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Genetic structure of wild chinook salmon populations of Southeast Alaska and northern British Columbia

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Synopsis

Allozyme variation was used to examine population genetic structure of adult chinook salmon, *Oncorhynchus tshawytscha*, collected between 1988 and 1993 from 22 spawning locations in Southeast Alaska and northern British Columbia. Thirty-five loci and two pairs of isoloci were variable, and of these, 25 loci and one pair of isoloci expressed the most abundant allele with a frequency of less than or equal to 0.95 in at least one collection. A neighbor-joining (NJ) tree of genetic distances defined five regional groups: (1) King Salmon River (the only island collection), which has large allelic frequency differences from other populations in this study; (2) heterogeneous coastal populations from southern southeast Alaska; (3) transmountain collections from the Taku and Stikine Rivers on the eastern side of the coastal mountain range; (4) Chilkat River in northern Southeast Alaska; and (5) northern coastal Southeast Alaska, which consists of the Situk River and the Klukshu River, a tributary of the Alsek River. A second NJ tree that included collections from the Yukon River and British Columbia did not reveal any strong genetic similarity between Southeast Alaska and the Yukon River. The data suggest that Southeast Alaska may have been colonized from both northern and southern refugia following the last glaciation – a period of sufficient time to allow for isolation by distance to occur.

Introduction

Chinook salmon, *Oncorhynchus tshawytscha*, is a valuable sport and commercial species in Southeast Alaska and northern British Columbia. It has also been the focus of Pacific Salmon Treaty negotiations because intercept fisheries on both sides of the border may harvest stocks from both countries. Recovery of coded-wire tagged chinook salmon caught as by-catch in domestic and foreign trawl fleets shows that fish from Southeast Alaska and northern British Columbia migrate well out into the North Pacific Ocean and the Bering Sea where they are vulnerable to both legal and illegal fishing (Healy 1991). Efforts are underway in both the U.S. and Canada to develop a genetic baseline for spawning populations of chinook salmon for use in determining the origins of fish harvested in interception fisheries near the U.S./Canada border (transboundary rivers, and between Southeast Alaska and northern British Columbia), and as by-catch in the North Pacific and Bering Sea trawling fleets (Teel et al.,[1] Wilmot et al.[2]).

Of the approximately 2 000 watersheds in Southeast Alaska, fewer than 40 support chinook salmon, and most of the major spawning areas are widely separated (Heard et al. 1995). Genetic similarities among populations of a species can be used to detect contemporary and historic relationships among them.

[1] Teel, D.J., P.A. Crane, C.M. Guthrie III, A.R. Marshall, D.M. Van Doornik, W.D. Templin, N.V. Varnavskaya & L.W. Seeb. 1999. Comprehensive allozyme database discriminates chinook salmon around the Pacific Rim. NPAFC document 440. 25 pp.

[2] Wilmot, R.L., C.M. Kondzela, C.M. Guthrie III, A. Moles, Jerome J. Pella & Michele Masuda. 2000. Origins of salmon seized from the F/V *Arctic Wind*. (NPAFC Doc. 471) Auke Bay Fisheries Laboratory, Alaska Fisheries Science Center, NMFS, NOAA, 11305 Glacier Highway, Juneau, AK 99801-8626. 18 pp.

The rivers of Southeast Alaska and the transboundary rivers that flow from northern British Columbia through Southeast Alaska support stocks of 'stream-type' chinook salmon that spend 1 year in freshwater before migrating to sea. A single 'ocean-type' population, which migrates to sea in its first year, occurs in the Situk River (Johnson et al. 1992). Teel et al. (2000) examined genetic structure of chinook salmon collections from British Columbia, and observed differences between inland stream-type and coastal ocean-type collections. Gharrett et al. (1987) found that chinook salmon populations from Southeast Alaska were genetically more heterogeneous than populations from elsewhere in Alaska, which presumably reflects the low gene flow among, or multiple founding sources for the Southeast Alaska populations. They also speculated that chinook salmon from Southeast Alaska may have originated from the Yukon River drainage through headwater capture of the transmountain rivers flowing out of northern British Columbia. There is geological evidence that the Taku River was blocked periodically and backed up into the Yukon River drainage (Kerr 1948). The Alsek River was also similarly connected to the Yukon River drainage in the past via Kluane Lake and the White River (Lindsey et al. 1981).

The objectives of this study were to use allozyme variation to describe the genetic structure of chinook salmon populations in Southeast Alaska and the transboundary rivers flowing out of northern British Columbia, and to investigate possible historic relationships among some Alaskan populations and chinook salmon from other areas.

Methods

Between 1988 and 1993, 37 collections of tissue samples were taken from adult chinook salmon at 22 spawning locations in Southeast Alaska and northern British Columbia (Table 1, Figure 1). Between late July and early September, fish were sampled at weirs, carcass weirs, stream spawning areas, and during egg takes for hatchery propagation. Ten locations were sampled in multiple years (Table 1). Whole eyes and samples of liver, cheek muscle, and heart were collected from each adult fish, cooled with frozen gel–ice, subsequently frozen at −20°C, and shipped to the Auke Bay Laboratory where they were stored at −80°C until analyzed. Liver was not collected from the Farragut River samples.

Table 1. Group designation (letters correspond to spawning areas listed in Figures 1 and 2 & Appendices 1 and 2 available at ftp.afsc.noaa.gov/sida/chinook/) location, date of collection, and sizes of chinook salmon collections (N), used for electrophoretic analysis. Districts are Alaska Department of Fish and Game Statistical Areas.

