

Postglacial genetic differentiation of reproductive ecotypes of kokanee *Oncorhynchus nerka* in Okanagan Lake, British Columbia

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Abstract

Okanagan Lake, south-central interior of BC, contains two reproductive ecotypes of kokanee *Oncorhynchus nerka*; individuals spawn in tributary streams ('stream-spawners') as well as on shoreline gravel areas ('beach-spawners'). We tested the hypothesis that these sympatric ecotypes comprise a single panmictic population by assaying variation in morphological traits and at allozyme, mitochondrial and minisatellite DNA loci in fish collected from three stream-spawning and two beach-spawning sites. No morphological traits consistently distinguished the reproductive ecotypes with the exception of the number of anal fin rays which was greater in stream-spawning kokanee. Four of 18 allozyme loci screened were polymorphic, but no significant allele frequency differences were detected among populations within ecotypes or between ecotypes. Similarly, allele frequencies at two minisatellite DNA loci were not significantly different among populations or between ecotypes. By contrast, significant differences in the frequencies of mitochondrial DNA restriction fragment length polymorphism (mtDNA RFLP) haplotypes were detected between stream- and beach-spawners, but not among populations within ecotypes. Further, two RFLPs that distinguished stream- and beach-spawning adults were found in juvenile kokanee sampled from the limnetic zone of Okanagan Lake. The two mtDNA RFLPs and a d-loop sequence variant appear to be unique to Okanagan Lake kokanee because we did not observe these haplotypes in sockeye salmon and kokanee sampled outside of Okanagan Lake. Our data suggest that: (i) there is restricted female-mediated gene flow between stream- and beach-spawning kokanee in Okanagan Lake, (ii) the forms have diverged within the lake basin since the retreat of the Wisconsinian glaciers (< \approx 11 000 years ago), and (iii) distinct reproductive niches may promote divergence in north temperate freshwater fish faunas.

Keywords: *Oncorhynchus nerka*, kokanee, morphology, mitochondrial DNA, allozymes, minisatellite DNA, reproductive ecotypes

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Introduction

Over the last 30 years it has become well established that many northern freshwater fish communities harbour a wealth of diversity in the form of sympatric populations of uncertain taxonomic status (e.g. Svardson 1961; Behnke

1972; Schluter 1996). Although such diversity was initially recognized through differences in coloration, body size, and morphological and meristic traits, concurrent or subsequent biochemical and molecular genetic investigations have usually revealed parallel genetic diversity (e.g. Larson 1976; McPhail 1992). In many cases, phenotypic and genetic variants have been recognized taxonomically as subspecies or, more ambiguously, as 'morphs' or 'forms' (e.g. Frost 1965; Behnke 1972). Increasingly, there is evidence from these ecological and genetic studies that many sympatric forms fulfil the criteria for recognition as

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biological species (*sensu* Mayr 1963; i.e. they are genetically and ecologically isolated in sympatry) even if they have not been designated as such taxonomically (e.g. McPhail 1984, 1992; Foote *et al.* 1989; Bernatchez & Dodson 1990; Bodaly *et al.* 1992; Taylor & Bentzen 1993). The tendency to show extensive, taxonomically unrecognized diversity at the species level is pronounced in salmonid fishes. Intraspecific forms, often occurring in sympatry and maintaining reproductive isolation from each other have been documented in Atlantic salmon, brown trout, lake whitefish, sockeye salmon and kokanee, and Arctic char (Fendersen 1964; Hindar *et al.* 1986; Foote *et al.* 1989; Verspoor & Cole 1989; Bernatchez & Dodson 1990; Ferguson & Taggart 1991; McVeigh *et al.* 1995; Taylor *et al.* 1996).

In the majority of these taxa, the intraspecific forms are not restricted to single watersheds, but are found throughout a large portion of each species' geographical range [e.g. sockeye salmon and kokanee (Taylor *et al.* 1996), Arctic char (Hindar *et al.* 1986)]. Furthermore, the bulk of evidence suggests that such diversity has arisen by multiple, independent episodes of divergence between forms throughout their ranges (e.g. Ryman & Stahl 1981; Hindar *et al.* 1986; Foote *et al.* 1989; Taylor *et al.* 1996). Also, ecotypic variation in salmonid fishes (as well as for other north temperate freshwater species) is often associated with alternative trophic niches and differentiation in feeding ecology appears to be a major ecological factor promoting genetic divergence in sympatry (Kurenkov 1977; Ferguson & Taggart 1991; Skulason *et al.* 1993; Schluter & McPhail 1992; Schluter 1996). By contrast, far less common in salmonids is the observation of intraspecific forms that share similar trophic ecology, but that are differentiated in terms of reproductive ecology, particularly in sympatry.

Oncorhynchus nerka is a salmonid fish native to watersheds tributary to both the north-western and north-eastern Pacific Ocean (Burgner 1991). Throughout its native range, *O. nerka* exists as sea-run or anadromous populations (sockeye salmon) or they may reside permanently in freshwater (kokanee). Often the two life-history types occur sympatrically, and the available phylogenetic and zoogeographical evidence suggests that kokanee have diverged repeatedly from sockeye salmon as the latter colonized emerging watersheds after the end of the most recent glaciation (Nelson 1968; Foote *et al.* 1989; Taylor *et al.* 1996). Typically, sockeye salmon and kokanee spawn in streams tributary to juvenile nursery lakes after maturing in the sea or lakes, respectively (Burgner 1991). There are, however, a few lake systems where *O. nerka* populations also spawn on lakeshore gravel beaches (Averett & Espinosa 1968; Blair *et al.* 1993; Burger *et al.* 1995). In such systems, 'beach-' and 'stream-spawning' sockeye salmon and kokanee appear to share the same pelagic feeding habitats, but their reproductive ecology is markedly

different. Stream-spawners typically ascend tributary streams to spawn in flowing water where females defend spawning territories. By contrast, beach-spawning *O. nerka* spawn on submerged lakeshore beaches that are usually associated with groundwater seepage areas and the fish may not defend individual territories (P. Dill, Okanagan University College, Kelowna, BC, personal communication). Furthermore, Blair *et al.* (1993) examined beach- and stream-spawning populations of sockeye salmon in an Alaskan lake and demonstrated morphological differences between ecotypes (e.g. beach-spawners were deeper-bodied). They attributed the morphological differences to adaptation to the differing energetic demands of spawning in still (beaches) vs. flowing (streams) waters. In none of the systems where beach- and stream-spawning populations of *O. nerka* have been reported, however, has the level of genetic distinctiveness or evolutionary origin of sympatric reproductive ecotypes been examined.

In this study, we addressed three questions regarding ecological differentiation and genetic relationships between beach- and stream-spawning kokanee found sympatrically in a lake in the south-central interior of British Columbia. First, do sympatric beach- and stream-spawning kokanee exhibit morphological differences similar to those in stream- and beach-spawners examined elsewhere (e.g. Blair *et al.*'s Alaskan populations) in response to similar spawning habitat differences? An affirmative answer to this question would provide support for Blair *et al.*'s adaptive explanation for morphological differentiation between sympatric reproductive ecotypes. Secondly, do beach- and stream-spawning kokanee represent distinct gene pools or environmentally induced 'ecophenotypes' that upon maturation assort randomly to beach or stream habitats for spawning? Thirdly, should they be genetically distinct, what is the likely evolutionary origin of the ecotypes? For instance, sympatric ecotypes in other northern freshwater fishes have been suggested to result from secondary contact between allopatrically derived forms (e.g. Bernatchez & Dodson 1990) or by sympatric divergence (Taylor & Bentzen 1993). To address these questions, we conducted a morphological analysis of sympatric reproductive ecotypes of kokanee and inferred relationships among kokanee populations from assays at protein-coding allozyme loci, mitochondrial DNA and minisatellite DNA loci.

