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MOLECULAR GENETIC EVIDENCE FOR PARALLEL LIFE-HISTORY EVOLUTION WITHIN A PACIFIC SALMON (SOCKEYE SALMON AND KOKANEE, *ONCORHYNCHUS NERKA*)

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Abstract.—The Pacific salmon *Oncorhynchus nerka* typically occurs as a sea-run form (sockeye salmon) or may reside permanently in lakes (kokanee) thoughout its native North Pacific. We tested whether such geographically extensive ecotypic variation resulted from parallel evolutionary divergence thoughout the North Pacific or whether the two forms are monophyletic groups by examining allelic variation between sockeye salmon and kokanee at two minisatellite DNA repeat loci and in mitochondrial DNA (mtDNA) *Bgl* II restriction sites. Our examination of over 750 fish from 24 populations, ranging from Kamchatka to the Columbia River, identified two major genetic groups of North Pacific *O. nerka*: a "northwestern" group consisting of fish from Kamchatka, western Alaska, and northwestern British Columbia, and a "southern" group consisting of sockeye salmon and kokanee populations from the Fraser and Columbia River systems. Maximum-likelihood analysis accompanied by bootstrapping provided strong support for these two genetic groups of *O. nerka*; the populations did not cluster by migratory form, but genetic affinities were organized more strongly by geographic proximity. The two major genetic groups resolved in our study probably stem from historical isolation and dispersal of *O. nerka* from two major Wisconsinan glacial refugia in the North Pacific. There were significant minisatellite DNA allele frequency differences between sockeye salmon and kokanee populations from different parts of the same watershed, between populations spawning in different tributaries of the same lake, and also between sympatric populations spawning in the same stream at the same time. MtDNA *Bgl* II restriction site variation was significant between sockeye salmon and kokanee spawning in different parts of the same major watershed but not between forms spawning in closer degrees of reproductive sympatry. Patterns of genetic affinity and allele sharing suggested that kokanee have arisen from sea-run sockeye salmon several times independently in the North Pacific. We conclude that sockeye salmon and kokanee are para- and polyphyletic, respectively, and that the present geographic distribution of the ecotypes results from parallel evolutionary origins of kokanee from sockeye (divergences between them) thoughout the North Pacific.

Key words.—Evolutionary genetics, life-history evolution, minisatellite DNA, mitochondrial DNA, *Oncorhynchus nerka*, sockeye salmon, zoogeography.

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One of the best known features of the biology of many salmonid fishes (salmon, trout, and char) is the often long distance migration made by fish from "sea-run" populations between the ocean, where they feed and grow to maturity, and the spawning and nursery habitats in freshwater lakes and streams (e.g., McDowall 1987). Many salmonid species, however, exhibit conspicuous polymorphisms in life history; some populations reside in freshwater thoughout their life, whereas others are sea-run, and both forms may coexist within the same freshwater habitats for part of the year (Hindar et al. 1986; Verspoor and Cole 1989; Cross et al. 1992). The sea-run and freshwater resident forms within species usually also differ in one or more morphological traits (body size, shape, or meristic characters) such that taxonomic distinctions have often been made between them (e.g., Wilder 1947; Behnke 1972). One of the recurring evolutionary problems associated with such conspicuous life-history polymorphism has been whether thoughout their range the forms represent two distinct lineages (and hence perhaps warranting taxonomic distinction) or whether they are polyphyletic and the result of multiple episodes of parallel evolution (e.g., Behnke 1972; Hindar et al. 1991).

Oncorhynchus nerka is native to watersheds tributary to the North Pacific from southern Kamchatka and Japan in the western Pacific to the Columbia River in North America. Thoughout this range, *O. nerka* may be sea-run (known as sockeye salmon) or may reside permanantly in lakes (known as kokanee). Sockeye salmon attain larger sizes at maturity and the two forms coexist broadly in many river/lake systems. There are also several instances where sockeye salmon and kokanee spawn in the same stream at the same time (Hanson and Smith 1967; McCart 1970; Foote and Larkin 1988). Foote et al. (1989) addressed the question of the evolutionary relationship between sockeye salmon and kokanee by surveying allelic variation at 3–5 polymorphic allozyme loci among populations from British Columbia. They concluded that the two forms were polyphyletic and that sockeye salmon had given rise to kokanee repeatedly thoughout British Columbia following the last glaciation. Patterns of relationship between forms within and between different watersheds, and the sharing of some rare alleles between forms within lakes, suggested that coexisting populations may have diverged sympatrically in each lake where they now co-occur (Foote et al. 1989). As acknowledged by Foote et al. (1989), however, common selective environments acting on the allozyme loci might also explain the closer genetic similarity of sympatric sockeye salmon and kokanee relative to comparable life-history types in other lakes (cf. Karl and Avise 1992).

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In this study, we had two objectives. First, we tested the hypothesis that the migratory life-history types within *O. nerka* are monophyletic groups by surveying variation in genetic systems evolving independently from allozyme loci. Assessing variation between taxa across multiple loci and genetic systems provides a powerful test of relationships even if the number of loci within systems is relatively small (Swofford and Olsen 1990; Degnan 1993). We also wished to broaden understanding of the evolutionary genetic relationship between forms by sampling sockeye salmon and kokanee from across their entire geographic range.

Most extant populations of *O. nerka* are of recent origin (< 15,000 years old) because the Wisconsinan glacial advance incorporated most of the present range of the species. Two major geographic areas ("Beringia" in the northwest and central Pacific and "Cascadia" in the lower Columbia River Valley and adjacent areas) are considered to be the main glacial refugia for sea-run and freshwater fishes in the North Pacific. Such historical isolations are thought to be major architects of patterns of population genetic structure within species (e.g., Avise et al. 1987; Bernatchez and Dodson 1990; Foote et al. 1992a). Our second objective, therefore, was to assay genetic variation in *O. nerka* from throughout the North Pacific to obtain evidence for at least two major genetic groupings of *O. nerka* as predicted by the "two refuge" hypothesis.

To these ends, we surveyed allelic variation at two minisatellite DNA loci and in mtDNA restriction sites resolved by digestion with the enzyme *Bgl* II. Minisatellite DNA is a class of repetitive nuclear DNA consisting of variable numbers of tandemly repeated copies (or VNTRs) of sequence that occur between restriction endonuclease restriction sites. The tandem repeats are composed of "core" sequences typically between 10–75 nucleotides in length (Jarman and Wells 1989; Wright 1993). Minisatellite DNA loci are highly polymorphic with heterozygosities typically ranging from 50–90% (Jeffreys et al. 1985; Taggart and Ferguson 1990). Minisatellite DNA variation has primarily been investigated in human populations or in pedigree studies of other animal populations (Nakamura et al. 1987; Georges et al. 1988). With such high levels of polymorphism, however, minisatellites are finding increasing application in the study of relationships and sociobiology in wild populations (Wayne et al. 1991; Prodöhl et al. 1992; Taylor and Bentzen 1993a). The usefulness of mtDNA for studying relationships among natural populations is well established (Avise et al. 1987; Harrison 1989; Meyer 1994). Its maternal mode of inheritance also makes it a useful adjunct (and independent) genetic system both from nuclear encoded allozymes and minisatellite DNA for the study of animal population relationships.

Materials and Methods

Study Populations and Tissue Sampling

From 1988–1993 we sampled from 10 to 61 sockeye salmon and kokanee from 24 populations ranging from Kamchatka in eastern Russia to the Columbia River (Fig. 1). These samples spanned most of the natural range of *O. nerka* except Japan, included populations from areas encompassing known glacial refugia for the species, and included replicate samples of life-history forms both within and among several river systems. From each fish we obtained blood samples as a source of genomic DNA by mixing 0.25–1.0 ml of blood with an excess of 95% ethanol. Ethanol stored samples of blood were kept at −20°C until DNA extraction. Liver samples from a few populations were frozen on dry ice and stored at −20°C for up to 4 yr before DNA extraction. Liver samples were also taken from 10 full-sib juvenile sockeye salmon and from their male and female parents which were mated artificially in the laboratory. The parent sockeye were from Weaver Creek a tributary of the lower Fraser River, British Columbia (R. H. Devlin, pers. comm., 1994). We analyzed the segregation of nuclear DNA polymorphisms between parent and offspring to verify their genetic inheritance. We sampled one population (lower Adams River sockeye salmon) both in 1990 and 1991 to test for interannual variation in allele frequency.

DNA Extraction

To extract genomic DNA, the blood was resuspended in ethanol and 250 μl of this mixture was centrifuged at 12,000 × g to pellet the blood cells. The ethanol was withdrawn and the blood cells were resuspended in 750 μl TE buffer (pH 8.0) and spun again at 12,000 × g. The TE was withdrawn and the cells were resuspended in 700 μl of lysis buffer containing 10 mM Tris, 400 mM NaCl, and 2 mM EDTA. The cells were lysed by the addition of SDS to 0.8% and the lysate was digested with proteinase K at a final concentration of 200 μg/ml. Digestion proceeded overnight at 55°C after which cellular debris and proteins were precipitated by the addition of 350 μl 6 M NaCl. Following centrifugation at 12,000 × g for 30 min, the supernatant was extracted once with phenol/chloroform (1:1) and high molecular weight DNA was precipitated by the addition of 0.6 volumes of isopropyl alcohol with gentle mixing. The DNA was recovered by centrifugation at 12,000 × g for 5 min, the pellets were washed with 70% ethanol, and dried under vacuum. For the ethanol-stored and frozen liver samples, we extracted DNA from 20 mg scrapings of tissue by digestion with Pronase followed by phenol/chloroform extraction and ethanol precipitation as outlined by Taggart et al. (1992). The DNA from the blood and liver extractions was resuspended in 50–200 μl TE, quantified by spectrophotometry, and stored at −20°C. These extraction procedures typically yielded 30–200 μg of high molecular weight DNA.

Restriction Enzyme Digestion and Southern Blotting

Approximately 5–8 μg of genomic DNA from each individual was digested with the restriction enzyme *Hae* III (5 units per ug genomic DNA) following the manufacturers instructions (New England Biolabs). The digested samples were size-fractionated by electrophoresis in 0.8% agarose gels in 0.5 χ TBE buffer (45 mM Tris-Borate, 1 mM EDTA, pH 8.0). DNase-free RNase was added to the molten agarose (0.2 μg ml^{-1}) before gel casting to digest low molecular weight RNA in each sample during electrophoresis. Lambda DNA restricted with *Hin*d III and *Eco*R I was included in four lanes of each gel to serve as a molecular weight size standard. After electrophoresis the agarose gels were depu-