Group	Location	Date	N
District 101–Boca De Quadra			
A.	Keta R.	Sep. 5–6, 89	15
West Behm canal			
B.	Chickamin R. (pooled)		151
	Chickamin South Fork	Aug. 24, 89	66
	Leduc R.	Aug. 24, 89	30
	Humpy C.	Aug. 24, 89	4
	Chickamin South Fork	Aug. 21, 90	15
	Leduc R.	Aug. 21, 90	25
	Chickamin R.	Aug. 21, 90	11
Unuk R. drainage			
C.	Clear C.	Aug. 30, 89	33
D.	Cripple C.	Aug. 16, 88	121
E.	Gene's Lake C.	Aug. 30, 89	67
District 107–Bradfield canal			
F.	Harding R.	Aug. 16,22,26, 89	45
District 108–Stikine R. drainage			
G./H.	Lower Stikine (pooled)		75
G.	North Arm C.	Aug. 17, 89	18
H.	Andrews C.	Aug. 17, Sep. 9, 89	57
I.	Shakes C.	1993	29
J.	Little Tahltan R. (pooled)		228
	Little Tahltan R.	Aug. 89	101
	Little Tahltan R.	Aug. 7–8, 90	50
	Little Tahltan R.	Jul., Aug. 91	77
District 110–Farragut Bay			
K.	Farragut R.	Aug. 22, 89	8
District 111–Northwest Admiralty Island			
L.	King Salmon R. (pooled)		86
	King Salmon R.	Jul. 25,30, 88	37
	King Salmon R.	Jul. 24,31, 89	31
	King Salmon R.	Jul. 24,31, 90	18
Taku R. drainage			
M.	Nakina R. (pooled)		198
	Nakina R.	Aug. 14–24, 89	104
	Nakina R.	Aug., 90	94
N.	Kowatua C.(pooled)		190
	Kowatua C.	Aug. 25,29, Sep. 1, 89	95
	Kowatua C.	Aug. 29, Sep. 6, 90	95
O.	Tatsatua C. (pooled)		228
	Tatsatua C.	Aug. 30, 89	112
	Tatsatua C.	Aug. 24, Sep. 6, 90	116
P.	Dudidontu R.	Aug. 13, 90	28
Q.	Tseta R.	Aug. 6,10, 89	81
R.	Nahlin R. (pooled)		129
	Upper Nahlin R.	Aug. 1–5, 89	81
	Upper Nahlin R.	Jul. 31, Aug. 5, 90	48

Table 1. (Continued)

Group	Location	Date	N
District 115–Chilkat R. drainage			
S.	Big Boulder C.	Aug. 5–15, 91	27
T.	Tahini R. (pooled)		83
	Tahini R.	Jul. 31, 89	26
	Tahini R.	Aug. 90	48
	Tahini R.	Aug. 2–5, 91	19
District 181–Alsek R. drainage			
U.	Klukshu R. (pooled)		250
	Klukshu R.	Aug. 89	105
	Klukshu R.	Aug. 8, 90	100
	Klukshu R.	Sep. 91	45
Situk R. drainage			
V.	Situk R. (pooled)		174
	Situk R.	Jul. 6, Sep. 90	31
	Situk R.	Jul. 12, Sep. 10, 91	65
	Situk R.	Aug. 29, 92	78

Protein electrophoresis was conducted as described by Aebersold et al. (1987). Specific enzyme activities were stained according to Harris & Hopkinson (1976) or Aebersold et al. (1987). Loci for which data were taken, the tissues in which they were expressed, and the buffer systems with which they were resolved are listed in Table 2.

Conformance to Hardy–Weinberg equilibrium expectations (e.g., Hardy 1908) was tested with Chi-square goodness-of-fit tests. For less variable loci, probabilities of the statistic were estimated using a Monte Carlo simulation [a FORTRAN program written by A.J. Gharrett (unpublished) analogous to Roff & Bentzen (1989)] using 2 000 iterations. Hardy–Weinberg tests were not conducted on co-migrating, duplicated loci (isoloci) or for three loci with dominant allele expression, *GPI-B2**60 allele, *GPIr** and *sMEP-2**, which were scored as dominant–recessive phenotypes. Variation at isoloci was treated as if all the variability was at one locus and the other was monomorphic. This is a conservative treatment for isoloci having relatively low allelic variability (Gharrett & Thomason 1987). We used log-likelihood ratio analysis (Sokal & Rohlf 1981) to test homogeneity of allele frequencies at four levels of hierarchy. For tests that included low-frequency alleles, probability levels were estimated by generating distributions of the G-statistic, a Monte Carlo method analogous to that described for the Hardy–Weinberg goodness-of-fit test (FORTRAN program from A.J. Gharrett, unpubl.). The relative amounts of variation at different levels of hierarchy were test using an approximate F-statistic:

$$F_{df\,among,\,df\,within} = \frac{G_{among}/df_{among}}{G_{within}/df_{within}}$$

Relationships among collections were examined descriptively with neighbor-joining (NJ) trees (Saitou & Nei 1987) constructed from chord distances (Cavalli-Sforza & Edwards 1967) between pairs of populations. Allelic frequencies were estimated for *sMEP-2** and *GPI-B2** from the alternate homozygotes (*GPIr** is monomorphic) assuming Hardy–Weinberg equilibrium. Significance of nodes in the NJ tree were inferred from test of heterogeneity among branches joined at the nodes (Chakraborty & Leimar 1987). Monte-Carlo simulations were conducted to assess the influence of sampling error of allele frequency estimates on the topology of trees (Hawkins et al. 2002). The entire data set was iteratively (20 000 trials) regenerated by resampling with replacement alleles from each population, and NJ trees were constructed for each iteration. A consensus tree was determined from these 20 000 trees using CONSENSE from PHYLIP (Felsenstein[3]). The stability of the topology is shown in the proportion of trees that share specific nodes. We used Arlequin (Schneider et al.[4]) to describe hierarchical genetic structure within (F_{SC}) and among (F_{CT}) regional groupings described by the NJ tree. The significance of the F-statistics was estimated from distributions of the statistics generated by 17 000 permutations. Three loci were dropped from this analysis: *GPI-2**, *GPIr** and *sMEP-2** since heterozygotes could not be detected for these loci.