Materials and Methods

Study populations and tissue sampling

The study populations were from Okanagan Lake, a large and deep (surface area = 351 km², average depth = 76 m) oligotrophic lake which is part of the Columbia River system and is located in the south-central interior of British

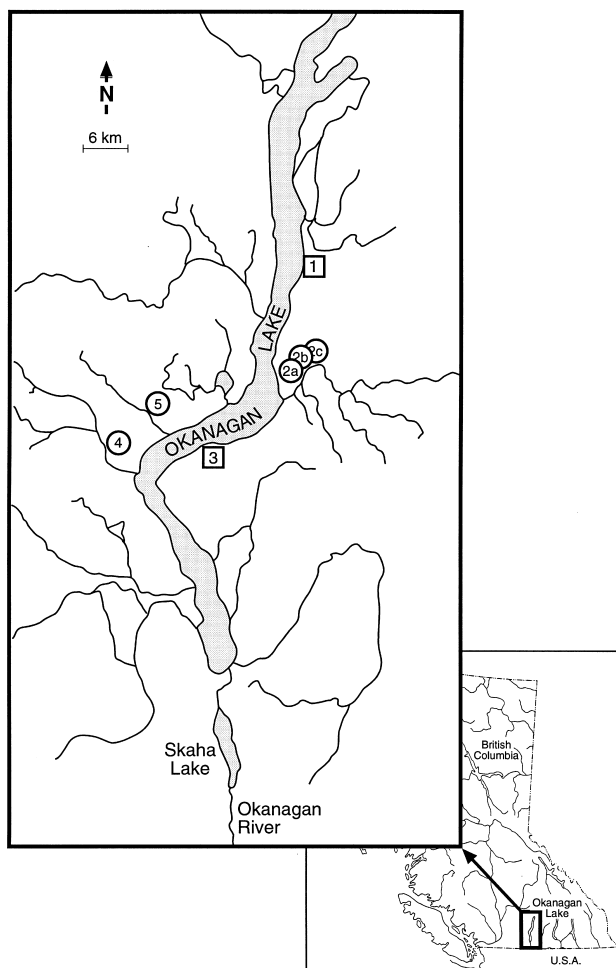


Fig. 1 Locations of spawning habitats in Okanagan Lake from which mature kokanee (*Oncorhynchus nerka*) were sampled. Circles, stream-spawning populations; squares, beach-spawning populations. 1, Okanagan Central; 2, Mission Creek (a, b and c represent lower, middle, and upper Mission Creek spawning sites, respectively); 3, Squally Point; 4, Peachland (Deep) Creek; 5, Powers Creek.

Columbia (Fig. 1). Limnological descriptions of the lake are given by Northcote & Larkin (1956) and Ashley & Shepherd (1996). The lake contains stream- and beach-spawning kokanee whose total adult population size has ranged from about 100 000 to 1 000 000 adults during the period 1971–94 (Ashley & Shepherd 1996). The relative proportion of stream- and beach-spawners has been $\approx 50 : 50$ over the same time period (Ashley & Shepherd 1996). Stream-spawning kokanee in Okanagan Lake spawn, on average, 2–4 weeks earlier than beach-spawners (B. Shepherd, BC Fish and Wildlife Branch, Penticton, BC, personal communication). Stream-spawning kokanee were collected during September 1994 using dipnets or seines from three tributaries: Mission, Peachland (Deep) and Powers creeks (Fig. 1). Three sites were sampled in

Mission Creek to assess intrapopulation heterogeneity in genetic and morphological characters. At beach sites, kokanee were collected after stream spawning was completed in mid-October 1994 by beach seine from two sites: Okanagan Central and Squally Point (Fig. 1). Only ripe individuals (sexually mature fish with no deterioration of body condition) were collected for morphological analysis because different states of maturity may confound morphological comparisons among populations (Quinn & Blair 1992). We also sampled 100 juvenile kokanee of unknown population or reproductive ecotype from the limnetic zone of the lake by mid-water trawling (B. Shepherd, BC Fish and Wildlife Branch, Penticton, BC, personal communication). In all cases, samples of liver were stored in 95% ethanol for DNA analysis, samples of liver, muscle, heart, and eye were frozen on dry ice and stored at $-20\text{ }^{\circ}\text{C}$ for allozyme analysis, and the carcasses were frozen for subsequent morphological analysis.

Morphological analysis

Kokanee sampled for morphological analysis were thawed and preserved in 10% formalin for 2 weeks, then rinsed in freshwater for 1 week before counts and measurements were recorded. Morphological analysis consisted of taking nine body measurements and counting the total number of gill rakers (TGR) and total anal fin rays (AFR). Morphological measurements recorded were: fork length (FL), orbit diameter (ORBD), upper jaw length (UPJL), snout length (SNTL), head length (HDL), head depth (HDD), pectoral fin insertion to insertion of dorsal fin (PECFD), insertion of anal fin to insertion of dorsal fin (AFDF), caudal peduncle depth (CPD). All counts and measures were taken on the left side of each fish. Gill rakers were counted under a dissecting microscope after dissection of the first gill arch and staining it in 1% KOH and alizarin red.

The morphological traits examined were those representing aspects of body depth, which Blair *et al.* (1993) suggested were the focus of divergent selection in beach- and stream-spawning sockeye salmon, and features likely to be related to trophic ecology (gill raker number, snout length), a common axis of divergence in sympatric fish populations (e.g. Schluter 1996).

Morphological data analysis

Mature salmonids often exhibit significant sexual dimorphism in body shape. Kokanee varied both within and between populations in body size and the sizes of body parts are usually positively correlated with general body size (fork length). Because our morphological analysis was performed on adult kokanee, our initial analyses consisted of analyses of covariance (ANCOVA) to test for

size-adjusted differences in body shape between sexes within populations. Homogeneity of slopes between sexes within populations was confirmed for each test and significance levels were adjusted for multiple simultaneous tests using the sequential Bonferroni procedure (Rice 1989). Traits that showed significant sexual dimorphism for at least one population were then analysed separately by sex using analysis of covariance with significance levels adjusted as above. The two meristic traits, gill raker and anal fin ray number, showed no correlation with body length. Tests for sexual dimorphism within populations were, therefore, conducted using *t*-tests and tests of differences among populations were conducted by analysis of variance (ANOVA). A posteriori means tests followed Tukey's procedure (Steel & Torrie 1980).

Allozyme analysis

All fish used for allozyme analyses were killed immediately and held on ice for a maximum of 4 h until tissue collection. Tissues were frozen in liquid nitrogen and then stored at -80°C until analysis. Standard horizontal starch gel and cellulose acetate electrophoresis were conducted on tissue samples following the methodology described by Allendorf *et al.* (1977) and Hebert & Beaton (1989), respectively. Nomenclature follows that recommended by Shaklee *et al.* (1990). Eighteen enzyme systems commonly found to be polymorphic in sockeye salmon and kokanee (Foote *et al.* 1989; Wood & Foote 1990; Bickham *et al.* 1995) were screened for variation: mAAT-1*, sAAT-1,2*, sAAT-3*, ADA-2*, sAH-3*, ALAT*, GPI-B1,2*, sIDHP-2*,

LDH-B2*, sMEP-1*, sMDH-A1,2*, mMDH-B1,2*, MPI*, PGDH*, PEP-C*, PEP-LT*, PGM-1* and PGM-2* (Table 1). Most fish samples were screened for all allozymes except for PGM-1* and PGM-2* where only a subsample from each site was analysed.