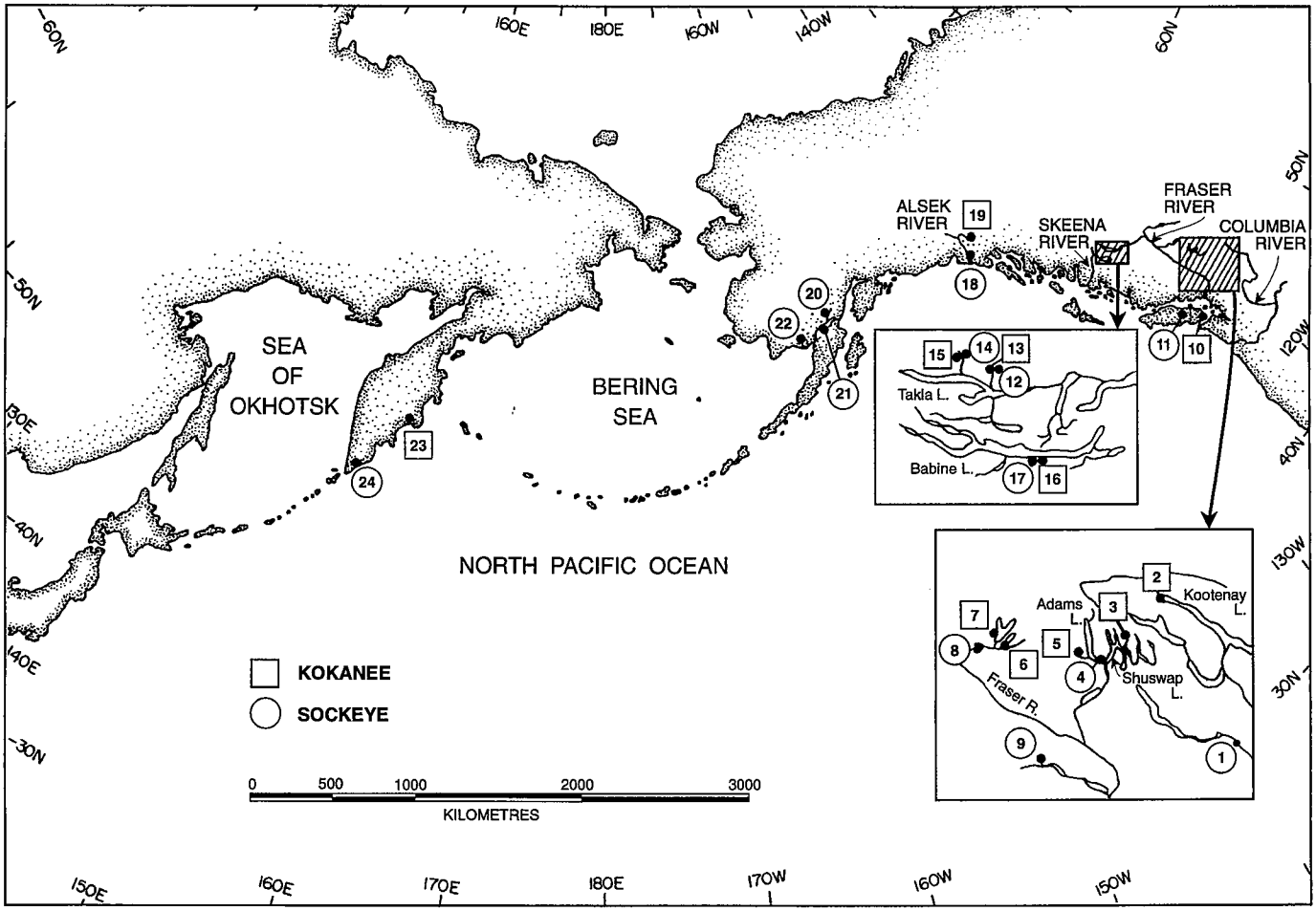

FIG. 1. Geographic locations of sockeye salmon (circles) and kokanee (squares) populations sampled tributary to the North Pacific Ocean. 1, Okanagan River; 2, Kootenay Lake; 3, Eagle River; 4, Lower Adams River; 5, Sinmax Creek; 6, Little Horsefly River; 7, Quesnel Lake; 8, Horsefly River; 9, Birkenhead River; 10, Cowichan Lake; 11, Great Central Lake; 12–13, Narrows Creek; 14–15, Shale Creek; 16–17, Pierre Creek; 18, Klukshu River; 19, Kathleen Lake; 20, Tazimina River; 21, Iliamna Lake; 22, Hansen Creek; 23, Kronotskiy Lake; 24, Kuril Lake.

rinated, alkali denatured and the DNA transferred under vacuum (Pharmacia Vacu-Gene) to nylon hybridization membranes (Amersham Hybond-N). After transfer, the membranes were washed briefly in 2 × SSC (0.3 M NaCl, 0.03 M sodium citrate) and fixed to the membranes by cross-linking under ultraviolet illumination for 3 min.

Hybridization Studies

We assayed variation at two variable number tandem repeat (VNTR) loci in sockeye salmon and kokanee by hybridization with two minisatellite repeat sequence probes, "Ssa1-rep" (Ssa1) and "3.15.34" (T34). Both probes were derived from Atlantic salmon (*Salmo salar*) genomic libraries as described by Bentzen et al. (1993) and Taggart and Ferguson (1991), respectively. Ssa1 hybridizes with homologous sequences in a number of salmonids (Bentzen et al. 1993) and detects variation in the number of tandemly arranged motifs containing a "core" 16 base pair (bp) sequence and that occur between restriction enzyme sites. Approximately 100 ng of Ssa1 was labeled nonradioactively with the nucleotide analog digoxigenin-11-dUTP by random priming (Feinberg and Vogelstein 1983; Höltke et al. 1992). The membranes containing *Hae* III-digested genomic DNA were prehybridized in 1% BSA, 7% SDS, 0.26 M Na_2PO_4, 1 mM EDTA for at least 1 h at 65°C in a rotisserie style hybridization oven (Robbins Scientific) then hybridized by the addition of denatured probe and incubated at 65°C for 16 h. Following hybridization, the membranes were washed in 2 × SSC/0.1% SDS at 65°C for 30 min and once in 1 × SSC/0.1% SDS under the same conditions. Hybridizations with T34 (12.5 ng) were completed under similar conditions but also included Atlantic salmon competitor DNA (120 ug) and final stringency washes were performed with 0.2 × SSC/0.1% SDS. For both probes the DNA hybrids were detected by chemiluminescence (Bronstein and McGrath 1989; Höltke et al. 1992) followed by exposure of the membranes to x-ray film (Kodak XOMAT-AR) at room temperature for between 2 and 16 h.

Scoring of VNTR Variation

High levels of polymorphism and the limitations imposed by current techniques for resolving VNTR alleles coupled with the essentially continuous distribution of the molecular

weights of such alleles make comparisons of restriction fragments within and between gels subject to measurement error (see Galbraith et al. 1991). We minimized this potential error in four ways. First, restricted DNA of individuals from two or thee populations was always electrophoresed on common gels. Estimates of band molecular weights of individuals within each population would thus not be limited to a single gel. Second, four λ size standards were included on each gel and the mean migration distance of each fragment of known molecular weight was used to estimate the molecular weight of sockeye and kokanee fragments resolved with the two probes. This procedure served to integrate mobility differences due to physical changes across single gels in the size standards. To estimate the molecular weights of unknown *O. nerka* restriction fragments, we calculated least-squares regressions between the known molecular weights of the lambda size standards and the reciprocal of their migration distances visualized on the autolumigraphs (Schaffer and Sederoff 1981). Third, and as recommended by Galbraith et al. (1991), "standard individuals" were included on each gel to serve as controls for running samples on different gels. These standards were two fish possessing the most common alleles at Ssa1 and T34 (range of allelic molecular weights were 0.8–5.1 kilobase pairs, kbp). Finally, where any ambiguities remained regarding allelic distinctions, the individual DNAs in question were restricted and hybridized with Ssa1 or T34 in side-by-side comparisons. The VNTR alleles resolved with Ssa1 and T34 were designated by single letter codes (e.g., "A," "B," etc.) in order of appearance.

Mitochondrial DNA

We also examined restriction site variation in the mitochondrial DNA genomes of most of the sockeye salmon and kokanee populations assayed for VNTR variation. The restriction endonuclease *Bgl* II resolves three restriction fragment length polymorphisms (RFLPs) in *O. nerka* that differ from one another by one or two site changes. The "A" pattern consists of 10.4, 3.6, and 2.6 kbp fragments, "B" 10.4, 2.6, 2.0, and 1.6 kbp, and "C" consists of 10.4, 2.3, 2.0, 1.6, and 0.3 kbp fragments (Brannon et al. 1992; E. B. Taylor, unpubl. data). We examined variation in these *Bgl* II mtDNA "alleles" as an independent (from the minisatellite DNA) genetic marker and because they were simply interpreted (in terms of site gains or losses). We had, however, no a priori knowledge of the extent or pattern of restriction site variation among populations or life-history types.

Five μg of total DNA (nuclear and mitochondrial) were digested with *Bgl* II, size-fractionated on 0.8% agarose gels and transferred under vacuum to nylon hybridization membranes as outlined above. Mitochondrial DNA RFLPs were resolved by hybridization to digoxigenin-labeled rainbow smelt (*Osmerus mordax*) mtDNA fragments cloned into PUC 18. The smelt mtDNA probes resolved the same set of *Bgl* II fragments in sockeye salmon and kokanee that Brannon et al. (1992) resolved probing with purified *Oncorhynchus* mtDNA. Hybridizations were carried out as described for Ssa1 above except that the temperature was lowered to 58°C and stringency washes were reduced to 2 × SSC/0.1% SDS for 10 min at room temperature and then for 20 min at 58°C. The probe-mtDNA hybrids were detected by chemiluminesence and autolumigraphy as described above.

Statistical Analysis of VNTR Allele mtDNA Genotype Frequency Data

Our analysis of VNTR allele variation in sockeye salmon and kokanee populations consisted of calculating allele frequencies and estimates of single locus heterozygosity using BIOSYS (release 1.7, Swofford and Selander 1981). Tests of the fit of observed genotype frequencies within populations with Hardy-Weinberg equilibrium expectations were evaluated using chi-square tests with probability levels adjusted for multiple comparisons within loci (Lessios 1992). The goodness of fit tests were conducted by pooling genotypes into three classes with all alleles but the most common considered as one allele because the expected frequencies of some genotypes were so small as to make the standard chi-square test unreliable (Swofford and Selander 1981). Tests of the significance of allele frequency, or genotype frequency for mtDNA, differences between *O. nerka* from different geographic regions and between sympatric sockeye and kokanee were evaluated using the chi-square randomization procedure outlined by Roff and Bentzen (1989) using the MONTE program of the REAP software package (McElroy et al. 1992).

Relationships among sockeye and kokanee populations were inferred from the allelic frequency data for the two minisatellite loci and also for mtDNA by treating the restriction site genotypes as "alleles." Numerous alternative phylogenetic inference methods are available for analysis of allele frequency matrices, few of which are immune from biological and/or numerical limitations (see Rogers 1985; Swofford and Olsen 1990). We estimated relationships among sockeye salmon and kokanee populations from the allele frequency matrix using a variety of distance measures, e.g., Nei's (1972), Rogers' (1972), Cavalli-Sforza and Edwards' (1967), and Reynolds et al.'s (1983) distances; and clustering methods, e.g., UPGMA (Sneath and Sokal 1973), Neighbor-Joining (Saitou and Nei 1987), Fitch-Margoliash (Fitch and Margoliash 1967), as well as by maximum-likelihood solutions using the raw allele frequency matrix (Felsenstein 1981) using programs in PHYLIP (version 3.4, Felsenstein 1991) or BIOSYS-1. All analytical techniques were identical in their identification of the major groups of *O. nerka* described below. Given this concordance when using different distance measures and/or tree-building methods, we elected to summarize relationships using Felsenstein's (1981) maximum-likelihood solution (using CONTML of PHYLIP) because (1) this analysis assumes that genetic divergence proceeds by drift alone (not unreasonable for our populations which are postglacial in origin, i.e., about 10,000–15,000 years old) following a Brownian model of divergence (Felsenstein 1981), and (2) under a drift only evolutionary model, maximum-likelihood algorithms outperform parsimony and phenetic clustering of drift-only genetic distances, particularly when evolutionary rates vary across populations [also likely in our case; (Felsenstein 1991; Kim and Burgman 1988)].

Our confidence in the maximum-likelihood topology was assessed by bootstrap resampling of the allele frequency matrix (Felsenstein 1985). To do this, we first used the SE-

QBOOT program in PHYLIP to generate 100 bootstrapped samples of the allele frequency data. For each such matrix we then calculated the maximum-likelihood solution using CONTML. A majority-rule consensus tree for the 100 maximum-likelihood analyses was then constructed using CONSENSE in PHYLIP.

By their very nature, agglomerative clustering techniques will tend to join populations of sockeye salmon and kokanee into groups regardless of the "true" population structure of samples. As an adjunct to our maximum-likelihood/bootstrapping analyses, therefore, we also visualized relationships among populations by multidimensional scaling analysis on a matrix of Nei's (1972) genetic distances (MDS, Pimentel 1979). This ordination analysis computes coordinates for a set of points (populations) in multidimensional space such that the spatial relationships between pairs of points fit as closely as possible to measured dissimilarities (genetic distances) among the corresponding set of populations (Pimentel, 1979). Lessa (1990) and Roy et al. (1994) provide recent examples and discussion of MDS analysis of genetic distance data. For visual simplicity and because the results of ordination along two and three dimensional axes were qualitatively very similar, we projected the ordination results on two axes (Dimensions 1 and 2). To incorporate possible information concerning relationships along additional axes we also calculated a minimum spanning tree (MST) which was superimposed on the projection of populations in bidimensional space. If populations are connected in the MST, then this may indicate a closer affinity than revealed by positions in bidimensional space if further (i.e., ≥ 3) dimensions are considered (Rohlf 1970). The MDS and MST analyses were conducted using NTSYS-pc (Rohlf 1990).