A second NJ tree, using the 32 loci and three isolocus pairs available for all three data sets (Table 2; *GPI-B2**60 allele was pooled with the common allele) and incorporating 13 additional populations from British Columbia (Teel et al. 2000) and 12 from the upper Yukon River (Wilmot et al.,[5] Wilmot unpubl. data), was constructed to examine the genetic

[3] Felsenstein, J. 1993. PHYLIP (*Phylogeny Inference Package*) Version 3.60a, Seattle WA, Department of Genetics, University of Washington.

[4] Schneider, S., J.-M. Kueffer, D. Roseli & Laurant Excoffier. 2000. Arlequin version 2.0: A Software for Population Genetic Data Analysis, Genetics and Biometry Laboratory, University of Geneva, Switzerland.

[5] Wilmot, R.L., R.J. Everett, W.J. Spearman & R. Baccus. 1992. Genetic stock structure of Yukon River chum and chinook salmon 1987 to 1990. Progress Report, Alaska Fish and Wildlife Research Center, U.S. Fish and Wildlife Service, U.S. Department of Interior, 1011 East Tudor Road, Anchorage AK, 99503. 132 pp.

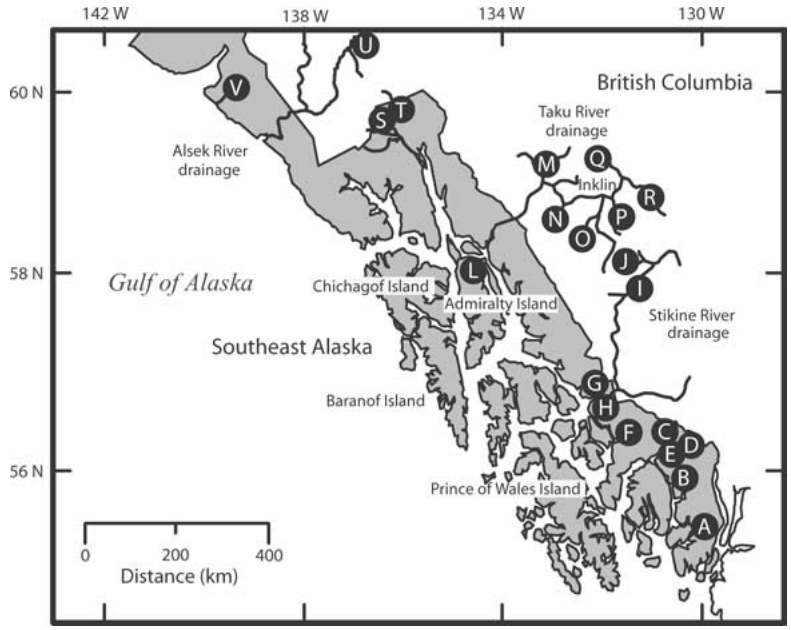

Figure 1. Sampling sites for chinook salmon in Southeast Alaska and northern British Columbia. Letters correspond to collection sites listed in Table 1.

Table 2. Protein coding loci (Shaklee et al. 1990) for enzymes resolved in this study, Enzyme Commission (EC) numbers (IUBMBNC 1992), and the tissues and buffer in which they were resolved. Peptidase loci are designated according to their substrate specificity. L = liver, H = heart, M = muscle, E = eye.

Enzyme	EC number	Locus	Tissue	Buffer[a]	Variability level[b]
Aconitate hydratase	4.2.1.3	*sAH-1**	L	B	4, 5
Adenosine deaminase	3.5.4.4	*ADA-1**	M,H	G,F	4, 5
		*ADA-2**	M,H	G,F	3, 5
Alanine aminotransferase	2.6.1.2	*ALAT**	M,H	G,E	4
Aspartate aminotransferase	2.6.1.1	*mAAT-1**	M,H	B,C,F	4, 5
		*sAAT-1,2**	M,H	B,C	4, 5
		*sAAT-3**	E	A	4
Creatine kinase	2.7.3.2	*CK-C1**	E	G,E	1
		*CK-C2**	E	G,E	4
		*CK-B**	E	G,E	1
Formaldehyde dehydrogenase (glutathione)	1.2.1.1	*FDHG**	H,E,L,M	G	4, 5
Fructose-biphosphate aldolase	4.1.1.13	*FBALD-3**	E	A	1
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-B1**	H	D,G	1, 5
		*GPI-B2**	H	D,G	4, 5
		*GPI-A**	H	D,G	4, 5
		*GPI-r**	H	D,G	1
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH-2**	H	C	3
		*GAPDH-3**	H	C	3

Table 2. (Continued)

