, **650**: 133–144. doi:10.1007/s10750-010-0104-3.

Goudet, J. 2001. FSTAT, version 2.9.3.1. [Updated from Goudet, J. 1995.] J. Hered. **86**: 485–486.

Günther, A.C.L.G. 1866. Catalogue of the fishes of the British Museum. *In* Catalogue of the Physostomi, containing the families Salmonidae, Percopsidae, Galaxidae, Mormyridae, Gymnarchidae, Esocidae, Umbridae, Scombresocidae, Cyprinodontidae, in the collection of the British Museum, Vol. 6. pp. 1–368.

Haas, G.R., and McPhail, J.D. 1991. Systematics and distributions of Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in North America. Can. J. Fish. Aquat. Sci. **48**(11): 2191–2211. doi:10.1139/f91-259.

Hammer, Ø., Harper, D.A.T., and Ryan, P.D. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica, **4**.

Hoffman, J.I., Matson, C.W., Amos, W., Loughlin, T.R., and Bickham, J.W. 2006. Deep genetic subdivision within a continuously distributed and highly vagile marine mammal, the Steller's sea lion (*Eumetopias jubatus*). Mol. Ecol. **15**: 2821–2832. doi:10.1111/j.1365-294X.2006.02991.x. PMID:16911203.

Klemetsen, A. 2010. The charr problem revisited: exceptional phenotypic plasticity promotes ecological speciation in postglacial lakes. Freshw. Rev. **3**: 49–74. doi:10.1608/FRJ-3.1.3.

Kowalchuk, M.W., Sawatzky, C.D., and Reist, J.D. 2010. A Review of the taxonomic structure within Dolly Varden, *Salvelinus malma* (Walbaum 1792), of North America. Department of Fisheries and Oceans Canadian Science Advisory Research Document 2010/013.

Krueger, C.C., Wilmot, R.L., and Everett, R.J. 1999. Stock origins of Dolly Varden collected from Beaufort Sea coastal sites of Arctic Alaska and Canada. Trans. Am. Fish. Soc. **128**: 49–57. doi:10.1577/1548-8659(1999)128<0049:SOODVC>2.0.CO;2.

Mallet, J. 2007. Subspecies, semispecies, superspecies. *In* Encyclopaedia of Biodiversity, Vol. 5. pp. 1–5.

May-McNally. S.L. 2014. Biodiversity of Arctic char (*Salvelinus alpinus*): sympatric morphs and hybridization with Dolly Varden (*S. malma*) in Alaska. M.Sc. thesis, The University of British Columbia, Vancouver, B.C.

McPhail, J.D. 1961. A systematic study of the *Salvelinus alpinus* complex in North America. J. Fish. Res. Board Can. **18**(5): 793–816. doi:10.1139/f61-053.

McPhail, J.D. 2007. The freshwater fishes of British Columbia. University of Alberta Press, Edmonton, Alta.

McPhail, J.D., and Lindsey, C.C. 1986. Zoogeography of the freshwater fishes of Cascadia (the Columbia system and rivers north to the Stikine). *In* The zoogeography of North American freshwater fishes. *Edited by* C.H. Hocutt and E.O. Wiley. John Wiley & Sons. pp. 615–637.

Moritz, C.R., and Faith, D.P. 1998. Comparative phylogeography and the identification of genetically divergent areas for conservation. Mol. Ecol. **7**: 419–429. doi:10.1046/j.1365-294x.1998.00317.x.

Moritz, C., Hoskin, C.J., MacKenzie, J.B., Phillips, B.L., Tonione, M., Silva, N., Van, der, Val, J., Williams, S.E., and Graham, C.H. 2009. Identification and dynamics of a cryptic suture zone in tropical rainforest. Proc. R. Soc. B Biol. Sci. **276**: 1235–1244. doi:10.1098/rspb.2008.1622.

Muller-Beck, H. 1967. On migrations of hunters across the Bering Land Bridge in the Upper Pleistocene. *In* The Bering Land Bridge. *Edited by* D.M. Hopkins. Stanford University Press, Stanford, Calif. pp. 373–409.

Nordeng, H. 1983. Solution to the "char problem" based on Arctic char (*Salvelinus alpinus*) in Norway. Can. J. Fish. Aquat. Sci. **40**(9): 1372–1387. doi:10.1139/f83-159.

O'Corry-Crowe, G.M., Suydam, R.S., Rosenberg, A., Frost, K.J., and Dizon, A.E. 1997. Phylogeography, population structure and dispersal patterns of the beluga whale *Delphinapterus leucas* in the western Nearctic revealed by mitochondrial DNA. Mol. Ecol. **6**: 955–970. doi:10.1046/j.1365-294X.1997.00267.x.

Oleinik, A.G., Skurikhina, L.A., and Brykov, V.A. 2007. Divergence of *Salvelinus* species from northeastern Asia based on mitochondrial DNA. Ecol. Freshw. Fish, **16**: 87–98. doi:10.1111/j.1600-0633.2006.00187.x.

Omelchenko, V.T., Salmenkova, E.A., and Shedko, S.V. 2002. Allozyme diversity and genetic divergence of the Dolly Varden *Salvelinus malma* Walbaum from the Kuril Islands. Russ. J. Genet. **38**: 1066–1075. doi:10.1023/A:1020295932643.