Results

Allelic Variation at the Ssa1 Locus

Ssa1 resolved seven alleles, ranging from 3.7–6.3 kbp in molecular weight, among the 751 sockeye salmon and kokanee examined (e.g., Fig. 2). Two alleles "A" and "B" (4.4 and 4.1 kbp, respectively), however, accounted for about 95% of all alleles scored (Fig. 3) and predominated in most populations from Kamchatka to the Columbia River. Only one population, kokanee from Kathleen Lake (upper Alsek River, northwestern British Columbia), did not have these two alleles as the most common. Although the "A" allele was present at a frequency of 0.81 in this population, the "B" allele was absent and a 4.5 kbp band ("E") accounted for the remaining 0.19 of alleles scored. The allelic combinations at Ssa1 are consistent with Mendelian inheritance at a single locus; in our family cross, Ssa1 alleles were inherited from parent to offspring (parent genotypes: AF (male) x BB (female), progeny genotypes: 7 AB, 3 FB) and no departures from Hardy-Weinberg expected genotypic frequencies were detected within any population (minimum $P > 0.1$). Single-locus expected heterozygosities averaged 0.47 (SE = 0.026, range: 0.32–0.60).

Allelic Variation at the 3.15.34 Locus

In 752 sockeye salmon and kokanee, 3.15.34 (T34) resolved five alleles ranging in molecular weight from 0.950–3.7 kbp (e.g., Fig. 2). As at the Ssa1 locus, two alleles ("A" = 0.980 kbp, "B" = 0.950 kbp) accounted for most (96%) of the variation scored within all populations (Fig. 3), and this variation was also consistent with Mendelian segregation at a single locus. For instance, sockeye salmon progeny from our full-sib mating inherited VNTR polymorphisms from their parents (parent genotypes: BB (male) x AB (female), progeny genotypes: 5 AB, 5 BB). Also, observed genotypic frequencies did not show any significant deviation from Hardy-Weinberg expectations and single-locus expected heterozygosity varied more at T34 than at Ssa1 and averaged 0.34 (SE = 0.049, range: 0.04–0.53).

Geographic Variation in Minisatellite Allele Frequencies

Although two alleles accounted for 95% of all those scored at both minisatellite loci in *O. nerka* populations from Kamchatka to the Columbia River, there were some striking allele frequency differences between major geographic regions at both Ssa1 and T34 (Table 1, Figs. 4–5). North and west of the Alsek River in northwestern British Columbia, the 4.4 kbp "A" allele at Ssa1 predominated in all sockeye and kokanee populations (mean $\pm$ SE frequency = 0.61 $\pm$ 0.04). By contrast, in the more southern Skeena, Fraser, and Columbia River and Vancouver Island populations, the 4.1 kbp "B" allele usually predominated (mean frequency of A = 0.40 $\pm$ 0.03, $P < 0.0001$, Fig. 4). Variation at Ssa1 within these putative "northwestern" and "southern" groups of sockeye and kokanee was more limited. There was no significant allelic variation among sockeye salmon populations from Kuril Lake (western Pacific) to the Alsek River (eastern Pacific, Fig. 4, $P = 0.8$). There was, however, significant allele frequency variation at Ssa1 among southern sockeye salmon and among southern kokanee populations (both $P < 0.0005$).

At the T34 locus, those populations north and west of the Alsek River had a significantly higher frequency of the approximately 0.98 kbp "A" allele (mean frequency = 0.78 $\pm$ 0.05, Fig. 5) than those south of this point (frequency of A = 0.38 $\pm$ 0.07, $P < 0.0001$). Allelic variation within each of the northwestern and southern groups was, however, greater than that observed at Ssa1. Significant allele frequency differences were observed among northwestern and southern sockeye salmon, and among southern kokanee populations (all $P < 0.0001$).

Allelic Variation between Sympatric Populations

The distinction between a northwestern and southern grouping of *O. nerka* at minisatellite loci was accompanied by significant variation between life-history forms inhabiting the same watersheds in various degrees of reproductive sympatry (Table 1, Figs. 4–5). In northwestern *O. nerka,* there was a striking difference in Ssa1 allele frequencies between Klukshu River sockeye salmon (lower Alsek River) and kokanee spawning in tributaries of Kathleen Lake (upper Alsek, $P < 0.0001$). Similarily, sockeye salmon and kokanee spawning in different tributaries of Adams Lake (Shuswap Lake system) and the Quesnel River system were sharply divergent at Ssa1 ($P < 0.0005$) as were the life-history types spawning in Pierre Creek in Babine Lake (but collected in two different

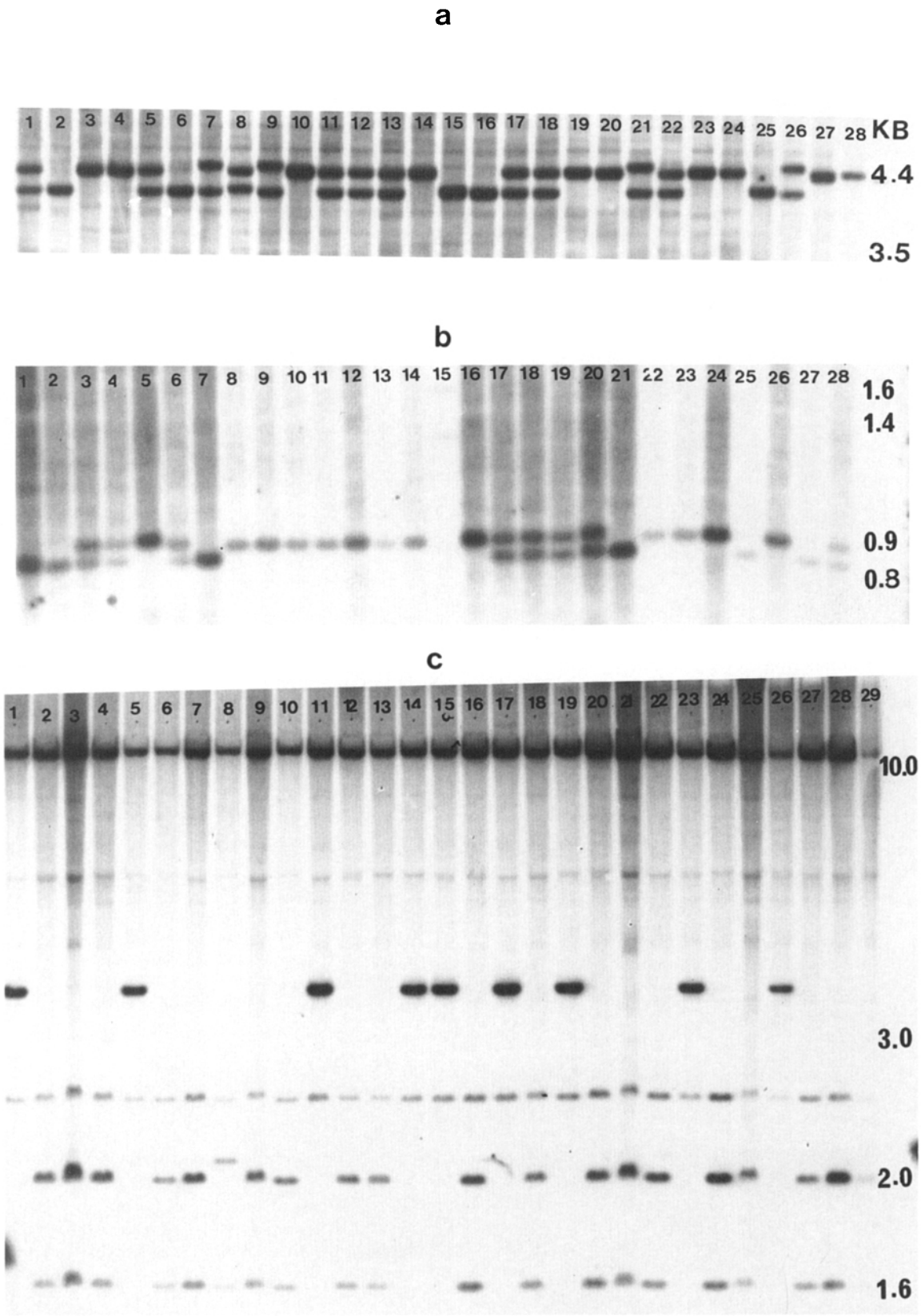

FIG. 2. Example autolumigram of alleles resolved at minisatellite loci (a) Ssa1 locus, (b) T34 locus restricted with *Hae* III, and (c) in mtDNA restricted with *Bgl* II. Hybridization and wash conditions were: 65°C, 2 × and 1 × SSC at 65°C for Ssa1, 65°C, 2 × and 0.2 × SSC at 65°C for T34, and 58 C, 2 × SSC at 58°C for mtDNA. In (a) lanes 1–3 and 7–8 exhibit genotypes a/b, b/b, a/a, b/e, and a/f, respectively. In (b) lanes 1–2 exhibit genotype b/b, lanes 3–4 exhibit a/b, and lane 5 exhibits genotype a/a. In (c) lanes 1 and 2 exemplify mtDNA genotypes A and B, respectively. Numbers at the far right of each autolumigraph indicate approximate locations of molecular weight markers in kilobase pairs.

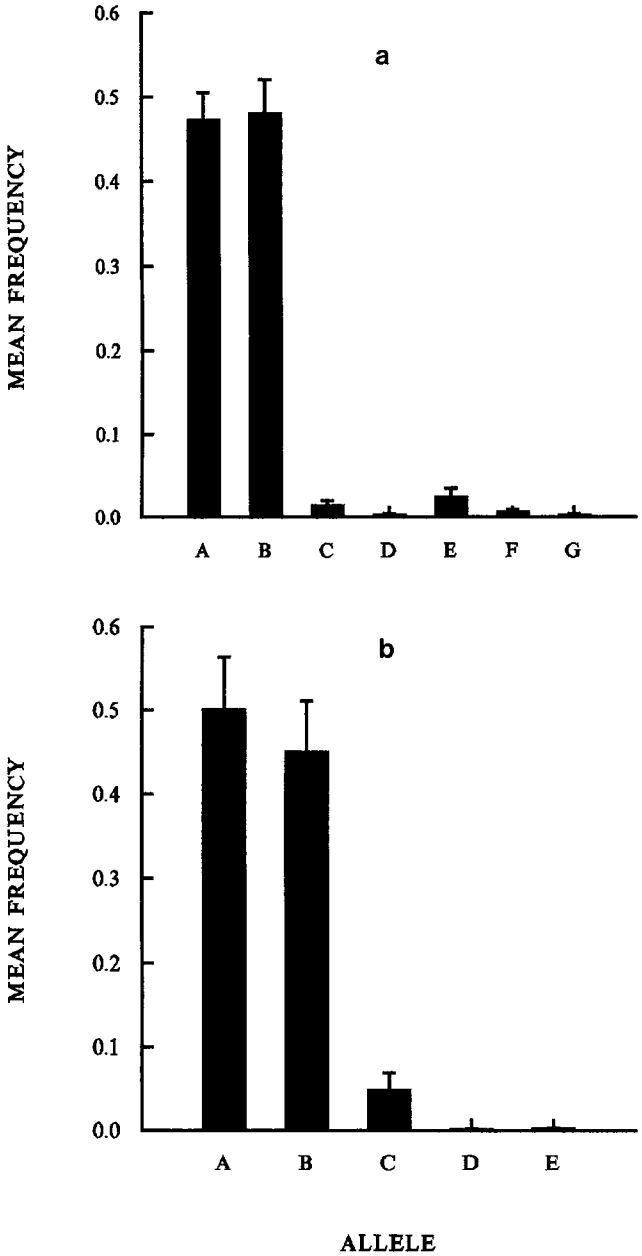

FIG. 3. Mean (+SE) frequency of alleles at two minisatellite loci resolved by hybridiation of *Hae* III-restricted sockeye salmon and kokanee genomic DNA with minisatellite probes Ssa1 (a) and T34 (b). The total sample sizes are 751 (Ssa1) and 752 (T34) fish from 24 populations from Kamchatka to the Columbia River. Molecular weights of Ssa1 alleles A–G are 4.4, 4.1, 3.7, 4.8, 4.5, 4.2, and 4.3 kilobase pairs, respectively. Molecular weights of T34 alleles A–E are 0.985, 0.950, 1.00, 1.10, and 3.65 kilobase pairs, respectively.

