Biochemical Genetic Comparison of Sockeye Salmon and Kokanee, the Anadromous and Nonanadromous forms of *Oncorhynchus nerka*

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Twenty-three anadromous (sockeye salmon) and nonanadromous (kokanee) *Oncorhynchus nerka* populations were sampled from throughout British Columbia and examined electrophoretically at three to five polymorphic loci to test whether the forms represent distinct genetic lineages or whether they are polyphyletic. Sockeye and kokanee which spawn sympatrically in three different lake systems were also examined to determine whether the two forms belong to a single panmictic population. Our results support the hypothesis that sockeye and kokanee are polyphyletic. No genetic characters were found by which the forms could be separated consistently. Greater differences exist among *O. nerka* populations from different drainages than between sockeye and kokanee forms. Sympatric sockeye and kokanee were significantly different in all systems examined, demonstrating that genetic differences can persist in the absence of geographic barriers to gene flow. While sympatric sockeye and kokanee were genetically divergent, they showed greater genetic similarity to one another (in allele frequency and/or allele types) than they did to their own forms in neighbouring lakes. We argue that this genetic similarity between sympatric forms is the result of sympatric divergence of sockeye and kokanee.

Vingt-trois populations de saumons rouges anadromes et de saumons kokanis non anadromes (*Oncorhynchus nerka*) réparties dans toute la Colombie-Britannique ont été échantillonnées dans le but de faire un examen électrophorétique portant sur de trois à cinq locus polymorphes afin de déterminer si ces formes représentaient des lignées génétiques distinctes ou si elles étaient polyphylétiques. Des saumons rouges et kokanis frayant de façon sympatrique dans trois bassins versants de lacs ont aussi été examinés pour déterminer s'il s'agissait de deux formes appartenant à une même population panmictique. Les résultats obtenus appuient l'hypothèse voulant que les saumons rouges et kokanis soient polyphylétiques. Aucun des caractères génétiques trouvés ne permettaient d'isoler les formes de façon constante. Il existait de plus importants écarts entre des populations de *O. nerka* de bassins versants différents qu'entre les formes rouges et kokanis. Les saumons rouges et kokanis sympatriques différaient de façon significative dans tous les bassins étudiés, ce qui montre que des écarts génétiques peuvent persister en l'absence de barrières géographiques au transfert de gènes. Les saumons rouges et kokanis sympatriques étaient génétiquement divergents, mais présentaient une plus grande similitude génétique l'un envers l'autre (fréquence des allèles ou type d'allèles) qu'avec leur propre forme se trouvant dans des lacs avoisinants. Les auteurs soutiennent que cette similitude génétique entre les formes sympatriques résultent de la divergence sympatique des saumons rouges et kokanis.

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The Pacific salmon *Oncorhynchus nerka* occurs in two distinct forms, the anadromous sockeye salmon and the nonanadromous kokanee. Sockeye salmon typically spend their first year of life (sometimes longer) in a lake before migrating to the ocean whereas kokanee remain in a lake throughout their lifetime. Sockeye usually attain at least twice the length of kokanee at maturity, largely because of the difference in productivity between the marine and freshwater environments (Foerster 1968). Sockeye salmon and kokanee populations occur either together or separately (Ricker 1940; Nelson 1968a). Where they occur sympatrically, spawning usually occurs in separate localities and at different times (Ricker 1940; Nelson 1968a). In a few localities, sockeye and kokanee spawn in the same place at the same time (Ricker 1940; Hanson and Smith 1967; McCart 1970).

It is generally accepted that kokanee have originated from sockeye on numerous independent occasions (Ricker 1940, 1959, 1972; Nelson 1968a; Behnke 1972). This conclusion is supported through an examination of the distribution of the two forms (Ricker 1940; Nelson 1968a) and by observations that nonanadromous populations of *O. nerka* have appeared after sockeye salmon were introduced to lakes previously barren of the species (Ricker 1959, 1972; Scott 1984). It seems more probable that kokanee in Japan, Siberia, and western North America (including Vancouver Island) have arisen largely from the marine dispersal of sockeye salmon following the retreat of the Wisconsin continental ice masses rather than solely through the freshwater dispersal of kokanee.

There is evidence of genetic divergence between sympatric sockeye and kokanee populations (Nelson 1968b; McCart 1970)