Mitochondrial DNA analysis

Genomic DNA was obtained from ethanol-stored liver samples using Pronase digestion and phenol/chloroform extraction procedures as outlined by Taylor *et al.* (1996). Genomic DNA (about 5 μg) was digested with six restriction enzymes (*BanI*, *EcoRI*, *HincII*, *HinfI*, *PvuII* and *StyI*) in overnight incubations. Digested DNAs were electrophoresed and Southern-blotted to nylon membranes as detailed by Taylor *et al.* (1996). Restriction fragment length polymorphisms (RFLPs) in kokanee mtDNA were resolved by hybridization of membrane-bound kokanee DNA with digoxigenin-labelled rainbow smelt (*Osmerus mordax*) mtDNA clones (Taylor *et al.* 1996). Probe-kokanee DNA hybrids were detected by chemiluminescence and autoluminography (Taylor *et al.* 1996).

A subsample of kokanee from Okanagan Lake and from some of the *O. nerka* populations studied by Taylor *et al.* (1996) were also subject to sequence analysis of a ≈ 240 base pair (bp) region of the mitochondrial control region located near the 3' end. The primers used for amplification ('t-Phe' and 'P2') of genomic DNA samples were described by Neilson *et al.* (1994) which they used to detect sequence polymorphisms in coho and chinook salmon and steelhead trout. Polymerase chain reactions were run in 40- μL

Tissue	Enzyme	E.C.N.	Locus	Buffer system
Eye	aspartate aminotransferase	2.6.1.1	sAAT-3*	AC
	peptidase gly-leucyl	3.4.1.1	PEP-C*	TB
	mannose-6-phosphate isomerase	5.3.1.8	MPI*	TB
Liver	adenosine deaminase	3.5.4.4	ADA-2*	AC
	aconitate hydratase	4.2.1.3	sAH*	AC
	isocitrate dehydrogenase (NADP+)	1.1.1.42	sIDHP-2*	AC
	lactate dehydrogenase	1.1.1.27	LDH-B2*	AC
	malate dehydrogenase	1.1.1.37	sMDH-A1,2*	AC
Heart	phosphoglucomutase	5.4.2.2	PGM-1*	TG
	phosphoglucomutase	5.4.2.2	PGM-2*	TG
Muscle	alanine aminotransferase	2.6.1.2	ALAT*	TB
	leucyl-tyrosine peptidase	3.4.1.3	PEP-LT* TB	
	aspartate aminotransferase	2.6.1.1	mAAT-1*	AC
	glucose-6-phosphate isomerase	5.3.1.9	GPI-B1,2*	TB
	malic dehydrogenase	1.1.1.37	sMDH-B1,2*	AC
	malic enzyme (NADP+)	1.1.1.40	sMEP-1*	AC
	phosphogluconate dehydrogenase	1.1.1.44	PGDH*	AC
	aspartate aminotransferase	2.6.1.1	sAAT-1,2*	AC

Table 1 Enzyme systems screened. Tissue source, enzyme commission number (according to IUBNC 1984), locus designation, and buffer system are included

*buffer systems: AC, amine-citrate buffer pH 7.0 (starch gel electrophoresis); TB, tris-EDTA-borate buffer pH 8.7 (starch gel electrophoresis); TG, tris glycine buffer pH 8.5 (cellulose acetate electrophoresis).

volumes with 800 μM total dNTPs, 1 μM each primer, 4 mM MgCl_2 , 1X Promega *Taq* polymerase buffer and 1.5 U of *Taq* polymerase. Cycling parameters were: 30 cycles of 94 °C denaturation for 30 s, 55 °C annealing for 30 s, and 72 °C extension for 30 s, followed by a single 5 min final extension at 72 °C. PCR products (about 240 bp in size) were purified from reaction components using Promega 'PCR preps' spin columns and eluted in 50 μL water. Dideoxy sequencing reactions (using the P2 primer) were performed using Sequenase Version 2.0 (U.S. Biochemicals) as outlined by Taylor & Dodson (1994) and reactions were electrophoresed on 6% 'Long Ranger' polyacrylamide gels. All mtDNA sequences have been deposited in GenBank under accession numbers U59926–U59928.

Minisatellite DNA analysis

Kokanee samples were assayed for variation at two minisatellite loci: 'Ssa1' and 'T34' (Taylor *et al.* 1996). Genomic DNAs restricted with *Hae*III were assayed by Southern hybridization using minisatellite probes and methods described by Taylor *et al.* (1996). Previous examination of over 700 sockeye salmon and kokanee at these minisatellite loci revealed single locus genetic variation on both a broad geographical scale (western and eastern North Pacific) as well as differentiation between sympatric sockeye salmon and kokanee (Taylor *et al.* 1996).

Genetic data analysis

Mitochondrial DNA RFLPs resolved with each enzyme were designated by single capital letter codes (e.g. *Ban*I-A) and haplotypes consisting of six-letter codes were used to represent the composite RFLPs across the six restriction enzymes. Estimates of sequence divergence among haplotypes and their standard errors were generated by the shared-fragment approach using programs in REAP (McElroy *et al.* 1992). Differences in the frequency of composite haplotypes among populations were assessed using χ^2 randomization tests (Roff & Bentzen 1989; 5000 replications) using MONTE of REAP. Estimates of nucleotide divergence among populations after accounting for nucleotide diversity within populations were generated using DA found in REAP. We also used analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) to estimate the extent of genetic differentiation resolved by RFLP analysis between ecotypes (ϕ_{CT}) relative to that among populations within ecotypes (ϕ_{SC}).

Control-region sequences were aligned by eye and estimates of sequence divergence among sequence haplotypes were generated using the Kimura two-parameter method using DNADIST of PHYLIP (Felsenstein 1993).

Allelic variation at the allozyme and minisatellite

loci was tested for departures from Hardy–Weinberg equilibrium using Yates' corrected χ^2 tests using BIOSYS (Swofford & Selander 1989). Because variation at PGM-1* is associated with a null allele making *-100/*-100 and *null/*-100 genotypes indistinguishable, Hardy–Weinberg proportions had to be assumed to calculate allele frequencies for this locus. Allelic frequencies were compared for single-locus and overall differences between populations within ecotypes and between ecotypes using an additive G-test (Sokal & Rohlf 1995). Tests for allele frequency differences among populations at the two minisatellite loci were conducted using the chi-square randomization procedure with MONTE and combined with allozyme data in additive G-tests. We also estimated the level of genetic differentiation between ecotypes by calculating the fixation index (F_{ST}) using BIOSYS. Tests of significance of F_{ST} were conducted as outlined by Taylor (1995). All genetic data analyses that involved multiple simultaneous comparisons incorporated the sequential Bonferroni procedure as outlined above for the morphological data analyses.

Results

Morphological variation: sexual dimorphism in morphology

Male and female kokanee were examined separately within populations for sexual dimorphism in morphological and meristic traits. In most populations, males tended to be significantly longer than females (Table 2). Consequently, we compared the sizes of individual body dimensions between the sexes after adjusting for differences in fork length by analysis of covariance. The following traits were sexually dimorphic for most of the populations: UPJL, SNTL, PECFD, AFDF, HDL and HDD (all $P < 0.01$). In all populations males tended to have larger body parts than did females of the same fork length (Table 2).