Enzyme	EC number	Locus	Tissue	Buffer[a]	Variability level[b]
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1**	M,H	B,C	1
		*G3PDH-2**	M,H,	B,C	1
		*G3PDH-3**	M,H,	B,C	3
		*G3PDH-4**	M,H	B,C	1
Glutathione reductase	1.6.4.2	*GR**	M,H	F	4, 5
β-N-Acetylhexosaminidase	3.2.1.30	*β-HEX**	L	F	4, 5
L-Iditol dehydrogenase	1.1.1.14	*IDDH-1**	L,H	D	4
		*IDDH-2**	L,H	D	1
Isocitrate dehydrogenase	1.1.1.42	*mIDHP-1**	M,H	B,F	2, 5
		*mIDHP-2**	M,H	B,F	3, 5
		*sIDHP-1**	M,H,L,E	A,B,F	4, 5
		*sIDHP-2**	H,L,E	A,B,F	4, 5
Lactate dehydrogenase	1.1.1.27	*LDH-B1**	M,H,E	G,D	3, 5
		*LDH-B2**	M,H,L,E	G,D	1, 5
		*LDH-C**	E	G,D	1, 5
Malate dehydrogenase	1.1.1.37	*sMDH-A1,2**	M,H	B,C	1, 5
		*sMDH-B1,2**	H,M,L	B,C	4, 5
		*mMDH-1**	H,M	B,C	1
		*mMDH-2**	H,M	B,C	4, 5
		*mMDH-3**	H,M	B,C	1
Malic enzyme	1.1.1.40	*sMEP-1**	M,H	F	4, 5
		*sMEP-2**	M,H	F	4
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	M,H,E	G,F	4, 5
Peptidases	3.4.*.*				
Dipeptidase	3.4.13.18	*PEPA**	M,H,E	D,G,C	4, 5
Glycl-leucine activity		*PEPC**	E	E	1
Tripeptide aminopeptidase	3.4.11.4	*PEPB-1**	M,H,E,L	F,G	4, 5
Leucyl-glycl-glycine activity		*PEPB-2**	M,H	F,G	3
Leucyl-tyrosine activity	3.4.11.*	*PEP-LT**	M,H	D,F	4, 5
X-Pro aminopeptidase	3.4.13.9	*PEPD-1**	M,H,E	C,D,F	1, 5
Phenylalanyl-proline activity		*PEPD-2**	M,H,E	C,D,F	1
Phosphoglucomutase	5.4.2.2	*PGM-1**	M,H	G	3, 5
		*PGM-2**	M,H	G	1, 5
6-Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	M,H,E,L	A,B	1, 5
Phosphoglycerate kinase	2.7.2.3	*PGK-1**	E,M,H	A,B	1, 5
		*PGK-2**	E,M,H	A,B	4, 5
Superoxide dismutase	1.15.1.1	*mSOD-1**	M,H	D,F,G	4, 5
		*sSOD-1**	M,H,E	D,F,G	4, 5
Triose-phosphate isomerase	5.3.1.1	*TPI-1**	M,E,H	G	1
		*TPI-2**	M,H	G	1
		*TPI-3**	M,E,H	G	4, 5
		*TPI-4**	M,E,H	G	4, 5

[a] A = CA 7.0 (Clayton and Tretiak 1972), B and C = CAME 7.0 and 7.0-NAD (Aebersold et al. 1987), D = R(Ridgeway et al. 1970), E = MF (Markert & Faulhaber 1965), F = TC-4 (buffer 'a' of Schaal and Anderson 1974), G = TG (Holmes and Masters 1970).
[b] 1 = monomorphic; 2 = rarely variable; most abundant allele ≥ 0.99; 3 = variable; most abundant allele < 0.99 but > 0.95; 4 = highly variable; most abundant allele ≤ 0.95; 5 = used in broad region analyses.

relationship with populations from Southeast Alaska. To test for isolation by distance and to assess the effects of gene flow and drift, the F_{ST} between all pairs of 10 coastal populations was calculated by the method of Weir & Cockerham (1984). The effective number of migrants ($N_e m$) per generation, was calculated between all pairs of populations from Wright's (1931) equation $F_{ST} = 1/(4N_e m + 1)$. The $\log_{10}(N_e m)$ was then regressed against the $\log_{10}$(distance) measured in kilometers between each pair of populations (Slatkin 1993). Distances were measured by water between the mouths of each drainage. For populations with g subdivisions the equation:

$$F_{ST} = \frac{1}{4N_e m\,(g/(g-1))^2} - 1$$

was used to assess divergence among subdivisions.

Results

We obtained data from 62 protein coding loci in the adult chinook salmon samples (Table 2). Variation was observed at 35 loci and two isolocus pairs, with the frequency of the most abundant allele was less than or equal to 0.95 in at least one collection at 25 loci and one isolocus pair. The frequency of the most abundant allele at four loci and one isolocus pair was between 0.95 and 0.99 in at least one collection, and was less than 0.99 in at least one collection at six loci. Twenty-one loci and one isolocus pair were monomorphic. Out of 703 tests possible for these collections, 22 did not conform to Hardy–Weinberg expectations, three of which remained significant when a sequential Bonferroni adjustment was applied (Rice 1989). These were: *LDHB1** in [Q] Tseta Creek 1989; *PEPLT** in [J] Little Tahltan River 1989; and *mAAT-1* in [D] Cripple Creek 1988. The departures from Hardy–Weinberg were due to a paucity of heterozygotes. Allele frequency appendices for polymorphic and monomorphic loci are available for download at ftp.afsc.noaa.gov/Sida/chinook/.

Multiple collections from different years were taken from 10 locations. *G*-tests indicated that seven of these locations were temporally heterogeneous (Table 3). We followed Waples (1990) recommendation for pooling temporal samples from the same location, when the differences were not too large to be attributed to sampling error or genetic drift. Temporal collections from the same location were pooled for analysis (Table 1), and we also pooled multiple, homogeneous collections within the Chickamin River drainage, as well as Andrews and North Arm Creeks, which are on opposite banks of the lower Stikine River (Tables 1 and 3).

Combined heterogeneity for among river drainages exceeded heterogeneity within drainages at 26 loci and two isolocus pairs (Table 3), indicating that divergence between rivers within drainages was less than divergence among drainages ($F_{333,962} = 10.66$; $p = 2.11 \times 10^{-182}$). Significant heterogeneity ($p < 0.05$) was observed within every river drainage except the Chickamin River. The Taku, Stikine, and Chilkat rivers exhibited heterogeneity at three, two, and one additional levels of hierarchy, respectively. A complete appendix of the hierarchal log-likelihood analysis is available for download at ftp.afsc.noaa.gov/Sida/chinook/.