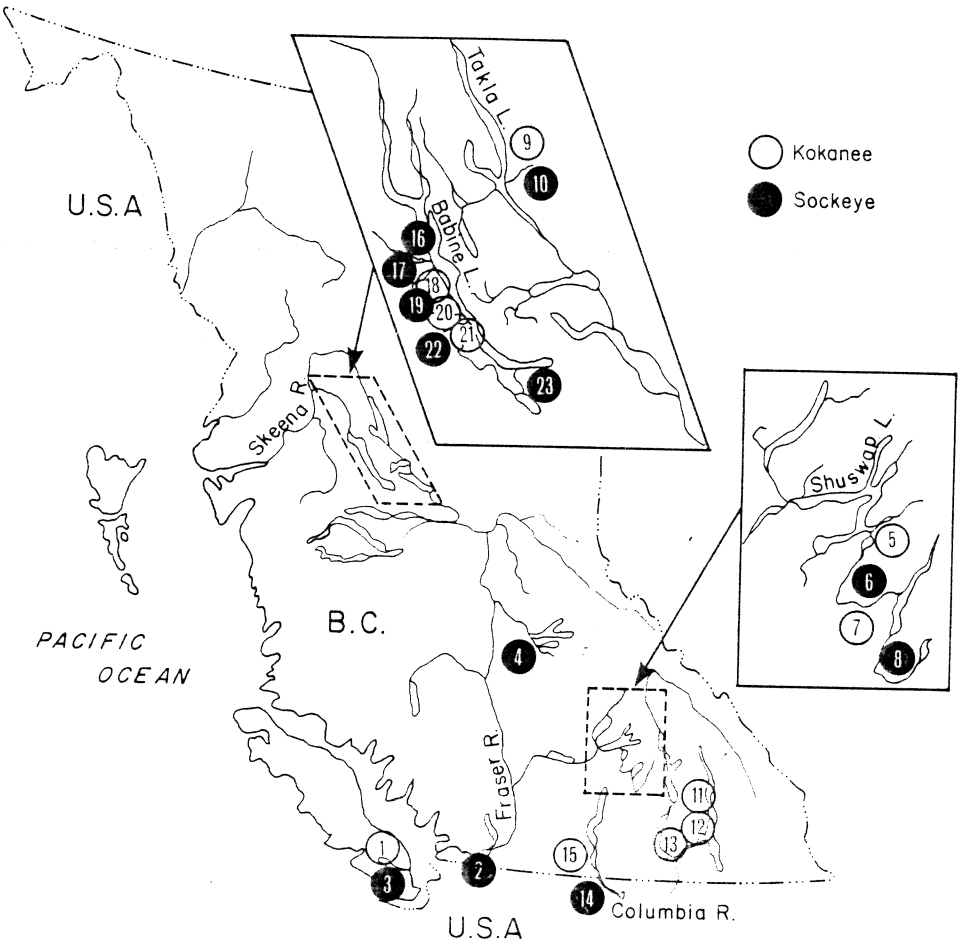

FIG. 1. Location and form of *O. nerka* populations sampled for electrophoresis. Population numbers correspond to those in Table 1.

and various mechanisms have been proposed to account for this. Selective pressures in freshwater and marine environments probably differ greatly, promoting genetic differentiation between the forms (Ricker 1940). Genetic differentiation is further promoted by the tendency of sympatric sockeye and kokanee to spawn in separate locations at different times, limiting gene flow between the forms (Ricker 1940). Where spawning is coincident, gene flow is probably greatly restricted through assortative mating by form, associated with the great size difference between mature sockeye and kokanee (Hanson and Smith 1967; McCart 1970; Foote and Larkin 1988). Sockeye males mate almost exclusively with sockeye females whereas kokanee males mate either with kokanee females or attempt to fertilize the eggs of sockeye females by "sneaking."

In this study, the results of an electrophoretic comparison of sympatric and allopatric sockeye and kokanee populations in British Columbia are used to address questions concerning the recent ancestry and genetic similarity of the two forms. If differentiation has been recent, sockeye and kokanee should be very similar genetically. If one or both forms have given rise to the other form on numerous occasions, then sockeye and kokanee should be genetically interrelated rather than members of two distinct genetic lineages. Additionally, if kokanee have arisen in sympatry with sockeye, sympatric sockeye and kokanee may be expected to be genetically more similar to each other than they are to their respective forms in other lakes.

We sampled sockeye and kokanee from throughout British Columbia, paying particular attention to three localities where the forms spawn sympatrically. Sympatric genetic differentiation would seem to be the least likely in these locations because of the great opportunity for gene flow between the forms. Genetic differentiation in these localities would suggest that mechanisms in addition to segregation to different spawning grounds are important in the genetic differentiation of salmonid populations.

Methods

Kokanee and sockeye were each collected from 11 and 12 localities, respectively, in British Columbia (Fig. 1; Table 1). Five populations were sampled annually for 2 or 3 yr. All samples, with the exceptions of those from Cultus and Cowichan lakes and Okanogan River, were collected with gaffs and small seines in streams where fish were spawning. Sockeye smolts were collected by trap in the outlet of Cultus Lake. Kokanee were captured with a midwater trawl in Cowichan Lake (Rutherford et al. 1988). Electrophoretic data for the Okanogan River sockeye were taken from Utter et al. (1984). Where spawning populations were small (<1000 fish), mainly moribund or freshly dead specimens were collected. Where sockeye and kokanee spawned sympatrically, there were distinguished by size; most sockeye were greater than 45 cm fork length whereas kokanee ranged from 16 to 28 cm. Jack sockeye (precocious

TABLE 1. Allele frequencies at polymomrphic loci for sockeye and kokanee populations sampled in British Columbia. *K* = kokance; *S* = sockeye.