Variation within populations

Adult kokanee from three localities within Mission Creek were examined for morphological variation: Mission Creek lower, Mission Creek middle, and Mission Creek upper sites (Fig. 1). For none of the mensural characters or anal fin ray and gill raker counts was a significant difference detected among sites within Mission Creek (all $P > 0.05$). Consequently, samples from the three sites were pooled for subsequent analysis among populations.

Variation among populations

Body size (fork length FL) varied significantly among male kokanee from the five Okanagan Lake populations (ANOVA $P < 0.001$) and, in general, stream-spawning

Table 2 Morphological variation among populations of kokanee sampled from Okanagan Lake. Sexually-dimorphic traits are standardized to an overall mean fork length of 25.4 and 25.1 cm for males and females, respectively. Non sexually-dimorphic traits were standardized to an overall mean fork length of 25.4 cm. Meristic traits were not standardized and all values are reported in cm (means \pm SE). Trait codes are: fork length (FL), orbit diameter (ORBD), snout length (SNTL), upper jaw length (UPJL), distance from pectoral fin insertion to dorsal fin insertion (PFDF), distance from anal fin insertion to dorsal fin insertion (AFDF), head length (HDL), head depth (HDD), caudal peduncle depth (CPD), anal fin ray number (AFR), and total gill raker number (TGR). Those means not annotated by the same letter are significantly different from one another (Tukey's test, $P < 0.05$)

Trait	Stream spawners			Beach spawners	
	Peachland Creek	Mission Creek	Powers Creek	Okanagan Central	Squally Point
Sexually-dimorphic traits: males					
<i>n</i>	19	65	24	21	9
FL	29.7 ^a (1.42)	25.4 ^b (0.38)	25.7 ^b (0.76)	22.9 ^b (0.24)	23.2 ^b (0.26)
UPJL	3.37 ^a (0.07)	3.42 ^a (0.02)	3.45 ^a (0.02)	3.50 ^a (0.03)	3.51 ^a (0.03)
SNTL	1.76 ^a (0.04)	1.87 ^a (0.02)	1.98 ^{bc} (0.02)	2.02 ^{bc} (0.02)	1.78 ^a (0.04)
PFDF	7.34 ^a (0.06)	7.45 ^a (0.03)	7.52 ^a (0.03)	7.51 ^a (0.03)	7.43 ^a (0.03)
HDL	6.31 ^a (0.04)	6.08 ^{bc} (0.02)	6.07 ^{bc} (0.02)	6.19 ^{ac} (0.03)	6.37 ^a (0.04)
HDD	3.46 ^a (0.04)	3.27 ^c (0.01)	3.24 ^c (0.01)	3.04 ^b (0.02)	2.98 ^b (0.02)
Sexually-dimorphic traits: females					
<i>n</i>	12	32	12	9	21
FL	29.5 ^a (1.66)	25.4 ^b (0.59)	25.1 ^b (0.95)	23.4 ^b (0.23)	23.1 ^b (0.24)
UPJL	2.77 ^a (0.06)	2.68 ^a (0.03)	2.66 ^a (0.03)	2.66 ^a (0.04)	2.76 ^a (0.04)
SNTL	1.36 ^a (0.08)	1.39 ^a (0.05)	1.53 ^b (0.05)	1.42 ^a (0.06)	1.32 ^a (0.06)
PFDF	7.03 ^a (0.06)	6.83 ^a (0.03)	6.94 ^a (0.03)	6.91 ^a (0.04)	6.80 ^a (0.04)
HDL	5.54 ^a (0.06)	5.40 ^{ab} (0.03)	5.31 ^{bc} (0.03)	5.33 ^{abc} (0.04)	5.56 ^a (0.04)
HDD	3.08 ^a (0.09)	2.94 ^b (0.07)	2.90 ^b (0.07)	2.83 ^b (0.07)	2.85 ^b (0.07)
Non-sexually dimorphic traits					
<i>n</i>	31	97	36	30	30
FL	29.6 ^a (1.06)	25.4 ^c (0.32)	25.5 ^{bc} (0.59)	22.4 ^d (0.76)	23.1 ^{bd} (0.19)
ORBD	1.06 ^a (0.11)	1.10 ^a (0.11)	1.02 ^a (0.11)	0.97 ^a (0.11)	0.99 ^a (0.11)
AFDF	7.34 ^a (0.04)	7.34 ^a (0.04)	7.49 ^b (0.03)	7.52 ^b (0.03)	7.33 ^a (0.04)
CPD	1.87 ^a (0.05)	1.97 ^b (0.08)	1.97 ^b (0.05)	1.94 ^b (0.05)	1.82 ^a (0.05)
AFR	14.9 ^{ab} (0.09)	15.1 ^a (0.07)	15.0 ^a (0.11)	14.4 ^b (0.41)	14.5 ^b (0.14)
TGR	35.9 ^a (0.24)	35.0 ^{ab} (0.12)	35.1 ^{ab} (0.29)	34.5 ^b (0.53)	35.2 ^{ab} (0.18)

kokanee were larger than beach-spawning fish (Table 2). When adjusted to an overall mean male fork length of 25.4 cm, SNTL, HDL and HDD also differed significantly among populations (minimum $P < 0.001$), but no clear differences in sizes of these body parts based on spawning ecotype (stream- vs. beach-spawning) were evident (Table 2).

For females, again, stream-spawning kokanee were larger than beach-spawners (ANOVA $P < 0.001$, Table 2). After adjustment to a common fork length of 25.1 cm, female kokanee differed significantly in four measures of body shape; however, the only trait that appeared to be consistently different between spawning ecotypes was HDD which was smaller for beach-spawning female kokanee (Table 2).

Non-sexually dimorphic traits CPD and AFDF (adjusted to a common fork length of 25.4 cm) and TGR and AFR varied significantly among populations (minimum

$P < 0.015$). Only AFR, however, appeared to differ consistently (albeit slightly) between spawning ecotypes; beach-spawning kokanee had, on average, about 0.6 fewer AFR than did stream-spawning kokanee (Table 2). Comparable results were obtained when the data were analysed by sex using multivariate techniques [principal components (PC) analysis], i.e. there were no significant differences in overall body form attributable to spawning ecotype (nested analysis of variance on PC scores, all $P > 0.05$).