An unrooted tree (Figure 2) constructed from chord distances by NJ identified five regional groupings of chinook salmon collections (letters from Table 1 and Figure 1): (1) southern coastal Southeast Alaska ([A] Keta River, [B] Chickamin River, [C] Clear Creek, [D] Cripple Creek, [E] Gene's Lake, [F] Harding River, and [G] Lower Stikine River); (2) [L] King Salmon River; (3) transmountain ([I] Shakes Creek, [J] Little Tahltan River, [M] Nakina River, [N] Kowatua Creek, [O] Tatsatua Creek, [P] Dudidontu River, [Q] Tseta Creek, and [R] Upper Nahlin River; (4) Chilkat River ([S] Big Boulder Creek, and [T] Tahini River); and (5) northern coastal Southeast Alaska ([U] Klukshu River and [V] Situk River). The same five regional groupings of chinook salmon were identified in a principal component analysis (not shown). The consensus tree shared the same topology with the point estimate of the NJ tree with one exception; Chickamin River and Clear Creek populations positions were reversed on the consensus tree. The consensus tree represents the stability of the position of nodes on the trees, but lacks the genetic distances of the point NJ tree. Because the point estimate and consensus trees were nearly coincidental we were able to label the nodes with the bootstrap values. The majority of the nodes had bootstrap values >50%, which indicate stability in the tree structure. A gene diversity analysis, based on five geographic areas inferred from the NJ tree, estimated that 29.5% ($F_{SC} = 0.01692$; $p \ll 1 \times 10^{-4}$) of the genetic diversity was within regions, while 70.5% ($F_{CT} = 0.04051$; $p \ll 1 \times 10^{-4}$) was among regions, which suggests greater gene flow within than among regions.

A second NJ tree (Figure 3) was used to examine the relationships among Southeast Alaska/northern

Table 3. Log-likelihood ratio analysis of regional genetic structure of chinook salmon from Southeast Alaska and northern British Columbia for a subset of loci – the full analysis is available at ftp://ftp.afsc.noaa.gov/Sida/chinook/. Probability levels ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$) were based on the distribution of *G*-statistics for each test based on Monte-Carlo resampling. *G* is the log-likelihood statistic and d.f. the degrees of freedom. Values of d.f. in parentheses are in the totals for all alleles in all populations and the d.f. shown are from the alleles observed in each test.

Source	Locus															
	*ADA-1**		*GPIB-2**		*IDDH-1**		*sIDHP-1**		*sMEP-1**		*sMEP-2**		Total		Grand total	
	d.f.	*G*	d.f.	*G*	d.f.	*G*	d.f.	*G*	d.f.	*G*	d.f.	*G*	d.f.	*G*	d.f.	*G*
Within Situk River	2	5.330	2		2	2.639	2	4.370	2	15.469***	2	2.867	12	30.675***	(74) 42	64.241*
Within Klukshu River	2	6.190*	2		2	0.240	2	0.223	2	21.615***	2	5.450	12	33.718***	(74) 38	64.918***
Within Chilkat River	3		3		3	15.461**	3	1.704	3	3.293	3	2.876	18	23.334	(111) 57	92.78***
Within Tahini River					2	12.941**	2	0.828	2	1.737	2	2.687	8	18.193*	28	38.346
Between Big Boulder and Tahini					1	2.520	1	0.876	1	1.556	1	0.189	4	5.141	19	54.434***
Within Taku River	9	26.471**	9	4.531	9	34.822***	9	14.001	9	13.732	9	19.188*	54	112.745***	(333) 306	486.658***
Between Nakina and Inklin	1	0.401	1	2.976	1	0.022	1	1.171	1	1.414	1	0.016	6	6.000	34	65.774***
Within Nakina River	1	7.696**	1	1.555	1	2.547	1	0.736	1	1.059	1	3.459	6	17.052**	30	42.704
Within Inklin River	7	18.374*	7		7	32.253***	7	12.094	7	11.259	7	15.714*	42	89.694***	238	378.183***
Among Kowatua, Tatsatua, Nahlin	2	5.192	2		2	18.344**	2	0.948	2	5.256	2	3.914	12	33.654***	72	165.706***
Within Kowatua	1	1.389	1		1	1.378	1	0.002	1	0.163	1	5.312*	6	8.244	32	49.56*
Within Tatsatua River	1	0.378	1		1	5.682	1	0.635	1	3.297	1	2.614	6	12.606*	33	38.799
Within Nahlin River	3	11.415**	3		3	6.850	3	10.508*	3	2.542	3	3.874	18	35.189**	90	124.119**

Table 3. (Continued)

Source	Locus															
	*ADA-1**		*GPIB-2**		*IDDH-1**		*sIDHP-1**		*sMEP-1**		*sMEP-2**		Total		Grand total	
	d.f.	*G*	d.f.	*G*	d.f.	*G*	d.f.	*G*	d.f.	*G*	d.f.	*G*	d.f.	*G*	d.f.	*G*
Among Tseta, U. Nahlin, Dudidontu	2	10.509**	2		2	6.339*	2	10.322**	2	2.541	2	3.836	12	33.547***	60	91.522*
Within upper Nahlin River	1	0.906	1		1	0.511	1	0.186	1	0.001	1	0.039	6	1.643	29	32.597
Within King Salmon River	2	6.945*	2	3.671	2	9.609**	2	1.627	2	3.431	2		12	25.283*	(74) 26	46.801**
Within Stikine River	5	9.351	5		5	28.603***	5	21.723***	5	18.231**	5	2.027	30	79.935***	(185) 160	247.382***
Between upper and lower Stikine	1	5.335	1		1	22.533***	1	8.623	1	15.595***	1	0.030	6	52.116***	32	125.83***
Within lower Stikine	1	0.278	1		1	0.820	1	0.490	1	0.147	1	0.003	6	1.738	28	24.536
Within upper Stikine	3	3.738	3		3	5.251	3	12.610**	3	2.489	3	1.994	18	26.082	93	97.013
Between Shakes and Tahltan	1	1.209	1		1	0.531	1	2.185	1	0.038	1	1.012	6	4.975	30	32.898
Within Tahltan River	2	2.529	2		2	4.720	2	10.425**	2	2.451	2	0.983	12	21.108*	60	64.118
Within Unuk River	2	4.191	2		2	2.308	2	1.653	2	5.331	2	0.402	12	13.885	64	105.108***
Within Chickamin River	1	0.418	1		1	0.017	1	0.445	1	1.839	1	0.828	6	3.547	(37) 32	32.296
Within rivers	26	58.895***	26	8.201	26	93.699***	26	45.746**	26	82.941***	26	33.638	156	323.120***	962	1140.184***
Among rivers	9	849.843***	9	192.383***	9	168.845***	9	41.136***	9	245.704***	9	48.867***	54	1546.778***	333	4204.841***
Total (within + among)	35	908.738***	35	200.584***	35	262.544***	35	86.882***	35	328.645***	35	82.505***	210	1869.898***	1295	5345.024***