Site	Form	Year	*Pgm-1* 100	*N*	*Pgm-2* 100	*N*	*Ldh-4* 100	115	85	*N*	*Gl-2* 100	*N*	*Alat* 91	100	108	95	*N*
Coastal populations																	
1. Cowichan L.	K	85	0.460	150	0.840	150	1.000	0.000	0.000	150	—	—	—	—	—	—	—
2. Cultus L.	S	85	0.147	158	0.837	95	1.000	0.000	0.000	99	—	—	—	—	—	—	—
3. Cheewhat L.	S	85	0.375	100	0.865	100	1.000	0.000	0.000	100	—	—	—	—	—	—	—
Upper Fraser River populations																	
4. Horsefly R.	S	85	0.624	93	0.805	100	1.000	0.000	0.000	100	0.995	96	0.330	0.569	0.000	0.101	94
5. Eagle R.	K	86	–348	99	0.828	99	1.000	0.000	0.000	99	1.000	99	0.505	0.111	0.354	0.030	99
6. L. Shuswap R.	S	83	0.414	99	0.842	101	0.995	0.005	0.000	97	1.000	80	0.172	0.754	0.030	0.045	67
7.	K	83	0.132	76	0.842	76	0.987	0.006	0.006	77	1.000	78	0.355	0.158	0.480	0.007	99
	K	86	0.142	53	0.783	53	0.991	0.009	0.000	58	—	—	0.336	0.052	0.586	0.026	58
8. M. Shuswap R.	S	86	0.583	144	0.870	142	0.993	0.007	0.000	145	1.000	141	0.132	0.688	0.066	0.115	144
9. Takla L.	K	82	0.357	77	0.903	77	1.000	0.000	0.000	76	—	—	—	—	—	—	—
	K	83	0.340	381	0.865	381	1.000	0.000	0.000	300	—	—	—	—	—	—	—
	K	85	0.367	124	0.846	123	1.000	0.000	0.000	124	0.992	119	0.721	0.119	0.000	0.160	122
10. Takla L.	S	83	0.467	83	0.837	83	1.000	0.000	0.000	79	—	—	—	—	—	—	—
	S	85	0.588	97	0.813	96	1.000	0.000	0.000	97	1.000	100	0.149	0.819	0.000	0.032	94
Columbia River populations																	
11. Meadow Cr.	K	83	0.586	151	0.870	151	0.993	0.003	0.003	151	—	—	—	—	—	—	—
12. Redfish Cr.	K	83	0.595	42	0.964	42	0.977	0.023	0.000	38	—	—	—	—	—	—	—
13. Arrow L.	K	85	0.575	100	0.910	100	1.000	0.000	0.000	98	—	100	0.360	0.615	0.000	0.025	100
14. Okanogan R.	S	—	0.493	71	0.782	71	1.000	0.000	0.000	72	0.995	62	0.285	0.590	0.007	0.000	72
15. Skaha L.	K	83	0.284	88	0.875	88	1.000	0.000	0.000	85	1.000	—	—	—	—	—	
Skeena River populations (Babine Lake)																	
16. Fulton R.	S	85	0.158	291	0.776	290	0.973	0.017	0.010	293	—	—	—	—	—	—	—
17. Tachek Cr.	S	85	0.290	50	0.800	50	0.940	0.010	0.050	50	—	—	—	—	—	—	—
18.	K	85	0.238	103	0.607	103	0.861	0.000	0.139	102	1.000	35	0.500	0.390	0.000	0.110	41
19. Pierre Cr.	S	84	0.215	100	0.735	100	0.968	0.016	0.016	94	—	—	—	—	—	—	—
	S	85	0.202	94	0.766	94	0.953	0.010	0.036	92	0.989	91	0.529	0.382	0.006	0.082	85
20. Pierre Cr.	K	84	0.270	111	0.698	111	0.850	0.000	0.150	110	—	—	—	—	—	—	—
	K	85	0.228	114	0.702	1114	0.870	0.004	0.126	115	0.980	74	0.649	0.299	0.000	0.052	77
21. Twain Cr.	K	85	0.239	92	0.678	101	0.865	0.005	0.130	95	—	—	—	—	—	—	—
22.	S	85	0.214	103	0.830	103	0.971	0.010	0.019	103	—	—	—	—	—	—	—
23. Pinkut Cr.	S	85	0.231	193	0.725	193	0.972	0.005	0.025	198	—	—	—	—	—	—	—

TABLE 2. Enzymes and tissues used to investigate genetic variation in sockeye salmon and kokanee. Buffers used were an amine-citrate buffer (AC) described by Clayton and Tretiak (1972), a Tris, citric acid, lithium hydroxide, and boric acid buffer (RW) described by Ridgway et al. (1970), and a Tris, boric acid, EDTA buffer (MF) described by Markert and Faulhaber (1965).

Enzyme	Tissue	Locus	Buffer
Aspartate aminotransferase	Eye	*Aat-1, 2*	AC
Adenosine deaminase	Muscle	*Ada-2*	AC
Alanine aminotransferase	Muscle	*Alat* (*Gpt-2*)	MF
Peptitdase (glycyl leucine substrate)	Eye	*Gl-2*	MF
Lactate dehydrogenase	Liver	*Ldh-4*	RW
Phosphoglucoisomerase	Muscle	*Pgi*	RW
Phosphoglucomutase	Heart	*Pgm-1*	AC
Phosphoglucomutase	Muscle	*Pgm-2*	RW
Sorbitol dehydrogenase	Liver	*Sdh*	RW
Superoxide dismutase	Muscle	*Sod*	RW

males) were rare in the populations sampled and were greater than 30 cm fork length.

Heart, liver, eye, and muscle tissues were collected from freshly killed fish, or rarely, from those recently dead as judged from redness of the gills. Samples were placed on ice immediately and frozen as quickly as possible, usually within 12 h of collection, and stored at −40°C until assayed. Samples were assayed electrophoretically using standard extraction and gel techniques (e.g. May et al. 1979). The system of nomenclature suggested by Allendorf and Utter (1979) was used to designate loci and alleles (Table 2); the same nomenclature has been used previously for sockeye salmon populations (Grant et al. 1980; Utter et al. 1984; Wilmot and Burger 1985; Quinn et al. 1987). A few samples were assayed at 24 loci but the majority were assayed at a subset of four or eight loci known to be polymorphic in at least some sockeye and kokanee populations. All 27 samples were assayed at *Pgm-1*, *Pgm-2*, *Ldh-4*, and *Aat-3* and 16 were also assayed at *Ada-2*, *Pgi*, and *Sdh*. In addition, 12 of these samples were reassayed at *Gl-2* and *Alat* (previously designated *Gpt-2*; this locus had been difficult to score reliably

until a new staining procedure was developed by P. Aebersold (Northwest and Alaska Fisheries Center, 2725 Montlake Boulevard East, Seattle, WA 98112, pers. comm. to C. C. Wood)).