Allozyme variation

Only four of the 18 allozyme loci screened were considered polymorphic (frequency of most common allele was less than 99%) in at least one population: ALAT*, PGM-1*, PGM-2* and LDH-B2* (Table 3). Rare alternative alleles were noted, however, for GPI-B1,2*, MPI* and PEP-LT* (frequencies $< 5\%$). Only two of four possible alleles could

Table 3 Variation at polymorphic allozyme loci ($q \geq 0.01$) in Okanagan Lake kokanee. Sample size (n) and frequencies of all observed alleles are listed

Locus	Allele	Population					
		Stream spawners			Beach spawners		
		Mission Creek	Peachland Creek	Powers Creek	Okanagan Central	Squally Point	Juvenile trawl sample
ALAT*1	n	91	31	36	50	49	72
	*91	0.566	0.532	0.528	0.520	0.500	0.521
	*95/*100/*108	0.434	0.468	0.472	0.480	0.500	0.479
GPI-B1,2*	n	91	31	36	50	49	72
	*100	0.995	1.000	1.000	1.000	0.990	0.993
	*132	0.005	0.000	0.000	0.000	0.010	0.007
LDH-B2*	n	97	31	36	51	50	74
	*100	0.959	0.968	1.000	0.971	0.950	0.851
	*110	0.041	0.032	0.000	0.029	0.050	0.149
MPI*	n	96	31	36	51	50	71
	*100	0.995	0.984	1.000	0.990	1.000	0.993
	*105	0.005	0.016	0.000	0.010	0.000	0.007
PGM-1*	n	37	28	30	15	22	28
	*100	0.195	0.134	0.184	0.184	0.121	0.155
	null	0.805	0.866	0.816	0.816	0.879	0.845
PGM-2*	n	37	28	30	15	22	28
	*100	0.919	0.893	0.817	0.733	0.841	0.750
	*77	0.081	0.107	0.183	0.267	0.159	0.250

Only two alleles for ALAT* could be resolved based on mobility differences. Based on previous studies of kokanee (Foote *et al.* 1989, Wood *et al.* 1994; Winans *et al.* 1996) alleles *95, *100 and *108 are most likely the alleles that could not be distinguished from each other. The frequency of the active allele *100 was calculated as $1 - (\text{null allele homozygote})^2$ because *100/*100 cannot be distinguished from *100/null*. Calculations assumed that the populations were in Hardy-Weinberg equilibrium.

be resolved for ALAT* and heterozygote deficiencies could not be tested for at this locus. Significant deviations from Hardy-Weinberg expectations ($P < 0.05$) were observed in the Mission Creek and juvenile trawl samples for LDH-B2*.

Based on allelic frequencies at polymorphic loci, there was no evidence of genetic differentiation among populations within ecotypes ($P > 0.05$). All populations within ecotypes were combined and the beach-spawning component was compared to the stream-spawning component. There were, however, no significant frequency differences at any of the polymorphic loci, or pooled across loci, between the ecotypes ($P > 0.05$).

Mitochondrial DNA variation

Of the six enzymes used to restrict kokanee DNA, only two were polymorphic: *BanI* and *HinfI*. Both RFLPs involved single restriction site changes relative to the common ('A') haplotype (Table 4). The *BanI*-B and C variant haplotypes were recorded in both of the beach-spawning populations, but not in the stream-spawners, and the *HinfI*-B variant haplotype was found only in the stream-

spawning populations (Table 5). There were no significant differences in the frequencies of mtDNA haplotypes between or among populations within reproductive ecotypes (all $P > 0.05$) so the data were pooled by population within ecotypes. Considering the total of 140 fish scored for *BanI* and *HinfI* polymorphisms, beach- and stream-spawning kokanee had significantly distinct haplotype frequencies ($P < 0.0001$, Table 5). Levels of sequence divergence between stream- and beach-spawning ecotypes (estimated from 70 fish assayed for variation with all six enzymes) were low and averaged 0.01%, but was relatively much greater than variation among populations within ecotypes ($< 0.002\%$). Analysis of mtDNA variation using AMOVA indicated that 95.4% of the total diversity was accounted for by variation within populations, 2.4% by variation among populations within ecotypes, and 2.3% by variation between ecotypes ($\phi_{CT} = 0.023$, $P = 0.073$).

We scored 54 juvenile kokanee sampled from the limnetic zone of Okanagan Lake for the *BanI* and *HinfI* RFLPs. Of these juveniles, two exhibited the *HinfI*-B RFLP found in stream-spawning adults and three other juveniles exhibited the *BanI*-B RFLP that was recorded in beach-spawners. All other fish from the trawl sample were

characterized by the common *BanI* and *HinfI* 'A' RFLPs (Table 5).

We also scored 28 Okanagan River sockeye salmon and 28 kokanee from a beach- and stream-spawning site within the Quesnel River system (upper Fraser River drainage) for RFLP variation using the six enzymes used on Okanagan Lake kokanee DNA. All Okanagan River sockeye salmon and all kokanee from the Quesnel system were characterized by the most common 'A' haplotypes resolved in Okanagan Lake kokanee. Neither the variant *BanI* or *HinfI* mtDNA haplotypes were found in the Quesnel kokanee or Okanagan River sockeye salmon.

We were able to consistently read 211 bp from the 3' end of the mtDNA d-loop from kokanee from a total of 32 fish. Kokanee sequences appear to be highly conservative in this region; only three sequence haplotypes were resolved; one common haplotype and two variants (Fig. 2, Table 6). The common haplotype accounted for the 30/32 fish and was distributed from the Kamchatkan Peninsula in the western Pacific to the Columbia River (Table 6). One of the variant haplotypes differed from the common haplotype at two nucleotide sites (0.95%, Fig. 2) and was found in a single fish from Peachland Creek (Table 6). A second variant haplotype characterized a single fish from Iliamna Lake, Alaska. The Iliamna Lake haplotype differed from the most common haplotype by 0.95% (two nucleotides) in sequence and from the variant Okanagan Lake haplotype by 1.9% (four nucleotides). All substitutions were either T-C or G-A transitions (Fig. 2).

We surveyed additional kokanee and sockeye salmon for the presence of the two sequence haplotypes observed in Okanagan Lake (haplotypes 1 and 2, Fig. 2) by restricting the PCR product with the enzyme *NlaIV* (recognition sequence GGN/NCC). This enzyme cuts both sequences twice, but at different positions [62 and 142 bases (common haplotype) and 62 and 163 bases (variant haplotype)] such that the restricted products could be differentiated on 2% agarose gels. Only two further individuals from Okanagan Lake (one in Powers Creek, one in Okanagan Central kokanee) of those surveyed by the RFLP analysis ($n = 100$) were found to have the variant sequence (haplotype 2, Table 6) and the variant Okanagan Lake haplotype was not found in 76 *O. nerka* assayed from various populations outside of Okanagan Lake.

Minisatellite DNA variation

At the minisatellite locus *Ssa1*, kokanee from Okanagan Lake displayed similar patterns of variation as documented by Taylor *et al.* (1996) for *O. nerka* throughout its geographical range. For example, the same two alleles, 'A' (4.4 kilobase pairs, kbp) and 'B' (4.1 kbp) that together accounted for about 95% of the total allele frequencies for sockeye and kokanee populations from Kamchatka to the Columbia River also predominated in the Okanagan Lake kokanee. These two alleles accounted for an average of 97% of alleles across the five Okanagan Lake populations (Table 7). Furthermore, in all populations from Okanagan

Enzyme	Haplotype	Fragment molecular weight											
<i>BanI</i>	A	5160	2330	1750	1680	1620	1175	1070	975	650	200		
<i>BanI</i>	B	5160	2330	1750	1680	1620	1175	1070	975	850			
<i>BanI</i>	C	5160	2330	1750	1680	1620	1175	1070	975	700	150		
<i>EcoRI</i>	A	6850	6450	3950	880								
<i>HinfI</i>	A	2910	1060	1000	895	830	765	720	650	550	360	320	
<i>HinfI</i>	B	1470	1420	1060	1000	895	830	765	720	650	550	360	320
<i>HincII</i>	A	5441	3960	2850	720								
<i>PvuII</i>	A	6525	4660	2425	2300								
<i>StyI</i>	A	3015	1700	1380	1200	1180	1030	700	510				

Table 4 Approximate molecular weights (in base pairs) of restriction fragments resolved by digestion of kokanee mitochondrial DNA with enzymes *BanI*, *EcoRI*, *HindIII*, *HinfI*, *PvuII* and *StyI*