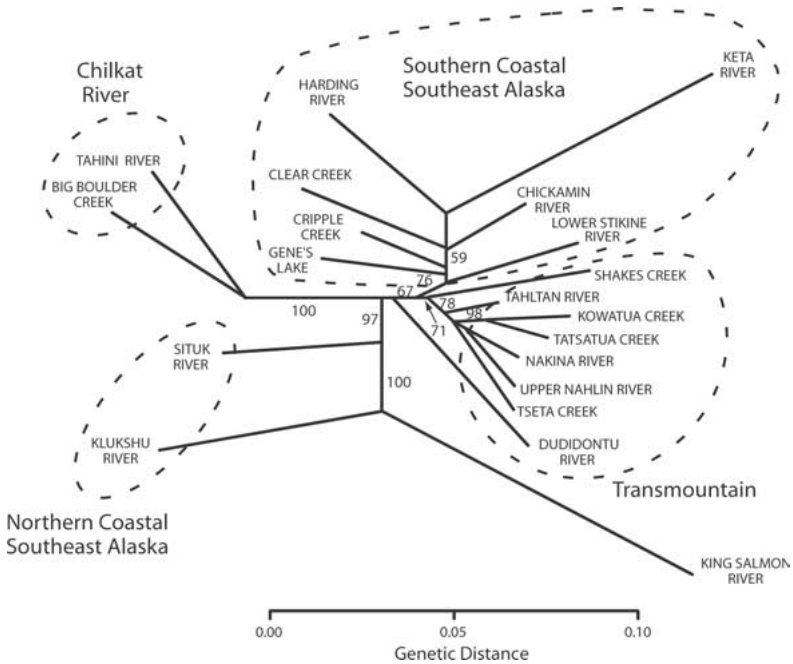

Figure 2. Neighbor-joining tree (Saitou & Nei 1987) using genetic distances (Cavalli-Sforza & Edwards 1967) estimated from allelic frequencies at 62 loci. Numbers on branches are percent consensus tree values from a bootstrap of alleles within populations.

British Columbia populations and those from the Yukon River (Wilmot et al.;[5] Wilmot unpubl. data) and other northern British Columbia populations (Teel et al. 2000). The tree illustrated eight regional groupings: (1) a coastal group which includes central coastal British Columbia (Kitimat, Atnarko, and Wannock) and southern coastal Southeast Alaska (identified from the previous tree); (2) Skeena River (Kitsumkalum, Cedar, Kitiwanga, Bulkey, Morice, Kispiox, Babine, and Bear; (3) Nass River (Damdochax and Cranberry); (4) Yukon River (North Klondike 89 & 90, McQuesten, Blind, Ross, Tatchun, Big Salmon, Little Salmon, Takhini, Stoney, Nitsutlin, and Nulato; and the four of the five previously identified regional groupings: (5) King Salmon River; (6) transmountain; (7) Chilkat River; and (8) northern coastal Southeast Alaska. Two collections are on branches outside their geographical groupings; the Lower Stikine arises on a branch with the Nass and Skeena Rivers, and the Bulkey, part of the Skeena River, is on a separate branch. Fifty percent of all nodes shared among bootstrap topologies had bootstrap values >50%. The consensus tree shared the same topology with the point estimate NJ tree on nodes with >51% bootstrap estimates.

A plot of all pairwise $N_e m$ values for central coastal British Columbia and southern coastal Southeast Alaska against geographic distance shows there is an apparent pattern of isolation by distance (Figure 4). The slope of the regression of $\log 10(N_e m)$ on $\log_{10}$(distance) is -0.299 and the regression explains 39% ($r^2 = 0.385$, $p < 0.001$) of the variation in the data. Estimation of the average numbers of migrants between rivers within drainages was about 14.5 fish per generation, whereas the number of migrants between drainages was about 3.8. Both estimates assume that the population systems are near a migration-drift equilibrium (Zhivotovsky et al. 1994).

Discussion

Descriptive analyses resolved five regional groups of chinook salmon in Southeast Alaska and northern British Columbia in this study. Four of the five groups remained intact when data from somewhat different sets of alleles were compared to data from other adjacent regions to the north and south. The clusters generally reflect geographic proximity, with several exceptions. The two northern coastal Southeast Alaska