Differences in genotype frequencies between years and among populations were tested by likelihood ratio (i.e. *G*-test, Sokal and Rohlf 1981) for individual loci and all loci considered simultaneously (using modified critical values for multiple comparisons). Genotype frequencies within populations were compared with those expected under Hardy–Weinberg equilibrium using the chi-square goodness-of-fit test. Allele frequencies at either three to five polymorphic loci, depending on the samples, were used to construct similarity phenograms using the unbiased genetic identity statistic (Nei 1978) and the unweighted pair group methods (Sneath and Sokal 1973).

Variation in allele frequencies (arcsine square-root transformed) was investigated using Method 3 of Henderson (1953) for the following analysis of variance (ANOVA) model:

$$Y_{ijklm} = \mu + T_i + D_j + T \times D_{ij} + L_{jk} + S_{jkl} + e_{ijklm}$$

where Y_{ijklm} is the transformed value of the individual observation of allele frequency, μ is the overall mean value, T_i is the fixed effect of life history type (i = 1–2, sockeye or kokanee), D_j is the random effect due to drainage (j = 1–4, Columbia, Skeena, Upper Fraser, and Coastal, including Lower Fraser River and Vancouver Island), $T \times D_{ij}$ is the effect of the interaction between type and drainage, L_{jk} is the random effect of lake within drainage, S_{jkl} is the random effect of spawning site within lake, and the error term e_{ijklm} is the effect of annual variability within spawning sites.

The significance of differences in allele frequencies between sockeye and kokanee was examined by an *F*-test of the "form" mean square divided by the "form–drainage" interaction, each with a single degree of freedom due to the unbalanced design. All other effects were considered random, and their individual contributions to the total phenotypic variance were estimated by division of the appropriate variance component by the sum of the drainage, type–drainage interaction, lake, site, and error variance components.

Allele frequency variation was also examined in a completely nested gene diversity analysis (GDA) (Nei 1973; Chakraborty 1980). In this model, sockeye and kokanee spawning sympatrically were treated as different "forms" of *O. nerka*, with form nested within spawning sites. Deviations from allele frequencies expected under panmixia due to each level of population subdivision were measured using Chakraborty's (1980) GDA model:

$$H_T = H_W + H_Y + H_F + H_S + H_L + H_D$$

where H_T is the total diversity among and within sites, H_W is the diversity within single samples, H_Y is the diversity among years within sites, H_F is the diversity between sockeye and kokanee within sites, H_G is the diversity among spawning sites within lakes, H_L is the diversity among lakes within drainages, and H_D is the diversity among drainages.

Results

Annual Variation in Allele Frequencies

Five of the loci assayed were polymorphic in some of the kokanee and sockeye populations examined (Table 1). These were *Pgm-1*, *Pgm-2*, *Ldh-4*, *Alat*, and *Gl-2*. The observed genotype frequencies did not differ significantly ($P > 0.05$) from those expected under Hardy–Weinberg equilibrium in any of the populations sampled. In addition, there were no significant differences in genotype frequencies over years at any of the individual loci examined, or for all loci combined, for the three kokanee (Takla Lake; Pierre Creek, Babine Lake; Lower Shuswap River) and two sockeye (Takla Lake; Pierre Creek, Babine Lake) populations sampled repeatedly (Table 1). This suggests that the genetic structure of *O. nerka* populations is stable, at least over relatively short time periods. Accordingly, the electrophoretic information within populations was pooled over years for most subsequent comparisons among populations.

Comparison of Sympatric Sockeye and Kokanee in Three Lake Systems

Sockeye from these streams in Babine Lake where sockeye spawn sympatrically with kokanee (Pierre, Twain, and Tachek creeks) and from two larger systems where spawning kokanee are largely absent (Fulton River and Pinkut Creek) showed no evidence of genetic differentiation at any of the three polymorphic loci examined ($P > 0.05$). Similarly, there was no evidence of differentiation among the kokanee populations at any of the three to five polymorphic loci examined ($P > 0.05$). In contrast, there were significant differences between sockeye and kokanee in all systems where they spawn sympatrically ($P < 0.05$). In Twain and Tachek creeks, sockeye and kokanee differed significantly in genotype frequencies at *Pgm-2* and *Ldh-4* whereas, in Pierre Creek, differentiation was significant at only *Ldh-4* (although the direction of difference at *Pgm-2* was similar to that observed in the others). Thus, there were high similarities within forms and consistent differences between forms across the Babine Lake spawning localities sampled (Fig. 2). Sockeye spawning in the presence of kokanee were no more similar to kokanee than were sockeye spawning in their absence.

Kokanee and sockeye were sampled from three localities in the Shuswap Lake system: the Middle Shuswap River where spawning kokanee are absent, the Lower Shuswap River where the forms spawn sympatrically, and Eagle River where the forms spawn sympatrically but sockeye are rare (and not sampled). Significant differences exist at *Pgm-1* between the Middle and Lower Shuswap River sockeye populations ($P < 0.05$). Similarly, significant differences exist at both *Pgm-1* and *Alat* between the Lower Shuswap River and Eagle River kokanee populations ($P < 0.05$). However, there were greater differences between sockeye and kokanee than between populations within form (Fig. 2). The frequency of *Pgm-1* 100 was consistently higher, and those of *Alat* 91 and 108 consistently lower, in sockeye as compared with kokanee populations in the Shuswap Lake drainage ($P < 0.01$).