years, $P < 0.0001$). Sockeye and kokanee collected while spawning at the same time in Narrows Creek (Takla Lake) also differed strikingly in allele frequencies at the Ssa1 locus ($P < 0.0001$). By contrast, although a difference in allele frequencies at Ssa1 between Shale Creek (Takla Lake) sockeye salmon and kokanee was in the same direction as for other sympatric pairs, including those in adjacent Narrows Creek (Fig. 4), it was not significant ($P = 0.17$). Within form variation in Takla Lake was, however, not significant: no differences were detected in allele frequencies between Narrows and Shale creeks sockeye salmon ($P = 0.17$) or kokanee ($P = 0.19$). In each of the four sympatric lake pairs of sockeye salmon and kokanee (Babine, Adams, Quesnel, and Takla Lakes) the difference in allele frequencies was in the same direction; sockeye salmon always had a higher frequency of the B allele (Table 1, Fig. 4). The only exception to this trend was the comparison between lower Adams River sockeye salmon and Eagle River kokanee (both streams empty into Shuswap Lake, Fig. 1) where Ssa1 allele frequencies were not significantly distinct between forms ($P > 0.1$, Table 1). Finally, there was no significant difference in Ssa1 allele frequencies between samples of sockeye salmon collected from the lower Adams River in 1990 and 1991 ($P > 0.1$, Table 1).

Some allele frequency differences between sympatric forms also were detected at T34, but in general differences were less striking than at Ssa1 (Table 1, Fig. 5). Alsek River sockeye salmon (Klukshu River) and kokanee (Kathleen Lake) were highly divergent at T34; kokanee lacked the "B" allele present at a frequency of 0.32 in sockeye salmon (Fig. 5; $P < 0.0001$). Quesnel River system sockeye salmon and kokanee differed significantly at T34 ($P < 0.005$) as did the forms in Babine Lake (spawning in Pierre Creek, $P = 0.028$), while differences between Sinmax Creek kokanee and Lower Adams River sockeye salmon (Adams Lake tributaries) approached significance ($P = 0.06$). By contrast, Eagle River kokanee and lower Adams River sockeye salmon (both empty into Shuswap Lake but approximately 30 km apart) had similar T34 allele frequency distributions ($P > 0.05$) and the forms collected spawning at the same time both in Shale and Narrows creeks (Takla Lake) had virtually identical T34 allele frequencies (maximum $P = 0.7$). As at Ssa1, no differences in T34 allele frequencies were detected between sockeye salmon or between kokanee from the two Takla Lake streams (both $P > 0.05$). Variation at T34 fluctuated significantly between annual samples of Lower Adams River sockeye ($P = 0.02$), but in both years the "B" allele was the most common (frequencies of 0.88 and 0.73 in 1990 and 1991, respectively, Table 1).

mtDNA Variation

The mtDNA restriction site polymorphisms resolved by digestion with *Bgl* II also showed striking geographic variation (Fig. 6). The "A" restriction site pattern (genotype) was found at moderate frequency (0.35 ± 0.13) in sockeye and kokanee from the Alsek River west to Kamchatka (Table 1). South of the Alsek River, however, the A genotype was less common (mean frequency $= 0.091 \pm 0.06$); the A genotype was found only in Babine Lake sockeye salmon and kokanee and in Great Central Lake sockeye (Table 1, $P < 0.0001$). A single Kootenay Lake kokanee displayed the "C" genotype (0.006 overall). No significant differences were detected in *Bgl* II genotype frequencies between any of the sympatric groupings of sockeye and kokanee in the Babine, Takla, Quesnel, or Adams Lake systems. Within the Alsek River system, however, the A genotype characterized 23% of Klukshu River sockeye but was absent in Kathleen Lake kokanee (Table 1, $P = 0.03$).

TABLE 1. Allele frequencies at minisatellite DNA loci (Ssa1 and T34) and frequencies of mtDNA *Bgl* II restriction site genotypes among populations of sockeye salmon and kokanee sampled in the study. Sample localities are numbered as in Figure 1, and are grouped by geographic region, major drainage area, life-history type (S = sockeye salmon, K = kokanee), and collection year.

			Ssa1								T34						mtDNA *Bgl* II			
Locality	Form	Year	*N*	A	B	C	D	E	F	G	*N*	A	B	C	D	E	*N*	A	B	C
Kamchatkan populations																				
Kuril Lake (24)	S	1989	33	0.667	0.258	0.076	0.000	0.000	0.000	0.000	61	0.984	0.016	0.000	0.000	0.000	42	0.643	0.347	0.000
Kronotskiy L. (23)	K	1991	10	0.500	0.500	0.000	0.000	0.000	0.000	0.000	10	0.750	0.250	0.000	0.000	0.000	10	0.111	0.889	0.000
Alaskan populations																				
Tazimina River (20)	S	1992	28	0.607	0.321	0.000	0.000	0.054	0.018	0.000	30	0.833	0.150	0.017	0.000	0.000	29	0.793	0.207	0.000
Iliamna L. (21)	S	1992	39	0.526	0.333	0.013	0.000	0.090	0.026	0.013	37	0.630	0.315	0.037	0.019	0.000	—	—	—	—
Hansen Creek (22)	S	1992	26	0.519	0.404	0.000	0.000	0.077	0.000	0.000	26	0.692	0.269	0.038	0.000	0.000	29	0.310	0.690	0.000
Northwestern British Columbia populations																				
Klukshu R. (18)	S	1992	27	0.648	0.296	0.056	0.000	0.000	0.000	0.000	27	0.630	0.204	0.167	0.000	0.000	26	0.231	0.769	0.000
Kathleen L. (19)	K	1992	34	0.779	0.000	0.000	0.000	0.221	0.000	0.000	28	0.946	0.000	0.054	0.000	0.000	28	0.000	1.000	0.000
Skeena River (Babine Lake) populations																				
Pierre Cr. (16)	K	1988	38	0.539	0.447	0.000	0.000	0.013	0.000	0.000	38	0.710	0.250	0.039	0.000	0.000	38	0.286	0.714	0.000
Pierre Cr. (17)	S	1991	25	0.220	0.780	0.000	0.000	0.000	0.000	0.000	25	0.500	0.460	0.040	0.000	0.000	25	0.320	0.680	0.000
Upper Fraser River (Takla Lake) populations																				
Narrows Cr. (12)	S	1991	30	0.250	0.750	0.000	0.000	0.000	0.000	0.000	26	0.692	0.288	0.019	0.000	0.000	28	0.000	1.000	0.000
Narrows Cr. (13)	K	1991	29	0.517	0.431	0.052	0.000	0.000	0.000	0.000	25	0.740	0.260	0.000	0.000	0.000	25	0.000	1.000	0.000
Shale Cr. (14)	S	1991	38	0.355	0.618	0.000	0.000	0.000	0.026	0.000	28	0.643	0.357	0.000	0.000	0.000	—	—	—	—
Shale Cr. (15)	K	1991	32	0.500	0.484	0.000	0.000	0.000	0.016	0.000	32	0.750	0.250	0.000	0.000	0.000	—	—	—	—
Upper Fraser River (Quesnel River) populations																				
Horsefly R. (8)	S	1992	31	0.274	0.677	0.000	0.000	0.000	0.048	0.000	31	0.429	0.375	0.196	0.000	0.000	30	0.000	1.000	0.000
Quesnel L. (7)	K	1992	26	0.577	0.404	0.019	0.000	0.000	0.000	0.000	26	0.135	0.442	0.423	0.000	0.000	26	0.000	1.000	0.000
Little Horsefly R. (6)	K	1992	26	0.558	0.442	0.000	0.000	0.000	0.000	0.000	26	0.212	0.673	0.115	0.000	0.000	26	0.000	1.000	0.000
Middle Fraser River (South Thompson River) populations																				
Lower Adams R. (4)	S	1990	43	0.291	0.698	0.000	0.000	0.012	0.000	0.000	39	0.115	0.885	0.000	0.000	0.000	—	—	—	—
Lower Adams R. (4)	S	1991	29	0.362	0.638	0.000	0.000	0.000	0.000	0.000	29	0.259	0.724	0.000	0.000	0.017	29	0.000	1.000	0.000
Sinmax Cr. (5)	K	1991	30	0.633	0.367	0.000	0.000	0.000	0.000	0.000	29	0.103	0.879	0.000	0.000	0.017	30	0.000	1.000	0.000
Eagle R. (3)	K	1989	25	0.300	0.700	0.000	0.000	0.000	0.000	0.000	26	0.077	0.923	0.000	0.000	0.000	—	—	—	—
Lower Fraser River populations																				
Birkenhead R. (9)	S	1992	33	0.333	0.652	0.000	0.000	0.015	0.000	0.000	33	0.318	0.636	0.045	0.000	0.000	33	0.000	1.000	0.000
Columbia River populations																				
Okanagan R. (1)	S	1992	25	0.348	0.609	0.000	0.022	0.000	0.000	0.022	24	0.190	0.810	0.000	0.000	0.000	25	0.000	1.000	0.000
Kootenay L. (2)	K	1991	33	0.483	0.467	0.000	0.033	0.017	0.000	0.000	33	0.091	0.909	0.000	0.000	0.000	33	0.000	0.970	0.030
Vancouver Island populations																				
Great Central L. (11)	S	1990	24	0.250	0.688	0.000	0.021	0.000	0.042	0.000	26	0.327	0.615	0.058	0.000	0.000	30	0.667	0.333	0.000
Cowichan L. (10)	K	1993	37	0.473	0.378	0.081	0.000	0.027	0.000	0.041	37	0.608	0.392	0.000	0.000	0.000	24	0.000	1.000	0.000