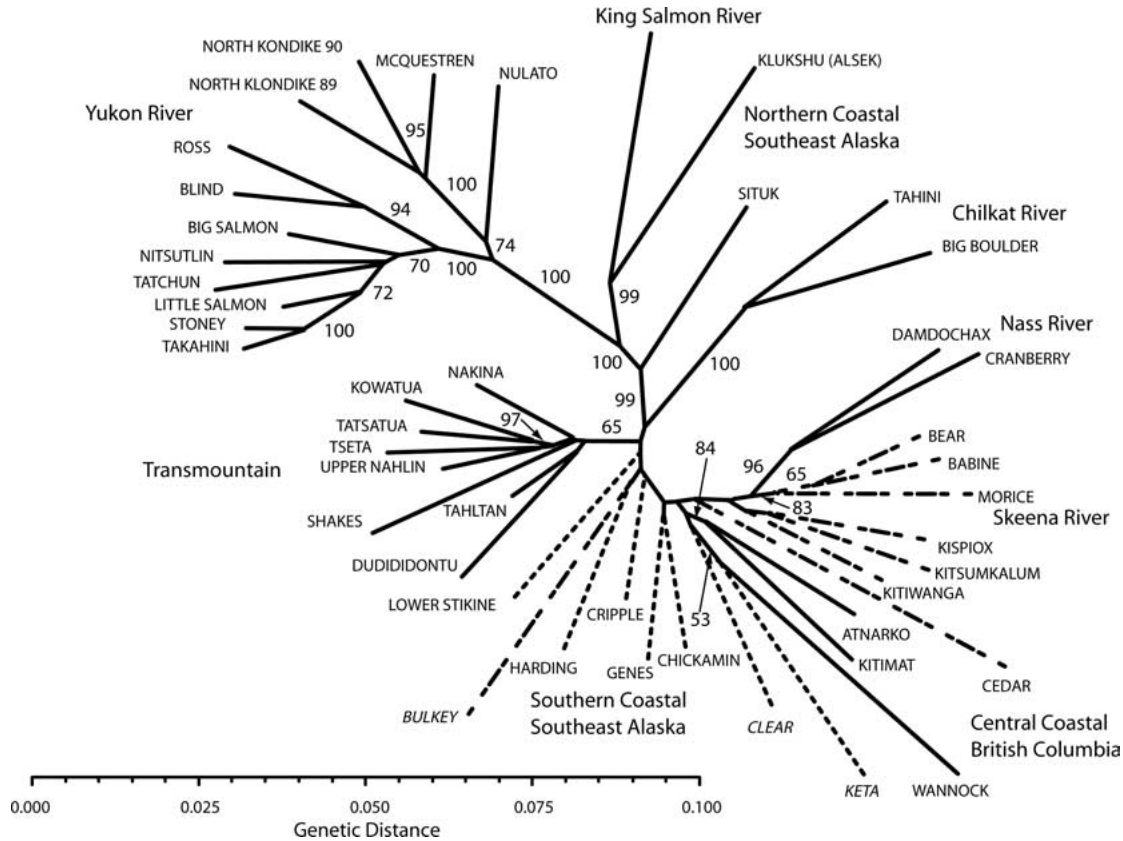

Figure 3. Neighbor-joining tree (Saitou & Nei 1987) using genetic distances (Cavalli-Sforza & Edwards 1967) estimated from allelic frequencies at 34 loci for 45 collections. Numbers on branches are percent consensus tree values from a bootstrap of alleles within populations. Collections which are italicized arise from branches outside their regional grouping.

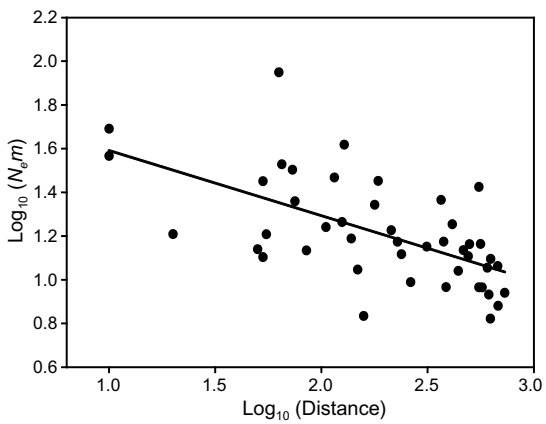

Figure 4. $\log_{10}(N_e m)$ plotted against $\log_{10}$(distance) measured in km for 10 coastal populations of chinook salmon from Southeast Alaska and central British Columbia. The linear regression equation is $\log_{10}(N_e m) = 1.892 - 0.299 * \log_{10}(\text{distance})$ with an adjusted $r^2 = 0.385$, $p < 0.001$.

collections do not form a strong cluster, which may be the result of the Situk chinook salmon having an ocean-type life-history (Johnson et al. 1992) with a relatively short distance for juvenile outmigration, whereas the chinook salmon in the Klukshu, a tributary far up the Alsek on the eastern side of the coastal mountain range, are stream-type. The King Salmon River clusters near the Klukshu River on both NJ trees (Figures 2 and 3), however they are separated by a large genetic distance. The King Salmon River which is the only significant (>100 spawners) wild island population in Southeast Alaska (Pahlke[6]), is unique in that its frequency of *GPIB-1**60 allele (58%) far exceeds the frequency in all other systems (the next highest

[6] Pahlke, K.A. 2000. Escapements of Chinook Salmon in Southeast Alaska and Transboundary Rivers in 1999, Alaska Department of Fish and Game, Division of Sport Fish, Fishery Data Series No. 00-34. 51 pp.

is 7.2% for Nakina River). The large genetic distance between the King Salmon River collection and the other collections may be a result of early run timing, unique habitat, small population size, and relative isolation from other chinook spawning areas. Wheeler and Greens creeks, drain western Admiralty Island, and may support small chinook salmon runs (Halupka et al.[7]). Although those in Wheeler Creek may be hatchery strays, there are anecdotal reports of historical Native harvests of chinook salmon at Wheeler Creek (Heard[8]). However, the wild population was probably eliminated by indiscriminate harvesting by a cannery at its mouth and its proximity to major fishing grounds (Pahlke[6]). The King Salmon River population is geographically isolated and may have escaped a similar demise.