Haplotype	Population					
	Stream spawners			Beach spawners		
	Mission Creek	Peachland Creek	Powers Creek	Okanagan Central	Squally Point	Trawl sample
AA	24	26	28	23	25	49
BA	0	0	0	3	2	3
CA	0	0	0	1	0	0
AB	5	3	0	0	0	2

Table 5 Frequency of mtDNA haplotypes resolved by restriction enzyme analysis. Letter codes refer to restriction fragment length polymorphisms resolved by restriction with *BanI* and *HinfI*. Additional restrictions with *EcoRI*, *HincII*, *PvuII*, and *StyI* resulted in monomorphic 'A' RFLPs for each enzyme

```

haplotype 1  gtctcaaatcagcgttatattttatatacattaataaacttttgatgc 50
haplotype 2  .....
haplotype 3  .....

actttatagcatttggcaccgacagcgtgtaatgcgtacactttcataa 100
.....
.....c.....

ttaaagtatacattaataaacttttcgccacttcatggcatccagcacc 150
.....g.....
.....a.....

gacaacgctatcatcggcaccattttaccggtgcaatccgctgctgggct 200
.....a.....
.....g...g.....

acgttaactaa 211
.....
.....

```

Fig. 2 Nucleotide sequence from the 3' end of the d-loop of *Oncorhynchus nerka* mtDNA. Identity among positions is indicated by '.'. Sequence haplotypes defined as 1–3 are those referred to in Table 6.

Lake, the 'B' allele was the most common (Table 7). There were no significant departures from Hardy–Weinberg expectations at Ssa1 in any of the Okanagan Lake populations (all $P > 0.1$, Table 7). Allele frequencies at Ssa1 were similar across all populations; there were no significant differences among the three stream-spawning populations or between the two beach-spawning populations (both $P > 0.5$). As there were no significant differences within reproductive ecotypes, we pooled the allele frequency data into beach- and stream-spawning groups. Although there was a tendency for beach-spawning kokanee to have a higher frequency of the Ssa1-B allele (Table 7), this apparent difference between forms was not significant ($P = 0.2$).

We also compared the Ssa1 allele frequency data for Lake Okanagan kokanee with the frequencies of alleles for Okanagan River sockeye salmon reported by Taylor *et al.* (1996). Although, there was a tendency for Okanagan Lake kokanee to have a higher frequency of the 'B' allele, the difference was not significant ($P = 0.09$).

At the T34 locus, those two alleles that were most common in North Pacific *O. nerka* (Taylor *et al.* 1996) were also most common in Okanagan Lake kokanee (Table 7). T34 alleles 'A' (0.98 kbp) and 'B' (0.95 kbp) accounted for an average total frequency of 0.92 compared with 0.95 reported for 24 populations by Taylor *et al.* (1996). Genotypic frequencies at T34 were consistent with Hardy–Weinberg

expectations in all populations from Okanagan Lake (all $P > 0.1$, Table 7). As at Ssa1, allele frequencies were homogeneous across populations within reproductive ecotypes (all $P > 0.05$). After pooling the T34 allele frequency data within ecotypes, there was a tendency for beach-spawning kokanee to have a higher frequency of the 'A' allele, but this apparent difference was not significant ($P > 0.1$, Table 7).

By contrast, however, to the results for Ssa1, kokanee from Okanagan Lake had a highly divergent T34 allele frequency distribution from that reported for Okanagan River sockeye ($P < 0.0001$); the 'A' allele predominated in kokanee whereas the 'B' allele predominated in Okanagan River sockeye (Table 7, cf. Taylor *et al.* 1996).

When data on minisatellite DNA allele frequencies were combined with that for allozyme loci, the level of genetic differentiation as measured by F_{ST} between ecotypes was low and nonsignificant (mean \pm SE $F_{ST} = 0.01 \pm 0.003$, $P > 0.05$).

Discussion

Genetic differentiation between spawning ecotypes of kokanee

Our combined genetic analyses of variation in mtDNA and nuclear (allozymes and minisatellite DNA) loci indicated low levels of differentiation between stream- and

Table 6. Geographical distribution of mtDNA d-loop haplotypes in kokanee. Haplotypes were resolved by: (i) sequencing 211 bp from the 3' end of a PCR-amplified 240 base pair fragment of the d-loop, or (ii) by *Nla*IV restriction of the PCR fragment. The number under each haplotype represents the total number of that haplotype in a particular population with the number resolved by sequencing, versus by RFLP, given in parentheses. Haplotypes 1, 2 and 3 are defined in Fig. 2

Location	mtDNA haplotype		
	1	2	3
Columbia River:			
Kootenay Lake	18 (1)		
Okanagan River	12 (1)		
Okanagan Lake:			
Peachland Creek	28 (8)	1 (1)	
Mission Creek	20 (2)		
Powers Creek	30 (4)	1	
Squally Point*	17 (2)		
Okanagan Central*	21 (2)	1	
Fraser River:			
Birkenhead River	4 (1)		
Little Horsefly River	5 (1)		
Quesnel Lake*	5 (1)		
Eagle River	4		
Takla Lake	5		
Vancouver Island:			
Cowichan Lake	5		
North-western BC			
Kathleen Lake	5 (1)		
Alaska:			
Iliamna Lake*	5 (2)		1 (1)
Hansen Creek	5 (2)		
Kamchatka:			
Kronoski Lake	3 (2)		

* Beach-spawning *Oncorhynchus nerka*.

beach-spawning kokanee ecotypes in Lake Okanagan. At the nuclear loci, allelic frequency differences between ecotypes (and among populations within ecotypes) were insignificant. By contrast, stream- and beach-spawning kokanee showed a significant difference in the frequency of mtDNA haplotypes (Table 5), which suggests that Okanagan Lake kokanee are not a single panmictic unit and that a fundamental restriction in gene flow occurs between beach- and stream-spawning populations. Allendorf & Phelps (1981) demonstrated that for neutral loci, significant genetic differentiation can occur among populations in the face of high levels of gene flow (i.e. $Nm > 1$). This is particularly true for populations of low effective size or when inferences about divergence among populations are inferred from genetic surveys of juveniles (Allendorf & Phelps 1981). Reproductive isolation therefore may not necessarily be the demographic cause of genetic divergence between populations at neutral loci. This caveat likely does not apply to our mtDNA evidence

of partial reproductive isolation between beach- and stream-spawning kokanee because: (i) of the historical and present high population sizes of kokanee in Okanagan Lake (i.e. at least 100 000 mature adults, B. Shepherd, BC Fish and Wildlife Branch, Penticton, BC), and (ii) we sampled adult fish for our genetic surveys. Even if effective population sizes of Okanagan Lake kokanee are only one-tenth the census size, the effective numbers of spawners are still well in excess of sizes where spurious differentiation could explain our mtDNA results (Allendorf & Phelps 1981).