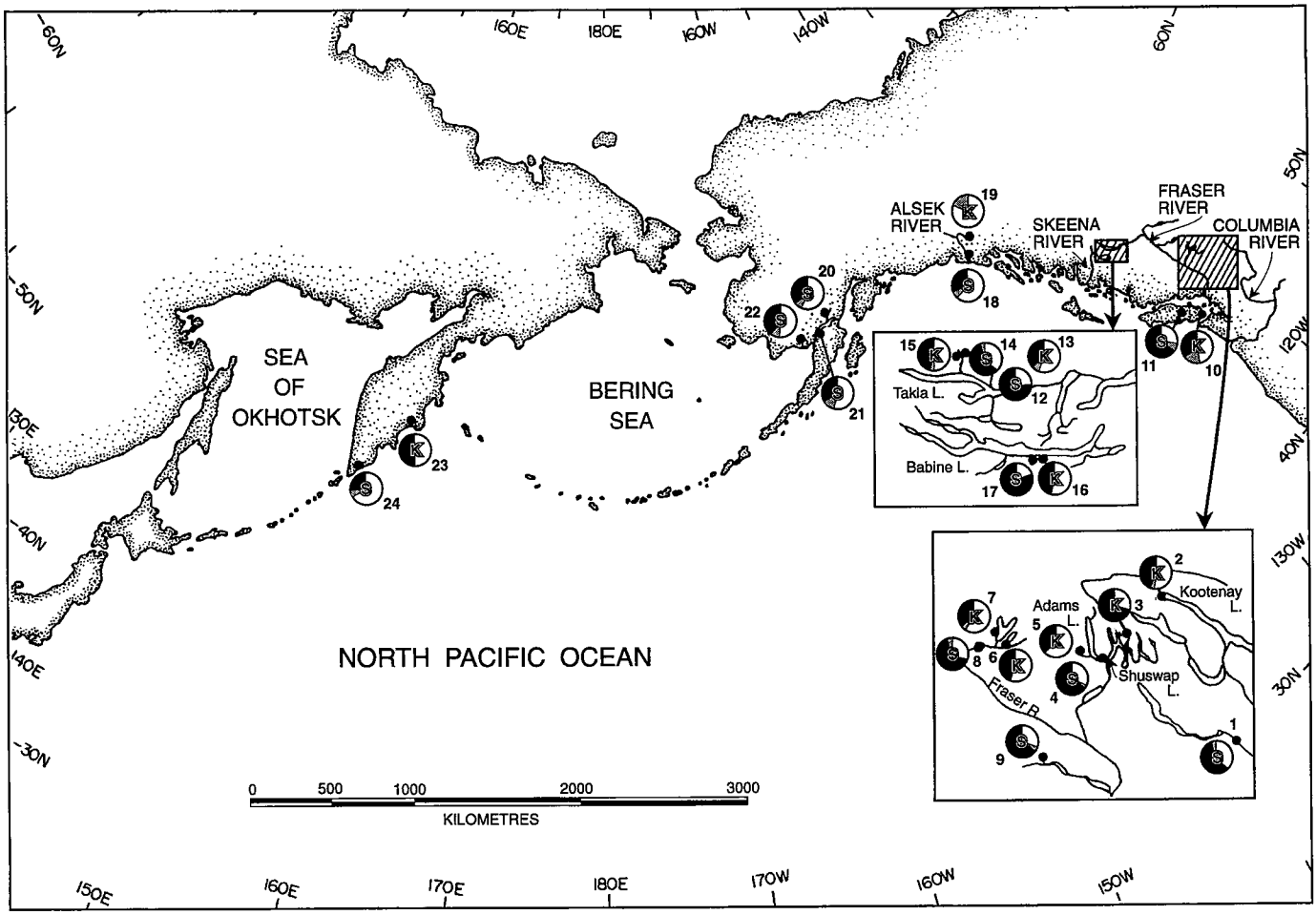

FIG. 4. Geographic variation in the frequency of minisatellite alleles resolved at Ssa1 locus among populations of sockeye salmon (S) and kokanee (K). Open shading = "A" allele (4.4 kilobase pairs, kbp), dark shading = "B" allele (4.1 kbp), cross-shading = other alleles. Numbers refer to population codes given in Figure 1.

Relationships among Populations and Life-history Forms

Bootstrapped (N = 100) maximum-likelihood (ML) analyses of the allele frequency matrix of minisatellite and mitochondrial DNA suggested that North Pacific *O. nerka* fell into two broad geographic groupings (Fig. 7). A "northwestern" grouping consisted almost entirely of sockeye salmon and kokanee from the Skeena River and north and west to Alaska and Kamchatka (minimum 64% bootstrap support, Fig. 7). The other, "southern" group, included *O. nerka* south of the Skeena River and consisted of populations from the middle Fraser and Columbia River systems (minimum 56% bootstrap support, Fig. 7). Alternative phylogenetic estimation procedures using various distance metrics and clustering algorithms (Nei's, Rogers', Reynolds et al.'s, Cavalli-Sforza and Edwards' genetic distances, UPGMA, Fitch-Margoliash, and Neighbor-Joining trees) yielded comparable results; a major distinction between northwestern and southern *O. nerka* was evident at between 53 and 80% bootstrap support.

Ordination analysis by multidimensional scaling (MDS) also suggested a distinction between "northwestern" and "southern" *O. nerka* (Fig. 8). For instance, most populations of sockeye salmon and kokanee from the Fraser/Columbia were close in multidimentional space and were closely connected by the minimum spanning tree (MST, e.g., BIS, QUK, LAS, SIK, OKS). Northwestern Pacific populations (e.g., TAS, KUS, KLS, KAK) were more interspersed amongst themselves, but were clearly separated from southern *O. nerka* and were connected by the MST (Fig. 8). The MDS also strongly suggested that within northwestern *O. nerka,* sockeye salmon and kokanee were more similar to each other then either form was to comparable life-history populations within southern *O. nerka,* and vice versa (Fig. 8).

DISCUSSION

Our study of minisatellite and mtDNA polymorphism provides strong support for the idea that historical geo-climatological events (e.g., Pleistocene glaciations) have played a prominent role in the organization of patterns of genetic variation (cf. Avise et al. 1987; Bernatchez and Dodson 1990; Foote et al. 1992a). For instance, our analyses indicate that North Pacific *O. nerka* can be broadly grouped into two major genetic groupings: a "northwestern" Pacific group consisting of sockeye salmon and kokanee from Kamchatka, western Alaska, and northwestern British Columbia and a "southern"

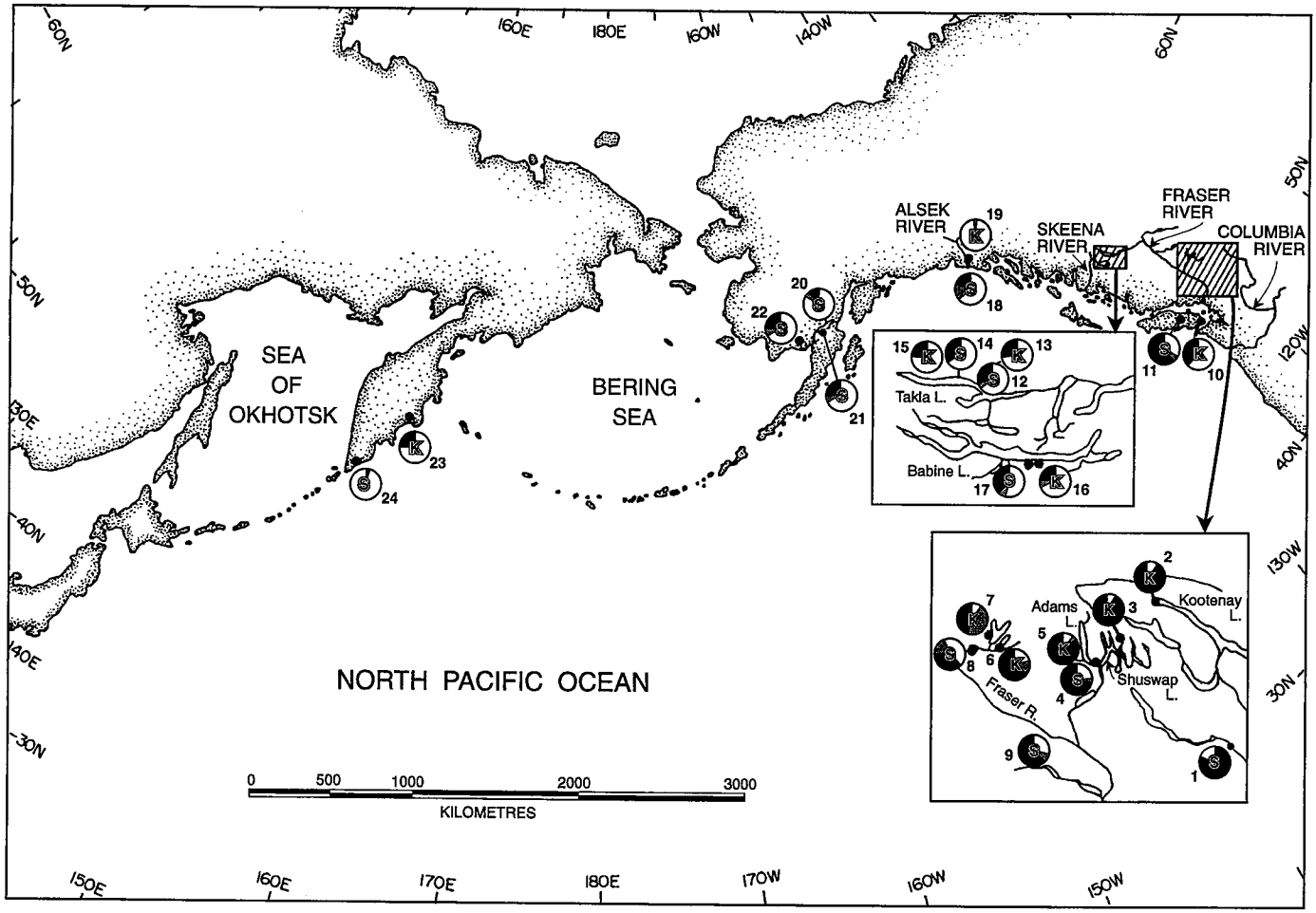

FIG. 5. Geographic variation in the frequency of minisatellite alleles resolved at T34 locus among populations of sockeye salmon (S) and kokanee (K). Open shading, "A" allele (0.98 kilobase pairs, kbp); dark shading, "B" allele (0.95 kbp); cross-shading, other alleles. Numbers refer to population codes given in Figure 1.

group of populations from the mid-lower Fraser River and the Columbia River (Figs. 4–8). These two groups of *O. nerka* were detected at each locus separately, although the exact position of the boundary between northwestern and southern groups varied among the Alsek, Skeena, or upper Fraser (Takla Lake) depending on the locus examined (cf. Wood et al. 1994).

The identification of two major genetic groupings of North Pacific sockeye salmon and kokanee at minisatellite and mitochondrial DNA loci is consistent with observations of variation at other loci in *O. nerka*. For instance, Utter et al. (1980) distinguished southern (Skeena River south through the Fraser and Columbia) sockeye and kokanee from those further north and west in Asia and Alaska based on variation at two allozyme loci. In a more extensive analysis of variation at 33 allozyme loci, Wood et al. (1994) also recognized "northern" and "southern" population groups among 80 spawning populations of sockeye salmon in British Columbia. The northern group resolved by Wood et al. (1994) included populations between the Alsek River south to the Skeena River whereas the southern group consisted of fish from the Fraser (including Takla Lake) and Columbia systems as found in our study of DNA-based polymorphism. Finally, Bickham et al. (1995) examined allozyme variation at three loci and restriction site and sequence variation within the mitochondrial DNA (mtDNA) cytochrome *b* gene in eight spawning populations of sockeye salmon from Kamchatka to the southern Fraser River. These authors also detected a major genetic distinction between the southern Fraser populations (Adams and Shuswap Rivers) and those from the Skeena, western Alaska, and Kamchatka (Bickham et al. 1995).