Sockeye and kokanee from Narrows Creek, Takla Lake, sampled over 2 and 3 yr, respectively, were consistently different at *Pgm-1* ($P < 0.05$) and *Alat* ($P < 0.01$) (*Alat* was examined only in 1985). Narrows Creek kokanee expressed the *Alat* 91 allele in the highest frequency observed in this study whereas sockeye had the highest observed frequency of *Alat* 100. As a result, genetic differentiation between forms in Takla Lake was greater than that for other sympatric sockeye and kokanee populations (Fig. 2).

In summary, there were significant and consistent genetic differences between sympatrically spawning sockeye salmon and kokanee in all systems examined. Within systems, the extent of genetic differentiation within forms was always less

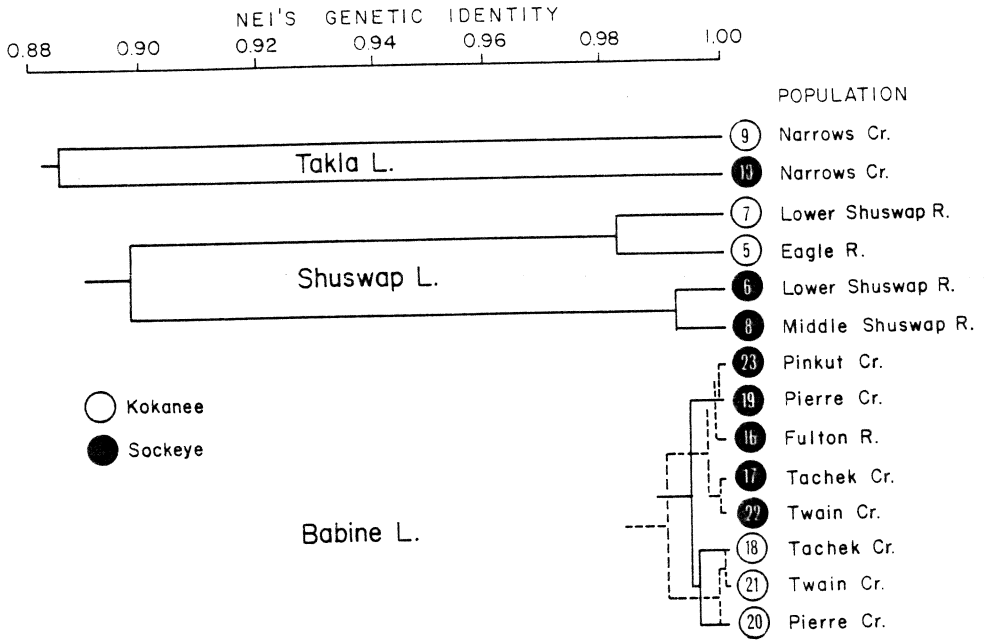

FIG. 2. Phenograms based on measurements of genetic similarity for each of the systems examined where sockeye and kokanee spawn sympatrically (Babine Lake, Shuswap Lake, and Takla Lake). Phenograms based on data taken from five polymorphic loci (all lakes) are drawn in solid lines; those based on three polymorphic loci (Babine Lake) are drawn in broken lines.

than that observed between forms, but the extent of this differentiation varied greatly between lakes. While sympatric sockeye and kokanee differed consistently in allele frequencies, they shared the same alleles at the loci examined. This indicates greater similarity between sympatric forms than that suggested through the use of Nei's genetic identity, which compares only differences in allele frequencies and not the presence or absence of alleles. For example, all sockeye and kokanee populations sampled from the Shuswap Lake system expressed the *Alat* 108 allele, which was largely absent from all other localities (Table 1). Similarly, all sockeye and kokanee populations sampled from Babine Lake expressed the *Ldh-4* 85 allele, also largely absent outside this system (Table 1; Withler 1985). Takla Lake sockeye and kokanee expressed the same allele types at the loci examined, although they displayed none unique to the system.

Overall Comparison of Sockeye and Kokanee in British Columbia

Oncorhynchus nerka populations from across British Columbia cluster into two broad groups (Fig. 3). One cluster includes all sockeye and kokanee populations sampled from the upper Fraser and Columbia River watersheds (with the exception of Skaha Lake and Lower Shuswap River kokanee). The other cluster includes the sockeye and kokanee populations of Babine Lake (Skeena River system). The three coastal *O. nerka* populations examined are split between the two clusters, with Cultus Lake sockeye clustering closely with Babine Lake sockeye and Cowichan Lake kokanee, and Cheewhat Lake sockeye clustering with upper Fraser and Columbia River *O. nerka* populations. There is no obvious separation among populations based on form (sockeye or kokanee). The broad grouping of Columbia and Upper Fraser River *O. nerka* populations, coupled with the separation of the Skeena River populations (Babine Lake) and the splitting of coastal populations between the groups, is very similar to that reported by Utter et al. (1984) in their extensive geographic examination of sockeye salmon at 50 loci.

The genetic relationship of sockeye and kokanee was investigated using ANOVA on allele frequencies at three loci. There were no significant differences between forms in allele frequencies at *Pgm-1*, *Pgm-2*, or *Ldh-4* alleles (Table 3). Differences among drainage systems and the interaction of drainage system and form accounted for most of the observed variance in allele frequencies. These results indicate that it is not possible to classify *O. nerka* populations as sockeye or kokanee solely from knowledge of the allele frequencies at the loci assayed, but classification might be possible given knowledge of the drainage system sampled. These conclusions are supported by the more limited data for *Alat* and *Gl-2*, which also displayed no consistent differences between sockeye and kokanee.

Gene diversity analysis (Nei 1973) yielded similar results. On average, over 90% of the variation in allele frequencies at the three polymorphic loci assayed was due to variation within populations (Fig. 4). Differences among drainage systems were consistently the second greatest source of variation at each locus, followed usually by differences among lakes, between forms, and among years (Table 4).