Greater differentiation observed at the mtDNA 'locus' relative to nuclear loci has been reported previously (e.g. Carr & Marshall 1991; Tessier *et al.* 1996) and could result from three processes: (a) gene flow between forms that is strong enough to prevent nuclear differentiation, but not so strong as to prevent some mtDNA differentiation (b) sex-biased gene flow between forms where males (contributing nuclear alleles, but not mtDNA) migrate between spawning habitats, but females (contributing nuclear and mtDNA genes) do not, and (c) no gene flow between ecotypes that have maintained large effective population sizes and/or have diverged so recently that genetic differentiation is apparent for only the more sensitive mtDNA genome. Explanations (a) and (c) are consistent with theoretical expectations of a greater effect of random drift on mtDNA diversity given the fourfold lower evolutionary effective population size of mtDNA (Birky *et al.* 1989). No tagging data is available to test the degree of migration between ecotypes, but two observations suggest that greater mtDNA differentiation may be due to the heightened sensitivity of mtDNA as a marker of population structure, limited migration between ecotypes, and recency of origin. First, the comparison of ϕ_{CT} and F_{ST} values suggest that if there is sex-biased migration, it is females that migrate between spawning habitats more than males because the ϕ_{CT} (based on maternally inherited mtDNA) was only twice that of F_{ST} (based on biparentally inherited nuclear loci). Assuming equal sex ratios among migrants, however, the fourfold lower effective population size of mtDNA should result in a ϕ_{CT} value four times as high as F_{ST} (Birky *et al.* 1989). Secondly, because beach-spawning kokanee in Okanagan Lake begin spawning at least 2 weeks after stream-spawning kokanee have finished (B. Shepherd, personal communication) the reproductive ecotypes in Okanagan Lake are isolated both spatially and temporally which limits the possibilities for migration between ecotypes (cf. Hendry *et al.* 1995).

Altogether, the significant mtDNA differentiation between spawning ecotypes of Okanagan Lake kokanee adds to the growing list of genetically distinct sympatric ecotypes found across many groups of north temperate freshwater fishes (reviewed by Robinson & Wilson 1994; Schluter 1996). For mtDNA sequences, sympatric ecotypes

Table 7 Variation at polymorphic minisatellite loci in Okanagan Lake kokanee. Sample size (n), frequencies of all observed alleles, and Hardy–Weinberg expected heterozygosities (H_E) are listed. Alleles listed are described in Taylor *et al.* (1996)

Locus	Allele	Population				
		Stream spawners			Beach spawners	
		Mission Creek	Peachland Creek	Powers Creek	Okanagan Central	Squally Point
Ssa1	n	25	29	33	23	25
	A	0.28	0.24	0.24	0.17	0.13
	B	0.72	0.72	0.72	0.80	0.83
	E	0.00	0.00	0.00	0.02	0.00
	F	0.00	0.02	0.02	0.00	0.02
	H_E	0.40	0.39	0.34	0.32	0.28
T34	n	25	30	33	26	25
	A	0.56	0.71	0.61	0.63	0.72
	B	0.35	0.28	0.32	0.24	0.22
	C	0.08	0.02	0.08	0.13	0.06
	H_E	0.53	0.41	0.53	0.47	0.43

may be highly distinct [e.g. up to 1.2% in sympatric brown trout (McVeigh *et al.* 1995), 2.3% in sympatric 'lake' and 'stream' forms of threespine sticklebacks (Thompson 1995)] or may show low levels of differentiation [e.g. < 0.5% mtDNA divergence between sea-run and freshwater resident Atlantic salmon (Birt *et al.* 1991), dwarf and normal lake whitefish (Bernatchez & Dodson 1990), dwarf and normal rainbow smelt (Taylor & Bentzen 1993)]. Our estimate of sequence divergence between beach- and stream-spawning kokanee (< 0.1%) is uncertain because: (i) we surveyed only about 1.2% of the mtDNA genome using the six restriction enzymes, and (ii) our estimates of divergence among haplotypes found in each populations had standard errors which equalled or exceeded the estimates themselves, a common limitation of restriction site surveys (Hillis & Moritz 1990). Notwithstanding this uncertainty, our data suggest that the Okanagan Lake ecotypes are at the low end of the scale of differentiation observed between sympatric ecotypes.

Our data support other evidence that *O. nerka* tends to be genetically conservative at biochemical and molecular loci relative to other *Oncorhynchus*. For instance, Allendorf & Utter (1979) reported that *O. nerka* had lower levels of allozyme heterozygosity than all other Pacific salmon and trout except coho salmon. Further, Taylor *et al.* (1996) reported that the same two alleles at each of two minisatellite DNA loci characterized *O. nerka* populations throughout their geographical range in the North Pacific. Neilson *et al.* (1994) documented high levels of d-loop sequence haplotype diversity in chinook and coho salmon and in steelhead trout in Northern California (number of haplotypes ranged from five to nine). Our analysis of the same region of the d-loop in sockeye salmon and kokanee,

however, revealed two haplotypes within Okanagan Lake, but only one additional haplotype in 76 fish assayed from Kamchatka to the Columbia River. The apparent relative lack of variation at biochemical and molecular loci in *O. nerka* stands in stark contrast to the remarkable level of behavioural and life-history variation within this species (reviewed by Wood 1995), much of which has been shown to have a large quantitative genetic component (Brannon 1972; Wood & Foote 1990). In fact, notwithstanding the low biochemical and molecular differentiation between forms within Okanagan Lake, differences in size and age at maturity, habitat choice, and spawning time are substantial and likely reflect, in part, quantitative genetic divergence between ecotypes (Foote *et al.* 1992; Wood & Foote 1996). We are assessing the level of quantitative genetic variation using experimental crosses within and between spawning ecotypes.

Evolutionary origin of kokanee ecotypes

Scenarios invoking allopatric and sympatric divergence have both been proposed to account for the evolutionary origin of co-existing, genetically distinct ecotypes in north temperate freshwater fishes (e.g. Hindar *et al.* 1986; Bernatchez & Dodson 1990; Ferguson & Taggart 1991; Foote *et al.* 1989; McPhail 1993; Taylor & Bentzen 1993). Similarly, stream- and beach-spawning kokanee could have diverged in allopatry during the many Pleistocene glaciation-mediated isolation events and come into secondary contact in Okanagan Lake. Alternatively, they may have diverged within the lake following colonization of the Okanagan watershed by a common ancestral *O. nerka*. Our data cannot unequivocally establish which model of

divergence describes the origins of Okanagan Lake kokanee ecotypes, but we favour a recent (postglacial) divergence that occurred within the lake basin as more likely for the following reasons.

First, Okanagan Lake kokanee of both spawning types possess allozyme alleles and mtDNA haplotypes that are either very rare or apparently absent from other surveyed *O. nerka* populations. For instance, Wood *et al.* (1994) and Winans *et al.* (1996) reported the sAH*117 allele at about 2–5% frequency both in stream- and beach spawners from Okanagan Lake and anadromous sockeye salmon from the Okanagan River. This variant allele, however, was found to be extremely rare outside the Okanagan watershed from surveys of over 100 other *O. nerka* populations in the Fraser and Columbia rivers (Winans *et al.* 1996). Similarly, the d-loop sequence variant that characterized three Okanagan Lake kokanee (haplotype 2, Fig. 1.) was not evident in 76 fish assayed from Kamchatka to the Columbia River (Table 5). The sharing of a rare allozyme allele and of the d-loop sequence variant between beach- and stream-spawning kokanee from Okanagan Lake is consistent with what would be expected if beach- and stream-spawners in Okanagan Lake were derived from a common ancestor. Furthermore, the *BanI* and *HinfI* mtDNA RFLPs that we have documented appear to be unique to Okanagan Lake kokanee; they were not recovered in Okanagan River sockeye salmon, from which Okanagan Lake kokanee were most likely derived, or in other stream- or beach-spawning kokanee from an adjacent watershed (E. B. Taylor, unpublished data). The apparent absence of the *BanI* and *HinfI* RFLPs and the sequence mtDNA variant from *O. nerka* surveyed outside Okanagan Lake suggests that these haplotypes may have arisen after the lake was colonized by postglacial dispersing *O. nerka*. Because, however, the whole molecule RFLPs were not found in both ecotypes (i.e. they do not represent shared derived traits) and because our surveys of the PCR-RFLP in the d-loop fragment (a shared derived trait) were limited in sample size, our data can not completely rule out separate colonizations of Lake Okanagan by beach- and stream-spawning fish that had diverged in allopatry.