The concordant pattern of variation in *O. nerka* resolved at independent allozyme, mtDNA, and minisatellite DNA loci provides strong evidence for at least two major genetic groups of sockeye and kokanee in the North Pacific. Similar genetic groupings of populations into northwestern and southern North Pacific groups have also been detected in chum, *O. keta,* (Taylor et al. 1994) and pink, *O. gorbuscha,* salmon (Varnavsakya and Beacham 1992) and in rainbow and steelhead trout, *O. mykiss* (Okazaki 1984). The two genetic groupings in sockeye salmon and kokanee and in these other sea-run fishes probably stems from the survival and subsequent dispersal of populations in two major refugia during the last (Wisconsinan) glaciation which ended about 15,000 years ago. McPhail and Lindsey (1986) and Lindsey and McPhail (1986) discussed the survival of North Pacific freshwater and

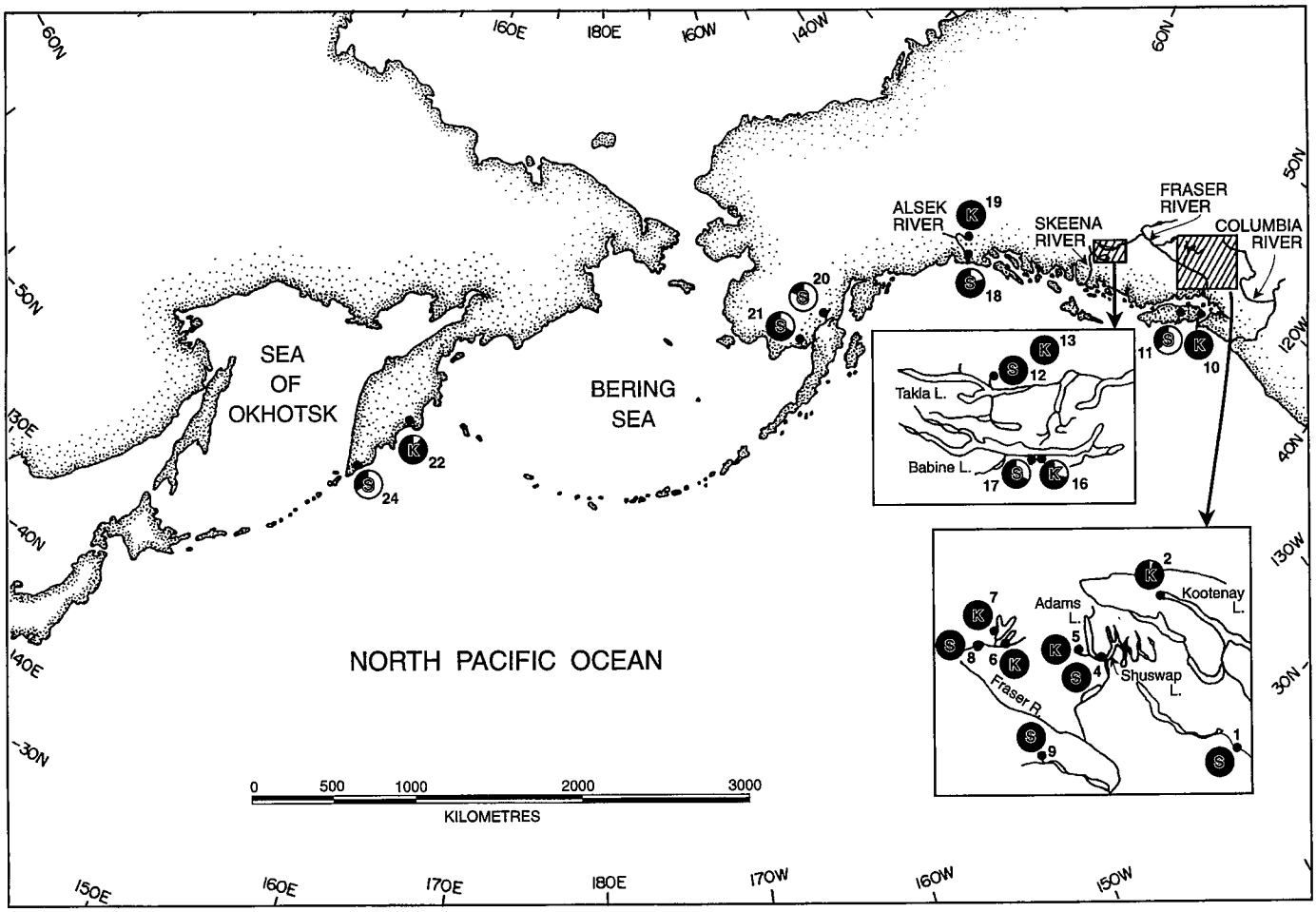

FIG. 6. Geographic variation in the frequency of mt DNA *Bgl* II restriction site genotypes among populations of sockeye salmon (S) and kokanee (K). Open shading, "A" genotype; dark shading, "B" genotype; cross-shading, "C" genotype. Numbers refer to population codes given in Figure 1.

sea-run fishes in a Bering Sea-Yukon River valley refugium ("Beringia") as well as south of the icesheet in the lower Columbia River valley ("Cascadia"). The genetic affinity between mid-lower Fraser and Columbia River *O. nerka,* thus, probably stems from their derivation from ancestral populations originating within the Columbia refuge. Similarily, the Bering-Yukon refuge included areas in eastern Siberia, southern Kamchatka, and western Alaska where postglacial dispersal in North America is thought to have extended south to at least the Skeena River (Lindsey and McPhail 1986).

Wood et al. (1994) presented evidence for a third genetic group of sockeye salmon consisting of Skeena River and "coastal islands" populations which tended to show closer affinity to northwestern *O. nerka.* In our study, Vancouver Island populations tended to be associated either with northwestern sockeye salmon and kokanee (e.g., Great Central Lake, Cowichan Lake). It should be noted, however, that Cowichan Lake kokanee lacked the *Bgl* II "A" genotype, a situation more characteristic of southern populations (Table 1). This apparent variability in affinities of Vancouver Island *O. nerka* depending on which loci were examined (VNTR or mtDNA) may indicate their founding by a mixture of northwestern and southern *O. nerka.* Alternatively, Vancouver Island *O. nerka* may have been founded by individuals that survived the last glaciation in a third, coastal islands refugium on northwestern Vancouver Island or the Queen Charlotte Islands (Pojar 1980; Warner et al. 1982; O'Reilly et al. 1993; Wood et al. 1994).

Sockeye salmon and kokanee may have existed both within northwestern and southern ancestral populations before the beginning of the last glaciation some 75,000 years ago. Within each region, however, most extant populations of kokanee probably have arisen by repeated, independent divergences from northwestern and southern anadromous refugial sockeye salmon following deglaciation beginning about 15,000 years ago. Alternatively, extant sockeye salmon and kokanee within each region may have arisen from a single, more ancient divergence and each form could have repeatedly colonized extant watersheds following deglaciation. We favor independent, multiple divergences of kokanee from sockeye salmon that colonized watersheds within northwestern and southern regions for the following reasons.

First, our maximum-likelihood and the multidimensional scaling analyses suggest that the two life-history forms of *O. nerka* are not monophyletic. For instance, there was no grouping of populations by life-history type (sockeye or kokanee)

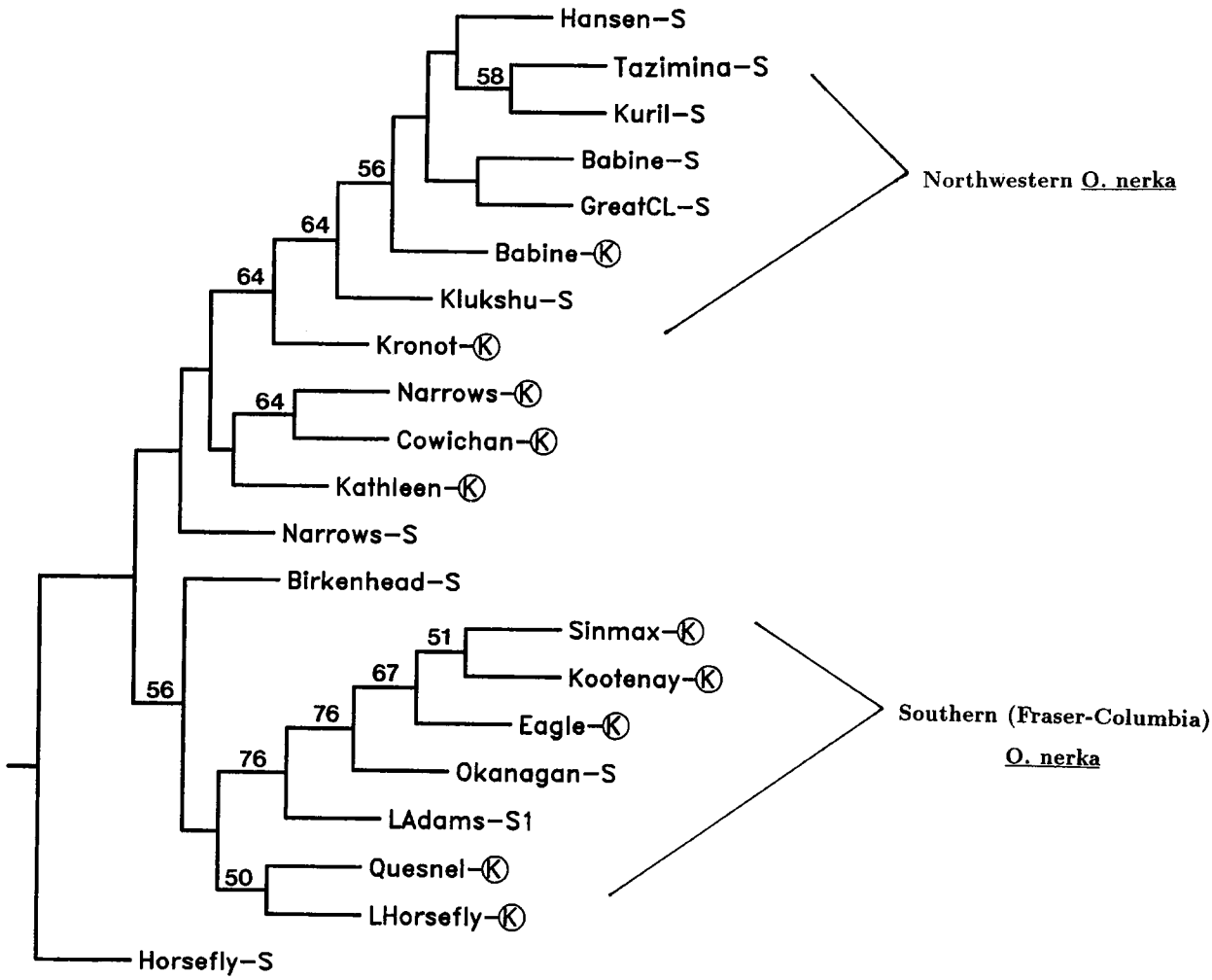

FIG. 7. Relationships among sockeye salmon (S) and kokanee (K) populations represented by the consensus tree from 100 bootstrap maximum-likelihood analyses of the allele frequencies at Ssa1 and T34 minisatellite loci and mtDNA *Bgl* II genotypic frequencies. Nodes receiving at least 50% support from 100 bootstrap replicates are indicated by numbers at branch points.

and there were several instances where kokanee populations were genetically more similar to sockeye salmon than to other kokanee and vice versa (Figs. 7–8). Our data, therefore, corroborate and extend the data of Foote et al. (1989) who concluded that sockeye salmon and kokanee from the Skeena, Fraser, and Columbia Rivers and Vancouver Island were not distinct lineages based on genetic distances inferred from variation at 3–5 polymorphic allozyme loci. Second, our suggestion that kokanee and sockeye salmon are poly- and paraphyletic, respectively, is consistent with the observation that kokanee populations have developed after the introduction of sockeye salmon into lakes open to the sea that were previously devoid of *O. nerka* (Ricker 1940; Scott 1984) and with sea-run (sockeye) individuals being observed after human introductions of kokanee (Kaeriyama et al. 1992). Also, the natural distribution of kokanee is contained *within* the distribution of sockeye salmon, and kokanee have not dispersed via freshwater routes beyond the geographic range of sockeye salmon (Nelson 1968). As well, the presence of kokanee in lakes on islands [e.g., Japan, Vancouver Island, islands in southeastern Alaska (Nelson 1968; Kaeriyama et al. 1992)] probably resulted via divergence from sea-run sockeye salmon that colonized the islands by marine dispersal following deglaciation.