Phillips, R.B., Gudex, L.I., Westrich, K.M., and DeCicco, A.L. 1999. Combined phylogenetic analysis of ribosomal ITS1 sequences and new chromosome

data supports three subgroups of Dolly Varden char (*Salvelinus malma*). Can. J. Fish. Aquat. Sci. **56**(8): 1504–1511. doi:10.1139/f99-103.

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

Redenbach, Z. 2000. Molecular evidence for current and historical introgresive hybridization between bull trout (*Salvelinus confluentus*) and Dolly Varden (*Salvelinus malma*). M.Sc. thesis, The University of British Columbia, Vancouver, B.C.

Redenbach, Z., and Taylor, E.B. 2003. Evidence for bimodal hybrid zones between two species of char (Pisces: *Salvelinus*) in northwestern North America. J. Evol. Biol. **16**: 1135–1148. doi:10.1046/j.1420-9101.2003.00619.x. PMID:14640405.

Reist, J.D., Power, M., and Dempson, J.B. 2013. Arctic charr (*Salvelinus alpinus*): a case study of the importance of understanding biodiversity and taxonomic issues in northern fishes. Biodiversity, **14**: 45–56. doi:10.1080/14888386.2012.725338.

Rexroad, C.E., Coleman, R.L., Hershberger, W.K., and Killefer, J. 2002. Rapid communication: Thirty-eight polymorphic microsatellite markers for mapping in rainbow trout. J. Anim. Sci. **80**: 541–542. PMID:11881939.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, **43**: 223–225. doi:10.2307/2409177.

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Seeb, L.W., and Crane, P.A. 1999. High genetic heterogeneity in chum salmon in western Alaska, the contact zone between northern and southern lineages. Trans. Am. Fish. Soc. **128**: 58–87.

Slaney, T.L., Hyatt, K.D., Northcote, T.G., and Fielden, R.J. 1996. Status of anadromous salmon and trout in British Columbia and Yukon. Fisheries, **21**: 20–35. doi:10.1577/1548-8446(1996)021<0020:SOASAT>2.0.CO;2.

Slettan, A., Olsaker, I., and Lie, Ø. 1997. Segregation studies and linkage analysis of Atlantic salmon microsatellites using haploid genetics. Heredity, **78**: 620–627. doi:10.1038/hdy.1997.101. PMID:9203354.

Taranetz, A. 1933. O nekotorykh novykh presnovodnykh rybakh iz dalnevostochnovo kraya. [Some new freshwater fishes from the Far East of USSR.] Doklady Akad. Nauk. SSSR, 1933, No. 2. pp. 83–84.

Taylor, E.B., Pollard, S., and Louie, D. 1999. Mitochondrial DNA variation in bull trout (*Salvelinus confluentus*) from northwestern North America: implications for zoogeography and conservation. Mol. Ecol. **8**: 1155–1170. doi:10.1046/j.1365-294x.1999.00674.x. PMID:10447856.

Taylor, E.B., Z., Redenbach, Z.A., Costello, A.B., Pollard, S.J., and Pacas, C.J. 2001. Nested analysis of genetic diversity in northwestern North American char, Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*). Can. J. Fish. Aquat. Sci. **58**(2): 406–420. doi:10.1139/f00-262.

Taylor, E.B., Lowery, E., Lilliestråle, A., Elz, A., and Quinn, T.P. 2008. Genetic analysis of sympatric char populations in western Alaska: Arctic char (*Salvelinus alpinus*) and Dolly Varden (*Salvelinus malma*) are not two sides of the same coin. J. Evol. Biol. **21**: 1609–1625.

Toews, D.P., and Brelsford, A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Mol. Ecol. **21**: 3907–3930. doi:10.1111/j.1365-294X.2012.05664.x. PMID:22738314.

van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., and Shipley, P. 2004. Micro-Checker: Software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes, **4**: 535–538.

Walters, V. 1955. Fishes of western Arctic America and eastern Arctic Siberia. Bull. Am. Mus. Nat. Hist. No. 106.

Waples, R.S. 2015. Testing for Hardy–Weinberg proportions: have we lost the plot? J. Hered. **105**: 1–19. doi:10.1093/jhered/esu0622.

Westlake, R.L., and O'Corry-Crowe, G.M. 2002. Macrogeographic structure and patterns of genetic diversity in harbor seals (*Phoca vitulina*) from Alaska to Japan. J. Mammol. **83**: 1111–1126. doi:10.1644/1545-1542(2002)083<1111:MSAPOG>2.0.CO;2.

Williamson, K.S., Cordes, J.F., and May, B. 2002. Characterization of microsatellite loci in chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Mol. Ecol. Notes, **2**: 17–19. doi:10.1046/j.1471-8286.2002.00129.x.

Yamamoto, S., Maekawa, K., Morita, K., Crane, P.A., and Oleinik, A.G. 2014. Phylogeography of the salmonid fish, Dolly Varden *Salvelinus malma*: multiple glacial refugia in the North Pacific Rim. Zool. Sci. **31**: 660–671. doi:10.2108/zs130266. PMID:25284385.