Secondly, most examples of secondary contact between allopatrically diverged forms exhibit a pattern where the ecotypes are partitioned between two major phylogenetic groups of alleles or haplotypes owing to extended periods of isolation in distinct glacial refugia (e.g. Hamilton *et al.* 1989; Bernatchez & Dodson 1990; Thompson 1995; but see Vuorinen *et al.* 1993 for a possible exception). The phylogenetic groups are usually distinguished by relatively large genetic distances or sequence divergences inferred from allozymes or mtDNA, respectively (e.g. Nei's $D = 0.04$ – 0.08 between sympatric brown trout (Ferguson & Taggart 1991); 2.0–3.0% mtDNA sequence divergence between stickleback clades (Thompson 1995) or 0.5%

between lake whitefish clades (Bernatchez & Dodson 1990). By contrast, Nei's genetic distance (0.001) and estimates of sequence divergence ($\ll 0.1\%$) between Okanagan Lake stream- and beach-spawners are not consistent with long-term divergence from isolation in distinct refugia. Indeed, patterns of allele frequency differentiation at allozyme and VNTR loci clearly indicate that stream- and beach-spawning kokanee from Okanagan Lake are not partitioned across the 'north-western' and 'southern' North Pacific phylogenetic groups of *O. nerka* described by Taylor *et al.* (1996), but are contained within the southern assemblage (cf. Wood *et al.* 1994).

Finally, the postglacial timescale ($< 11\,000$ years) is adequate to account for the level of phenotypic differentiation exhibited by beach- and stream-spawning ecotypes. For instance, Burger *et al.* (1995) suggested that differentiation of sockeye salmon into stream and 'shore' spawning phenotypes in an Alaskan lake has occurred since the lake became ice free less than 2000 years ago. Similarly, stream-spawning sockeye salmon from Baker Lake, WA were introduced into Lake Washington about 60 years ago, and since that time have diversified into 'small creek', 'large river' and 'beach-spawning' ecotypes (Hendry & Quinn 1996).

Phenotypic differentiation between spawning ecotypes

Our data indicate that there is little morphological differentiation between populations or spawning ecotypes of kokanee in Okanagan Lake. In fact, the most striking differences in morphology were between males and females within populations (Table 2); males tended to have larger body parts than females of the same length. Sexual dimorphism in morphology is common in mature salmonids (e.g. Vernon 1957; Blair *et al.* 1993) and is associated with exaggerated development of secondary sexual characteristics in male salmon which engage in aggressive interactions during competition for access to females (Quinn & Foote 1994).

Blair *et al.* (1993) documented greater body depths in beach-spawning sockeye salmon compared with stream-spawning salmon within Iliamna Lake, Alaska and suggested that the habitat-associated morphological differences resulted from divergent selection regimes in the two spawning environments. Deep bodies were suggested to be under strong positive selection in beach areas owing to the apparent advantage that males with deep bodies have in sexual competition (e.g. Quinn & Foote 1994) coupled with relaxed hydrodynamic constraints on body form associated with spawning in these low water-flow habitats, relative to fish from streams that must make upstream migrations and spawn in relatively fast water habitats (Blair *et al.* 1993). In Okanagan Lake, however, we did not find parallel morphological differences between

kokanee spawning in beach vs. stream habitats that would be expected if the hypothesis proposed to account for such variation between Iliamna Lake ecotypes spawning is generally correct. Hendry & Quinn (1996) also failed to find consistent morphological differences between stream- and beach-spawning sockeye salmon in Lake Washington, WA which mirrored those reported for the Alaskan spawning ecotypes. Our morphological data therefore suggest that variation in spawning habitat in *O. nerka* may not always promote morphological differentiation in a manner proposed by Blair *et al.* (1993), which, perhaps, stems from different magnitudes of divergent selection between beach and stream habitats in different watersheds (e.g. length and difficulty of freshwater migrations). For instance, stream spawning sockeye salmon in Iliamna Lake make upstream migrations of up to 43 km in rivers that are, in general, much larger (15–46 m wide) and faster flowing than the streams used by kokanee in Okanagan Lake (Blair *et al.* 1993). Peachland Creek kokanee, for example, migrate no more than 100 m from the lakeshore to spawn in a creek that is 2–3 m wide (E. B. Taylor, personal observations).

Relative morphological homogeneity between kokanee ecotypes was also observed for traits thought to be important in foraging ecology (gill raker number, snout length, eye diameter). Kokanee are usually specialized for planktivory in the limnetic zone of lakes, but Kurenkov (1977) reported the presence of 'few-rakered' (mean number of gill rakers = 32) and 'many-rakered' (mean gill rakers = 43) forms of kokanee in Lake Kronotskiy, Russia. The two morphotypes of kokanee also specialized on different food items; the low raker form on macrobenthos and the high raker form on plankton (Kurenkov 1977). The apparent lack of differentiation in trophic morphology of stream- and beach-spawning kokanee in Okanagan Lake suggests that they are similar in terms of trophic ecology and habitat. Indeed, our observation that the sample of juvenile kokanee obtained by trawling exhibited both the mtDNA RFLPs that distinguished beach- and stream-spawning adults suggests that the reproductive ecotypes share common limnetic habitats and feeding niches as juveniles. A similar result was obtained by Chouinard *et al.* (1996) for genetically distinct populations of dwarf and normal lake whitefish in Lac de l'Est, Quebec; no differences in morphological or meristic traits associated with feeding ecology were observed. Whereas genetic differentiation between kokanee ecotypes can be reasonably postulated to be promoted by their use of distinct spawning habitats (and spawning at different times), it is unclear what ecological factors have facilitated the observed molecular genetic differences between the sympatric whitefish because they spawn in the same streams at the same time (Chouinard *et al.* 1996). Notwithstanding morphological similarity between stream- and beach-spawning kokanee,

it is possible that some trophic differentiation may be present in terms of spatial or temporal aspects of limnetic habitat use.

The ecological differentiation observed between genetically (i.e. mtDNA) distinct populations of Okanagan Lake kokanee, therefore, stands in contrast to most other instances of sympatric populations in north temperate freshwater fishes where the availability of alternative trophic niches (e.g. benthic vs. limnetic, planktivorous vs. piscivorous) appears to be a prominent ecological factor promoting phenotypic and genetic differentiation (reviewed by Schluter 1996). The ecotypes in Okanagan Lake illustrate that other ecological axes may also play a role in promoting isolation of populations and, hence, evolutionary divergence in sympatric populations, i.e. the presence of distinct reproductive niches (beaches and streams) has facilitated the evolution of reproductive ecotypes that appear to share a common trophic resource.

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This study was a collaborative effort between the laboratory of EBT and the Conservation Section of the British Columbia Fish and Wildlife Branch. E. B. Taylor's interests are in the application of molecular genetic techniques to address evolutionary and ecological questions in population and conservation genetics of fishes. S. Pollard's interests and responsibilities involve designing and implementing management strategies to conserve wild populations and genetic diversity in BC fishes. J. Volpe is a PhD student interested in molecular studies of genetic/environment interactions. A portion of the data in this paper formed the basis of S. Harvey's BSc thesis at UBC.