Third, Foote et al. (1989) suggested that coexisting populations of sockeye salmon and kokanee (e.g., Babine, Takla, and Shuswap Lakes) may have arisen by independent within-lake divergences. Our VNTR and mtDNA data did not find sister group relationships between sympatric forms within individual lakes as would be predicted from a sympatric divergence scenario (e.g., Fig. 7). Our data, however, do suggest that sockeye and kokanee may have diverged independently in the different major river *systems,* i.e., on a microallopatric scale. For instance, a sockeye salmon and kokanee split from a common ancestor within the middle Fraser/Columbia River (e.g., Eagle River, Lower Adams River) distinct from divergence between forms in Takla Lake (upper Fraser) or Babine Lake (Skeena River) was found at between 64–76% bootstrap support (Fig. 7). The distribution of individual alleles is also consistent with separate origins of sockeye salmon and kokanee in different watersheds. At the T34 locus, for example, sockeye salmon and kokanee from the lower

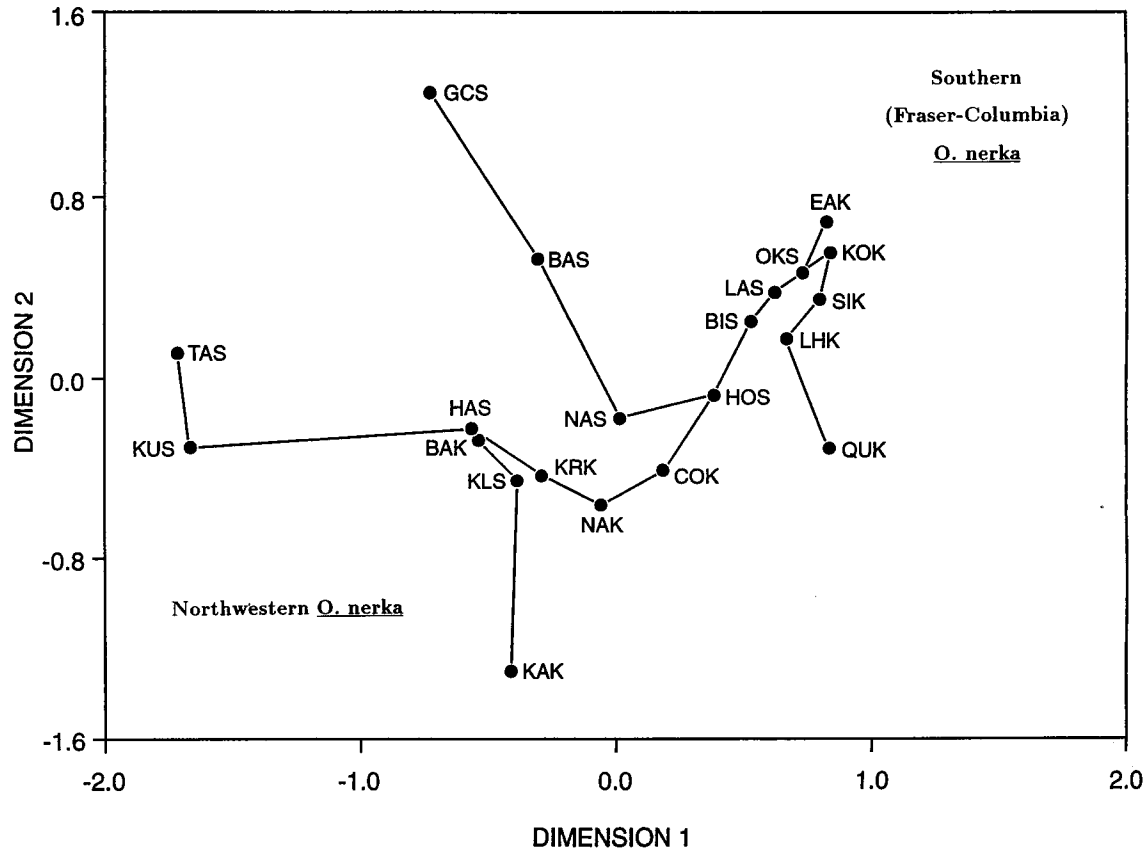

FIG. 8. Two dimensional scaling of 21 samples of North Pacific Ocean sockeye salmon and kokanee (*Oncorhynchus nerka*) based on Nei's (1972) genetic distance inferred from minisatellite and mtDNA variation. Populations are encoded by the first two letters of their names given in Table 1 and labels ending with "S" or "K" represent sockeye salmon or kokanee populations, respectively.

Adams River and Sinmax Creek (Adams Lake) shared a unique allele ("E," 3.7 kbp) not found in any other population. Similarily, at this same locus sockeye salmon and kokanee from the Quesnel River system shared the "C" allele at between 11 and 43% frequency. This allele was rare in populations outside the Fraser River system, and was found in only two other Fraser River populations (Takla and Birkenhead) at much lower frequencies (< 5%, Table 1). Further, the mtDNA *Bgl* II data argue strongly that Babine Lake sockeye salmon and kokanee have had a separate origin from other sympatric pairs because the Babine forms shared the "A" genotype (both at about 30% occurrence) that was not found in any of the Fraser or Columbia River system lake pairs (Table 1, Fig. 6).

Finally, in many other species of north temperate fishes, alternative migratory life-history types appear to have diverged repeatedly (Hindar et al. 1986; Stahl 1987; Hindar et al. 1991; Taylor and Bentzen 1993b). The repeated opportunities for geographic isolation associated with Pleistocene glaciations in north temperate aquatic environments, the excellent dispersal capabilities of euryhaline species such as *O. nerka,* coupled with the general depauperate aquatic faunas in postglacial environments, have probably contributed to parallel divergences occurring across independently evolving north temperate species (Rice and Hostert 1993).

We have argued for multiple divergences between sockeye salmon and kokanee that occurred independently among major geographic regions and watersheds. Within each of these large watersheds (e.g., Fraser, Skeena, Alsek Rivers), however, extant sockeye salmon and kokanee populations may, in some cases, stem from multiple colonizations of lakes by common ancestral populations of each form. For example, our data suggested close genetic affinity among kokanee populations and among sockeye populations from different lakes within major watersheds (e.g., Sinmax, Eagle, Kootenay kokanee, Figs. 7–8). There is good geological and biogeographic evidence for faunal exchanges among the upper Fraser and Columbia Rivers and among the upper Fraser and Skeena Rivers (Lindsey and McPhail 1986; McPhail and Lindsey 1986). The upper Fraser, for instance, used to drain to the sea via the upper Columbia when the lower Fraser was blocked by ice dams during deglaciation (McPhail and Lindsey 1986). The striking similarity in allele frequencies and mtDNA restriction sites among some kokanee and among sockeye salmon populations from the Fraser and Columbia Rivers, could, therefore, have resulted from multiple colonizations of lakes in the two systems by common ancestral populations of "southern" sockeye salmon and of kokanee that survived the Wisconsinan glaciation in streams and large proglacial lakes of the lower Columbia River Valley (McPhail and Lindsey 1986).

Our study has provided evidence that within each lake

where they co-occur, sockeye salmon and kokanee are genetically distinct demes, a finding consistent with that of Foote et al. (1989) and Wood and Foote (in press) based on allozymes. Within Babine and Takla Lakes, the genetic distinction between the life-history forms prevails even though they spawn at the same time in the same streams. Notwithstanding assortative mating by size (sockeye are larger than kokanee) which limits interbreeding, and hence potential gene flow, in sympatry (Foote and Larkin 1988), kokanee males often act as "sneaks" to spawn successfully with sockeye salmon females (Hanson and Smith 1967; Foote and Larkin 1988). Biochemical and molecular genetic differentiation in the face of low, but persistent, levels of interbreeding between sympatric forms implies that postmating selection against sockeye salmon-kokanee "hybrids" must be substantial. Selection against hybrids to promote genetic differentiation is likely given the distinct selective environments associated with sea-run (sockeye salmon) versus wholly freshwater (kokanee) life histories (Ricker 1940). Further, experimental evidence for the reduced performance of sockeye salmon-kokanee F_1 hybrids has been demonstrated for a variety of traits, including salinity tolerance, swimming performance, and growth and development, associated with sea-run and freshwater resident life histories (Wood and Foote 1990, Taylor and Foote 1991; Foote et al. 1992b). These studies suggest that adaptive divergence associated with distinct life histories and habitats maintains genetic differentiation between ocassionally interbreeding sympatric sockeye salmon and kokanee. More generally, adaptive diversification has been suggested to be a key mechanism for the maintenance of reproductive isolation in sympatry (Rice 1984; Bush 1994) and likely has been a significant factor promoting multiple divergences between life-history forms within several other species of north temperate fishes (cf. Schluter and McPhail 1992; Taylor and Bentzen 1993b).

In conclusion, our study has found evidence from two independent genetic systems that sockeye salmon and kokanee are para- and polyphyletic, respectively, across the entire range of *O. nerka.* In combination with concordant results from allozyme data over a smaller geographic range (Foote et al. 1989), our study provides compelling evidence that extant populations of *O. nerka* are derived primarily from two Pleistocene refuge races and that there have been multiple episodes of independent divergence between sockeye salmon and kokanee thoughout the postglacial dispersal range of *O. nerka.* More generally, parallel divergences between sockeye salmon and kokanee implicate common ecological factors in the divergences (i.e., migration to the sea versus freshwater residence). Our evidence for parallel life-history evolution within *O. nerka,* therefore, provides a further example of the potential for rapid (i.e., postglacial) replicate adaptive evolution as an important mechanism of evolutionary change (cf. Losos 1992; Schluter and McPhail 1993).

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Literature Cited

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18:489–522.

Behnke, R. J. 1972. The systematics of salmonid fishes in recently glaciated lakes. Journal of the Fisheries Research Board of Canada 29:639–671.

Bentzen, P., E. B. Taylor, and J. M. Wright. 1993. A novel synthetic probe for DNA fingerprinting salmonid fishes. Journal of Fish Biology 43:313–316.

Bernatchez, L., and J. J. Dodson. 1990. Allopatric origin of sympatric populations of lake whitefish (*Coregonus clupeaformis*) revealed by mitochondrial DNA restriction analysis. Evolution 44:1263–1271.

Bickham, J. W., C. C. Wood, and J. C. Patton. 1995. Variation in mitochondrial DNA cytochrome *b* sequences and allozymes in sockeye (*Oncorhynchus nerka*). Journal of Heredity 86:140–144.

Brannon, E. L., G. H. Thorgaard, and S. A. Cummings. 1992. Genetic analysis of *Oncorhynchus nerka.* Annual Progress Report, U.S. Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Portland, OR.

Bronstein, I., and P. McGrath. 1989. Chemiluminescence lights up. Nature 338:599–600.

Bush, G. 1994. Sympatric speciation: New wine in old bottles. Trends in Ecology and Evolution 9:285–288.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: Models and estimation procedures. Evolution 32:550–570.

Cross, T. F., C. P. R. Mills, and M. De C. Williams. 1992. An intensive study of allozyme variation in freshwater resident and anadromous trout, *Salmo trutta* L., in western Ireland. Journal of Fish Biology 40:25–32.

Degnan, S. M. 1993. The perils of single gene trees—mitochondrial versus single-copy nuclear DNA variation in white-eyes (Aves: Zosteropidae). Molecular Ecology 2:219–226.

Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Analytical Biochemistry 132:6–13.

Felsenstein, J. 1981. Evolutionary trees from gene frequencies and quantitative characters: Finding maximum-likelihood estimates. Evolution 35:1229–1241.

———. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

———. 1991. PHYLIP: Phylogeny inference package, Version 3.4. Department of Genetics, SK-50, University of Washington, Seattle.

Fitch, W. M., and E. Margoliash. 1967. Construction of phylogenetic trees. Science 155:279–284.

Foote, C. J., and P. A. Larkin. 1988. The role of mate choice in the assortative mating of anadromous and non-anadromous sockeye salmon (*Oncorhynchus nerka*). Behaviour 106:43–62.

Foote, C. J., C. C. Wood, and R. E. Withler. 1989. Biochemical

genetic comparison between sockeye salmon and kokanee, the anadromous and nonanadromous forms of *Oncorhynchus nerka*. Canadian Journal of Fisheries and Aquatic Sciences 46:149–158.

Foote, C. J., J. W. Clayton, C. C. Lindsey, and R. A. Bodaly. 1992a. Evolution of lake whitefish (*Coregonus clupeaformis*) in North America during the Pleistocene: Evidence for a Nahanni glacial refuge race in the northern Cordillera. Canadian Journal of Fisheries and Aquatic Sciences 49:760–768.

Foote, C. J., C. C. Wood, W. C. Clarke, and J. Blackburn. 1992b. Circaannual cycle of seawater adaptability in *Oncorhynchus nerka*: Genetic differences between sympatric sockeye salmon and kokanee. Canadian Journal of Fisheries and Aquatic Sciences 49:99–109.

Galbraith, D. A., P. T. Boag, H. L. Gibbs, and B. N. White. 1991. Sizing bands on audoradiograms: A study of precision for scoring DNA fragments. Electrophoresis 12:210–220.