Discussion

Sockeye and kokanee occur naturally in Japan, Kamchatka, Alaska, British Columbia (including Vancouver Island), and the northwestern United States (Nelson 1968a; Ricker 1972; Scott and Crossman 1973). It seems likely that sockeye originally colonized these diverse regions and subsequently gave rise to nonanadromous populations (kokanee) (Ricker 1940; Nelson 1968a).

The present study supports these conclusions regarding the polyphyletic relationship between sockeye salmon and koka-

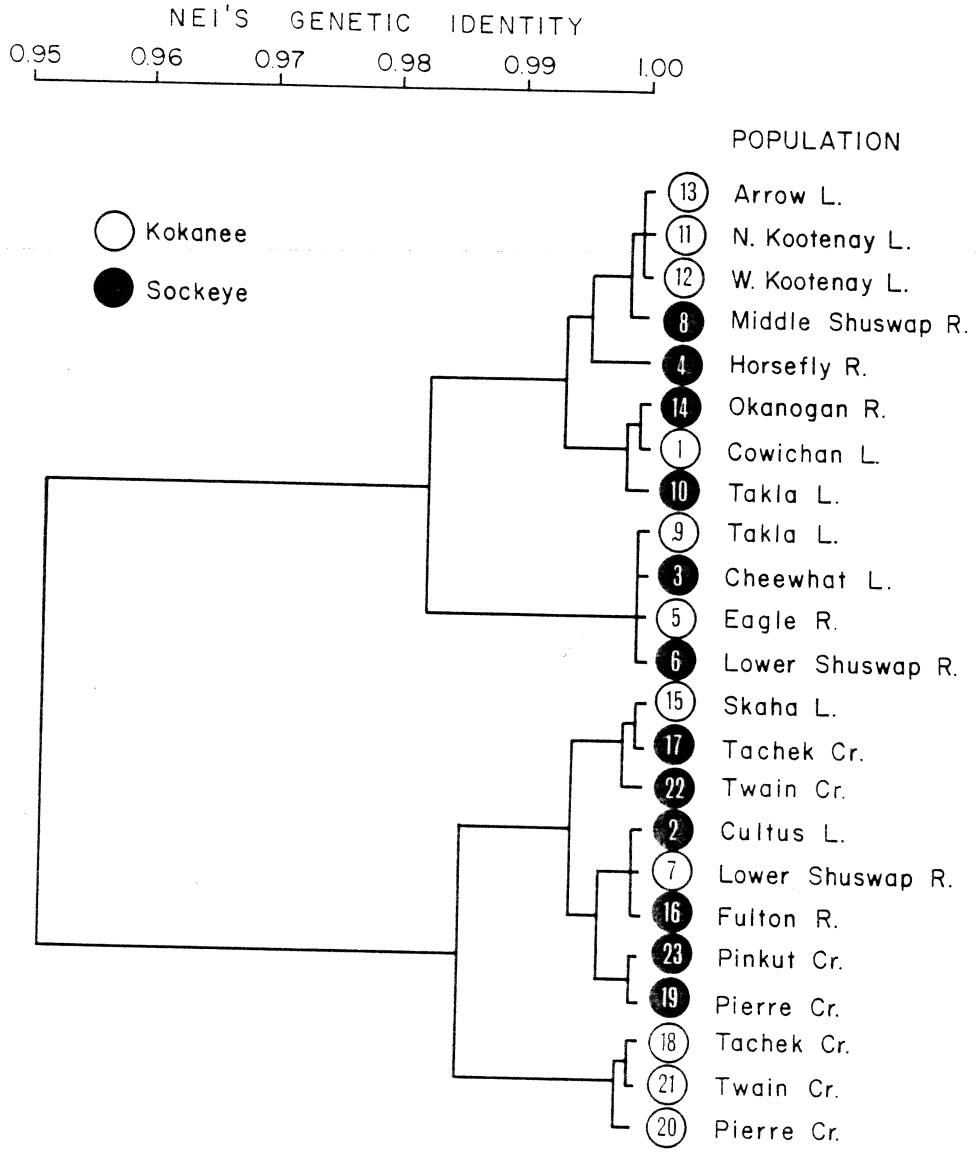

FIG. 3. Phenograms of the relationship among sockeye salmon and kokanee populations based on measurements of genetic similarity at three polymorphic loci.

TABLE 3. Results of a mixed-model ANOVA to determine the sources of variation in allele frequencies at three polymorhic loci. Percent of total variation and $F_{1,1}$ statistics are presented for the random effects and fixed effect, respectively.

	% of total variation				
Source	*Pgm-1* 100	*Pgm-2* 100	*Ldh-4* 100	*Ldh-4* 115	*Ldh-4* 85
Random effects					
error					
(among years)	5.31	18.26	1.87	8.91	2.35
Sites	12.67	0.00	1.22	46.55	0.56
Lakes	25.14	0.00	0.00	1.55	0.00
Drainage	17.05	38.14	29.35	0.00	14.96
Form – drainge	39.84	43.60	67.56	42.99	82.13
Fixed effect					
Form	0.78	0.50	1.09	0.68	1.04
	$P > 0.25$	$P > 0.25$	$P > 0.25$	$P > 0.25$	$P > 0.25$