Georges, M., A.-S. Lequarre, M. Castelli, R. Hanset, and G. Vassart. 1988. DNA fingerprinting in domestic animals using four different minisatellite probes. Cytogenetics and Cell Genetics 47: 127–131.

Hanson, S. J., and H. D. Smith. 1967. Mate selection in a population of sockeye salmon (*Oncorhynchus nerka*) of mixed age groups. Journal of the Fisheries Research Board of Canada 24:1955–1977.

Harrison, R. G. 1989. Animal mitochondrial DNA as a genetic marker in population biology and evolutionary biology. Trends in Ecology and Evolution 4:6–11.

Hindar, K., N. Ryman, and G. Stahl. 1986. Genetic differentiation among local populations and morphotypes of Arctic charr, *Salvelinus alpinus*. Biological Journal of the Linnean Society 27: 269–285.

Hindar, K., B. Jonsson, N. Ryman, and G. Stahl. 1991. Genetic relationships among landlocked, resident, and anadromous Brown Trout, *Salmo trutta* L. Heredity 66:83–91.

Höltke, H. L., G. Sagner, C. Kessler, and G. Schmitz. 1992. Sensitive chemiluminescent detection of digoxigenin-labelled nucleic acids: A fast and simple protocol and its applications. Biotechniques 12:104–112.

Jarman, A. P., and R. A. Wells. 1989. Hypervariable minisatellites: Recombinators or innocent bystanders? Trends in Genetics 5: 367–371.

Jeffreys, A. J., V. Wilson, and S. L. Thein. 1985. Hypervariable "minisatellite" regions in human DNA. Nature 316:76–79.

Kaeriyama, M., Urawa, S., and T. Susuki. 1992. Anadromous sockeye salmon (*Oncorhynchus nerka*) derived from nonanadromous kokanees: Life history in Lake Toro. Scientific Reports of the Hokkaido Salmon Hatchery No. 46:157–174.

Karl, S. A., and J. C. Avise. 1992. Balancing selection at allozyme loci in oysters: Implications from nuclear RFLPs. Science 256: 100–102.

Kim, J., and M. A. Burgman. 1988. Accuracy of phylogenetic estimation methods under unequal evolutionary rates. Evolution 42:596–602.

Lessa, E. 1990. Multidimensional analysis of geographic genetic structure. Systematic Zoology 39:242–252.

Lessios, H. A. 1992. Testing electrophoretic data for aggreement with Hardy-Weinberg expectations. Marine Biology 112:517–523.

Lindsey, C. C., and J. D. McPhail. 1986. Zoogeography of fishes of the Yukon and Mackenzie Basins. Pp. 639–674 *in* C. H. Hocutt and E. O. Wiley, eds. Zoogeography of North American freshwater fishes. Wiley and Sons, New York.

Losos, J. B. 1992. The evolution of convergent structure in Caribbean *Anolis* communities. Systematic Biology 41:403–420.

McCart, P. J. 1970. A polymorphic population of *Oncorhynchus nerka* in Babine Lake, British Columbia. Ph.D. diss. Department of Zoology, University of British Columbia, Vancouver, Canada.

McDowall, R. M. 1987. The occurrence and distribution of diadromy among fishes. American Fisheries Society Symposium 1: 1–13.

McElroy, D., P. Moran, E. Bermingham, and I. Kornfield. 1992. REAP: An integrated environment for the manipulation and phylogenetic analysis of restriction data. Journal of Heredity 83: 157–158.

McPhail, J. D., and C. C. Lindsey. 1986. Zoogeography of the freshwater fishes of Cascadia (the Columbia system and rivers north to the Stikine). Pp. 615–637 *in* C. H. Hocutt and E. O. Wiley, eds. Zoogeography of North American freshwater fishes. Wiley and Sons, New York.

Meyer, A. 1994. Molecular phylogenetic studies in fishes. Pp. 219–249 *in* A. Beaumont, ed. Genetics and evolution of aquatic organisms. Chapman and Hall, London.

Nakamura Y., M. Leppert, P. O'Connell, R. Wolff, T. Holm, M. Culver, C. Martin, E. Fuimoto, M. Hoff, E. Kumlin, and R. White. 1987. Variable number of tandem repeat (VNTR) markers for human gene mapping. Science 235:1616–1622.

Nelson, J. S. 1968. Distribution and nomenclature of North American kokanee, *Oncorhynchus nerka*. Journal of the Fisheries Research Board of Canada 25:409–414.

Nei, M. 1972. Genetic distance between populations. American Naturalist 106:283–292.

Okazaki, T. 1984. Genetic divergence and its zoogeographic implications in closely related species *Salmo gairdneri* and *Salmo mykiss*. Journal of Ichthyology 31:297–311.

O'Reilly, P., T. E. Reimchen, R. Beech, and C. Strobeck. 1993. Mitochondrial DNA in *Gasterosteus* and Pleistocene glacial refugium on the Queen Charlotte Islands. Evolution 47:678–684.

Pimentel, R. A. 1979. Morphometrics: The multivariate analysis of biological data. Kendall/Hunt Publishing, Dubuque, IA.

Pojar, J. 1980. Brooks Peninsula: Possible Pleistocene glacial refugium on northwestern Vancouver Island. Botanical Society of America Miscellaneous Publication 158:1–89.

Prodöhl, P. A., J. B. Taggart, and A. Ferguson. 1992. Genetic variability within and among sympatric brown trout populations: multi-locus DNA fingerprint analysis. Hereditas 117:45–50.

Reynolds, J., B. S. Weir, and C. C. Cockerham. 1983. Estimation of the coancestry coefficient: Basis for a short-term genetic distance. Genetics 105:767–779.

Rice, W. R. 1984. Disruptive selection on habitat preference and the evolution of reproductive isolation: A simulation study. Evolution 38:1251–1260.

Rice, W. R., and E. E. Hostert. 1993. Perspective: Laboratory experiments on speciation: What have we learned in forty years? Evolution 47:1637–1653.

Ricker, W. E. 1940. On the origin of kokanee, a fresh-water type of sockeye salmon. Transactions of the Royal Society of Canada 34:121–135.

Roff, D. A., and P. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small sample sizes. Molecular Biology and Evolution 6:539–545.

Rogers, J. S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics University of Texas Publ. 7213: 145–153.

———. 1985. Deriving phylogenetic trees from allele frequencies: A comparison of nine genetic distances. Systematic Zoology 35: 297–310.

Rohlf, F. J. 1970. Adaptive hierarchical clustering schemes. Systematic Zoology 19:58–82.

———. 1990. NTSYS: Numerical taxonomy and multivariate analysis system, version 1.6. Exeter, Setauket, NY.

Roy, M. S., E. Geffen, D. Smith, E. Ostrander, and R. K. Wayne. 1994. Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. Molecular Biology and Evolution 11:553–570.

Saitou, N., and M. Nei. 1987. The Neighbor-Joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406–425.

Schaffer, H. E., and R. R. Sederoff. 1981. Improved estimation of DNA fragment lengths from agarose gels. Analytical Biochemistry 115:113–122.

Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. American Naturalist 140:85–108.

———. 1993. Character displacement and replicate adaptive radiation. Trends in Ecology and Evolution 8:197–200.

Scott, D. 1984. Origin of the New Zealand sockeye salmon, *Oncorhynchus nerka* (Walbaum). Journal of the Royal Society of New Zealand 14:245–249.

Sneath, P. H. A., and R. R. Sokal. 1973. Numerical taxonomy. Freeman, San Francisco, CA.

Stahl, G. 1987. Genetic population structure of Atlantic salmon. Pp. 121–140. *In* N. Ryman and F.M. Utter, eds. Population genetics and fishery management. University of Washington Press, Seattle.

Swofford, D. L., and G. J. Olsen. 1990. Phylogeny reconstruction. Pp. 411–501 *in* D. M. Hillis and C. Moritz, eds. Molecular systematics. Sinauer Associates, Sunderland, MA.

Swofford, D. L., and R. B. Selander. 1981. BIOSYS-1. A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281–283.

Taggart, J. B., and A. Ferguson. 1990. Minisatellite DNA fingerprints of salmonid fishes. Animal Genetics 21:377–389.

———. 1991. Hypervariable single locus DNA probes for the Atlantic salmon, *Salmo salar* L. Journal of Fish Biology 37:991–993.

Taggart, J. B., R. A. Hynes, P.A. Prodöhl, and A. Ferguson. 1992. A simplified protocol for routine total DNA isolation from salmonid fishes. Journal of Fish Biology 40:963–965.

Taylor, E. B. 1995. Genetic variation at minisatellite DNA loci among North Pacific populations of steelhead and rainbow trout (*Oncorhynchus mykiss*). Journal of Heredity 86:354–363.

Taylor, E. B., and P. Bentzen. 1993a. Molecular genetic evidence for reproductive isolation between sympatric populations of smelt *Osmerus* in Lake Utopia, south-western New Brunswick, Canada. Molecular Ecology 2:345–357.

———. 1993b. Evidence for multiple origins and sympatric divergence of trophic ecotypes of smelt (*Osmerus*) in northeastern North America. Evolution 47:813–822.

Taylor, E. B., and C. J. Foote. 1991. Critical swimming velocities of juvenile sockeye salmon and kokanee, the anadromous and non-anadromous forms of *Oncorhynchus nerka*. Journal of Fish Biology 38:407–419.

Taylor, E. B., T. D. Beacham, and M. Kaeriyama. 1994. Population structure and identification of North Pacific Ocean chum salmon (*Oncorhynchus keta*) revealed by an analysis of minisatellite DNA variation. Canadian Journal of Fisheries and Aquatic Sciences 51:1430–1442.

Utter, F. M., D. Campton, S. Grant, G. Milner, J. Seeb, and L. Wishard. 1980. Population structure of indigenous salmonid species of the Pacific Northwest. Pp. 285–304 *in* W. J. McNeil and D. C. Himsworth, eds. Salmonid ecosystems of the North Pacific. Oregon State University Press, Corvallis.

Varnavskaya, N., and T. D. Beacham. 1992. Biochemical genetic variation in odd-year pink salmon (*Oncorhynchus gorbuscha*) from Kamchatka. Canadian Journal of Zoology 70:2115–2120.

Verspoor, E., and L. J. Cole. 1989. Genetically distinct sympatric populations of resident and anadromous Atlantic salmon, *Salmo salar*. Canadian Journal of Zoology 67:1453–1461.

Warner, B. C., R. W. Mathews, and J. J. Clague. 1982. Ice-free conditions on the Queen Charlotte Islands, British Columbia, at the height of the Wisconsin Glaciation. Science 218:675–677.

Wayne, R. K., S. B. George, D. Gilbert, P. W. Collins, S. D. Kovach, D. Girman, and N. Lehman. 1991. A morphologic and genetic study of the island fox, *Urocycon littoralis*. Evolution 45:1849–1868.

Wilder, D. G. 1947. A comparative study of the Atlantic salmon, *Salmo salar* Linneaus, and the lake salmon, *Salmo salar sebago* (Girard). Canadian Journal of Research 25:175–189.

Wood, C. C., and C. J. Foote. 1990. Genetic differences in the early development and growth of sympatric sockeye salmon and kokanee (*Oncorhynchus nerka*) and their hybrids. Canadian Journal of Fisheries and Aquatic Sciences 47:2250–2260.

Wood, C. C., and C. J. Foote. In press. Evidence for sympatric genetic divergence of anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). Evolution 50.

Wood, C. C., B. E. Riddell, D. T. Rutherford, and R. E. Withler. 1994. Biochemical genetic survey of sockeye salmon *Oncorhynchus nerka* in Canada. Canadian Journal of Fisheries and Aquatic Sciences 51 (Suppl. 1):114–131.

Wright, J. M. 1993. DNA fingerprinting of fishes. Pp. 59–71 *in* P. Hochachka and T. Mommsen, eds. Biochemistry and molecular biology of fishes. Vol. 2. Elsevier Science Publishers B.V., Amsterdam, Netherlands.

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