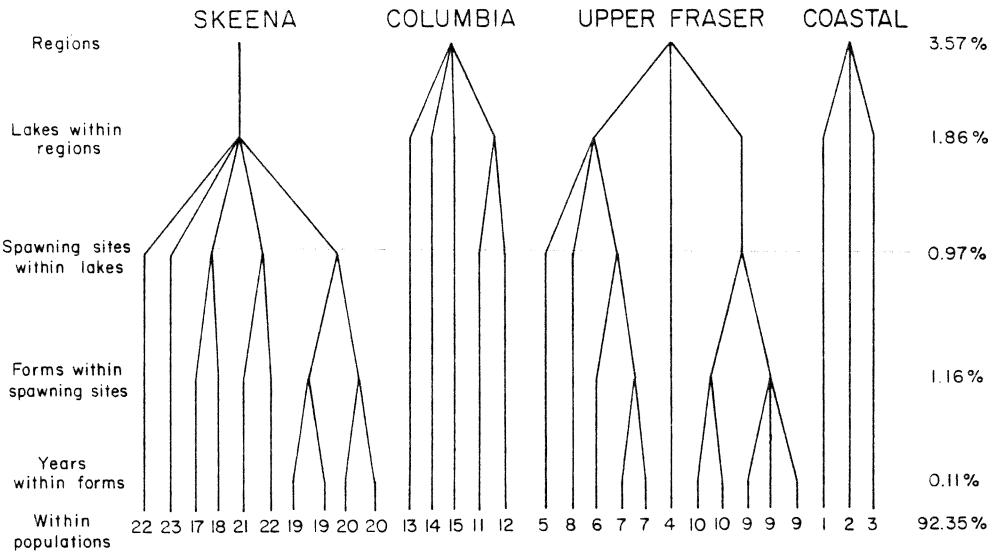

FIG. 4. Hierarchy used in gene diversity analysis of sockeye salmon and kokanee. Percentages at the far right are the average proportions over three loci of total genetic variation occurring at different levels. Population numbers correspond to those in Table 1.

TABLE 4. Percentage distribution of electrophorectically detectable gene diversity at three polymorphic loci among 29 collections of sockeye salmon and kokanee.

Source of variation	Relative gene diversity			
	Pgm-1	*Pgm-2*	*Ldh-4*	Mean
Within populations	89.35	96.10	96.13	92.35
Among years	0.12	0.09	0.02	0.11
Among forms	1.38	0.81	1.16	1.16
Among sites	1.55	0.20	0.44	0.97
Among lakes	3.07	0.40	0.04	1.86
Among drainages	4.52	2.39	2.22	3.57

nee. There were no consistent electrophoretic differences between sockeye and kokanee in British Columbia by which the forms could be separated. There were greater differences among *O. nerka* populations among drainages than there were between sockeye and kokanee. Therefore, it seems unlikely that the existing sockeye and kokanee populations in British Columbia are derived from separate genetic lineages.

The anadromous and nonanadromous forms of other salmonids also appear to have given rise to one another on numerous occasions throughout their range. Nordeng (1983) demonstrated that both anadromous and nonanadromous forms of Arctic char (*Salvelinus alpinus*) can produce anadromous and nonanadromous progeny, the propensity to do so depending on parental form and environmental conditions. Johnson (1980) compared the distribution of anadromous and nonanadromous stocks of Arctic char and concluded that one form gave rise to the other on numerous occasions throughout the species' range. Similarly, Osinov (1984) concluded that anadromous and nonanadromous forms of brown trout (*Salmo trutta*) in the U.S.S.R. were "interchangeable." Supporting this, Ryman (1983) demonstrated that anadromous and nonanadromous brown trout from Sweden were not members of distinct genetic lineages. Allendorf (1975) and Wilson et al. (1985) found that anadromous (steelhead) and nonanadromous rainbow trout (*Salmo gairdneri*) populations were genetically interrelated. McGlade and MacCrimmon (1979) showed that anadromous and nonanadromous brook trout (*Salvelinus fontinalis*) were genetically interrelated. Stähl (1987) demonstrated that nonanadromous and anadromous Atlantic salmon (*Salmo salar*) within broad geographic regions were genetically more similar to each other than they were to their respective forms between regions.

Genetic differentiation among local populations has been documented on numerous occasions in a variety of salmonid species (e.g. Vernon 1957; Frost 1965; Ryman et al. 1979; Ferguson and Mason 1981; Parkinson 1984; Osinov 1984; Wilmot and Burgner 1985; Crozier and Ferguson 1986; Hindar et al. 1986; Campton and Utter 1987). Such differentiation is commonly attributed to the virtual isolation among populations which results from accurate homing to separate spawning grounds (philopatry) (e.g. Vernon 1957; Hartman and Raleigh 1964; Frost 1965; Behnke 1972; Ryman et al. 1979; Crozier and Ferguson 1986; Quinn et al. 1987) or from temporal isolation within spawning grounds (Aspinwall 1974), coupled with the effects of genetic drift (Allendorf and Phelps 1981; Campton and Utter 1987). However, genetic differences between sympatric sockeye and kokanee are difficult to explain solely on these grounds. Genetic differences exist between the forms even where they spawn predominantly in the same place at the same time, indicating that isolation through homing to separate spawning areas cannot account for observed differences between forms. Because spawning populations of *O. nerka* at our study sites were usually large (>1000 to >500 000 individuals), differences between forms are not likely to have arisen from recent genetic drift, although historical bottleneck effects cannot be ruled out. If the genetic heterogeneity observed between sympatric sockeye and kokanee was solely the product of genetic drift (see Allendorf and Phelps 1981), we would expect to see significant heterogeneity within forms as well as between forms within lakes. This was not the case in our extensive survey of spawning populations in Babine Lake. There were no significant genetic differences among either the five separate sockeye spawning populations examined or among the three kokanee populations examined. Furthermore, it is improbable that the observed differences are due to sampling

error or yearly variation, as our sample sizes were large and replicated.

It is not yet clear how genetic differences between sympatrically spawning sockeye and kokanee originate or how they are maintained. McCart (1970) concluded that sockeye and kokanee spawning in the small tributaries to Babine Lake were part of the same panmictic population. He noted that sockeye and kokanee intermixed on the spawning grounds and that kokanee males (like jack sockeye males) showed a strong propensity to act as "sneaks" to spawning pairs, suggesting that significant interbreeding occurred between the forms. McCart (1970) demonstrated that kokanee males fertilized the eggs of sockeye females in the absence of competition and showed that the progeny of such crosses were fully viable in hatchery conditions. However, the present electrophoretic comparison of sockeye and kokanee clearly demonstrates genetic differentiation between forms in Babine Lake tributaries. Despite apparent interbreeding, there is an effective restriction in gene flow between sockeye and kokanee that indicates that they do not constitute a single panmictic population.

The observed genetic similarity and sharing of alleles between populations of sockeye and kokanee in Babine, Takla, and Shuswap lakes strongly indicates that, in each case, one form has arisen from the other in sympatry. The plausibility of sympatric divergence is supported by knowledge of the biology of *O. nerka*. Native and introduced populations of sockeye salmon are known to have given rise to nonanadromous individuals (Ricker 1938, 1940, 1959, 1972; Smirnov 1959; Krokhin 1967; Krogius 1981) and in some cases self-sustaining populations (Ricker 1959; Scott 1984). Similarly, kokanee can give rise to sockeye (Foerster 1947). However, there is no conclusive evidence that the genetic similarities observed between forms within lakes are the result of sympatric divergence. Other mechanisms can be proposed to account for the patterns of similarity observed. The forms may have evolved in allopatry and subsequently introgressed and/or have been subjected to convergent selection pressures in sympatry. However, neither of these alternative hypotheses appears to be tenable. If introgression between the forms had occurred, one would expect to find greater genetic similarity between sockeye and kokanee where they spawn sympatrically than where they spawn in separate localities within the same lake because of the opportunity for continuing introgression. The extensive comparison of Babine Lake sockeye and kokanee spawning populations provides no evidence of such continuing introgression. Sockeye that spawn in the absence of kokanee in Fulton River and Pinkut Creek were genetically indistinguishable from sockeye that spawn sympatrically with kokanee in Pierre, Twain, and Tachek creeks. Similarly, in the Shuswap lake system, sockeye that spawn sympatrically with kokanee in the Lower Shuswap River are genetically no more similar to kokanee than sockeye that spawn in the absence of kokanee in the Middle River system.

It seems even more improbable that convergent selection pressures within lakes could account for the observed genetic similarity of sympatric sockeye and kokanee, since alleles at the loci examined are generally considered to be neutral or nearly neutral to selection (e.g. Ryman 1983). Alternatively, if these alleles are not selectively neutral, and convergent selection pressures were responsible for the observed genetic similarity, one might expect *O. nerka* populations in neighbouring lakes to be genetically similar as well. Babine Lake, in the Skeena drainage, and Takla Lake, in the Fraser Drainage, are separated by a distance of less than 100 km and are morphometrically similar; they occur in the same climatic zone and have similar geological and colonization histories and fish species compositions (Lindsey and McPhail 1986). The physical and biotic similarities between lakes should give rise to similar selective regimes, and in the absence of founder effects, sockeye and kokanee in the two lakes might be expected to be genetically and phenotypically similar. In fact, very substantial differences were found both in allele frequencies and allele types among sockeye and kokanee populations in Takla and Babine lakes. Moreover, sympatric sockeye and kokanee in Takla Lake show greater differences in allele frequencies and gillraker counts (Nelson 1968b) than do those in Babine Lake. These results suggest that lacustrine selection pressures alone do not account for the genetic similarity observed between sympatric forms.

This study, together with previous studies of anadromous and nonanadromous *O. nerka* occurring in sympatry, suggests that genetically isolated populations of sockeye and kokanee have arisen in sympatry, probably on numerous occasions throughout the species' range. The sympatric origin of different forms followed by genetic divergence is theoretically possible (e.g. Maynard Smith 1966; Rosenzweig 1978; Pimm 1979; Rice 1984) but its occurrence in nature is extremely difficult to substantiate and is widely disputed (Mayr 1970; Futuyma and Mayer 1980; Templeton 1981). The biology of *O. nerka* meets two critical requirements of models for sympatric speciation. First, the selective pressures experienced by anadromous and nonanadromous individuals are probably very different and would be expected to promote the genetic divergence of the forms (Ricker 1940). Second, rather strict assortative mating by form would occur within a single generation of the sympatric origin of the forms because of the great size difference between them at maturity. Even within forms, assortative mating by size is conspicuous in wild populations (Hanson and Smith 1967; Foote 1988). Strong assortative mating greatly facilitates genetic differentiation caused by selection (Maynard Smith 1966). Once differences of selective value have accrued, "hybrids" would likely have lower fitness than either "pure" sockeye or kokanee. Such selection can lead to the evolution of premating ethological isolating mechanisms (Maynard Smith 1966), which may partially account for the premating isolation observed between sockeye and kokanee in Babine Lake tributaries (Foote and Larkin 1988). In Pierre Creek, kokanee males prefer to mate with kokanee females rather than with the larger, and more fecund, sockeye females.

In conclusion, recent work describing ecological, morphological, and biochemical differentiation of sympatric forms in salmonids suggests that divergence in sympatry is possible and may be widespread (Johnson 1980; Savvaitova 1980; Balon 1980; Balon and Penczak 1980; Hindar et al. 1986). This study provides further evidence to support this hypothesis.

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