The Chilkat River collections (Figures 2 and 3) cluster together on the NJ trees. However the large genetic distance between these two collections from this river is not unexpected because they are widely separated geographically (Figure 1). Big Boulder Creek [S], is a clearwater stream that flows into the Klehini River (about 15 km upriver from the confluence with the Chilkat mainstem) (Mecum & Kissner[9]), and the Tahini River [T] flows directly into the Chilkat mainstem ~25 km upriver from the Klehini. The southern Southeast Alaska coastal group clusters on a single branch on the NJ tree (Figure 2), but individual collections are separated by varying genetic distances. This group includes three collections from the Unuk River (Clear Creek, Gene's Lake, and Cripple Creek), which were significantly heterogeneous (Table 3) and could not be pooled, but cluster together in the descriptive analyses. The Keta River is an outlier of this group, but that could be due to the small sample size (N = 15).

The transmountain group consists of collections from the Taku and Stikine rivers east of the coastal mountain range. The genetic similarities of the transmountain group illustrated by the NJ tree (Figure 2) may have resulted from a headwater capture of the Taku River causing it to flow into the Stikine River. Two geological features of the Taku River support this idea: the Taku and Stikine rivers share the drainage of the Stikine Plateau, hence the upper reaches of these rivers lie close to each other (Kerr 1948). Other biological evidence for exchange between the Taku and the Stikine has been observed in sockeye salmon, which exhibit the same transmountain regional groupings (Guthrie et al.[10]). Kowatua and Tatstua both cluster strongly together, perhaps because they are both associated with glacial fed lakes, and have a later run timing than the other populations on the Inklin branch of the Taku River. Gharrett et al. (1987) suggested that a headwater capture (Kerr 1948, Lindsey et al. 1981) of some transmountain rivers (Taku, Stikine, Alsek [Klukshu], and Chilkat) might have been a mechanism for colonization from the Yukon River. The Alsek and Chilkat rivers do not cluster with the transmountain group in this study (Figures 2 and 3). The NJ tree (Figure 3) does not show evidence of gene flow between the Yukon River and the transmountain group. A study of mitochondrial DNA haplotypes may shed light on this possibility.

All the coastal groups (southern Southeast Alaska and central British Columbia) form a geographic cluster (Figure 3), but with large genetic distances between collections. Wannock Creek in central British Columbia is the most divergent, possibly because it is the most southern of all the collections and has ocean-type (Atnarko and Kitimat are intermediate) life-history (Teel et al. 2000). The Keta River is also divergent, but this may be due to the small collection size. The Nass and Skeena river collections, with the exception of Bulkey which was also a genetic outlier in Teel et al. (2000), clustered strongly together (Figure 3), and these populations would be inland groups as described by Teel et al. (2000). There appears to be a loose grouping of inland and coastal groups. Lower Stikine cluster with the Nass/Skeena group, but is a short distance from the coastal groups with weak topology.

The absence of chinook salmon on Prince of Wales Island in southern Southeast Alaska, which may have harbored some plant and animal populations during the

[7] Halupka, K.C., M.D. Bryant, M.F. Willson & F.H. Everest. 2000. Biological characteristics and population status of anadromous salmon in Southeast Alaska. General Technical Report PNW-GTR-468. Portland, OR, U.S. Deptartment of Agriculture, Forest Service, Pacific Northwest Research Station. 255 pp.

[8] William Heard, NMFS Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801 (pers. comm.).

[9] Mecum, R.D. & P.D. Kissner, Jr. 1989. A study of chinook salmon in Southeast Alaska, Alaska Department of Fish & Game, Fishery Data Series No. 117. 76 pp.

[10] Guthrie III, C.M., J.H. Helle, P. Aebersold, G.A. Winans & A.J. Gharrett. 1994. Preliminary report on the genetic diversity of sockeye salmon populations from southeast Alaska and northern British Columbia. U.S. National Marine Fisheries Service, AFSC (Alaska Fisheries Science Center) Processed Report 94-03, Seattle. 109 pp.

last ice age (Baichtal et al. 1997), suggests that colonization occurred from regions outside of Southeast Alaska. The overall genetic structure we observed suggests that Southeast Alaska was possibly colonized by chinook salmon from refugia to the north and the south. Their strong regional structure suggests that the Situk, Klukshu, King Salmon, and Chilkat rivers may have been populated by individual colonization events, isolated for a long period, or may have experienced several bottlenecks. Specifically, the King Salmon River may have been originally colonized via Wheeler Creek or Greens Creek on western Admiralty Island, which could explain why these fish arrive in ripe spawning condition. Both creeks may have been part of the same drainage as the King Salmon River since their headwaters are in close geographic proximity and may have been separated as the land rebounded after the last glaciation. The transmountain group may have been colonized from the south from the Nass/Skeena drainages, but has been isolated from these groups for some time. The southern coastal Southeast Alaska group may have been colonized more recently from the central coastal British Columbia populations, given the proximity of these groups on the NJ tree. Although there is genetic similarity between these two regions, there are life-history differences. The central coastal British Columbia populations have intermediate and ocean-type life histories, while the southern coastal Southeast Alaska populations have a stream-type life-history, possibly an example of the life-history plasticity in salmonids. Chinook salmon transplanted to New Zealand have shown life-history plasticity (Unwin & Quinn 1993). One could speculate that cooler northern climate may have caused the life-history change. This would be a useful adaptation to temperature regime shifts. New genetic markers (based on randomly amplified polymorphic DNA) have been developed that discriminate ocean-type and stream-type chinook salmon in the Columbia River basin (Rasmussen et al. 2003). It would be interesting to examine the southern coastal Southeast Alaska populations using these markers in future studies.

The isolation by distance analysis suggests chinook salmon in coastal areas of southern Southeast Alaska and central British Columbia area are at approximate migration-drift equilibrium with relatively high level of gene flow that is somewhat geographically restricted, and that long distance dispersal is not sufficient to prevent isolation by distance.

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References

Aebersold, P.B., G.A. Winans, D.J. Teel, G.B. Milner & F.M. Utter. 1987. Manual for Starch Gel Electrophoresis: A Method for the Detection of Genetic Variation, U.S. Department of Commerce, NOAA Technical Report NMFS 61. 19 pp.

Baichtal, J., G. Streveler & T. Fifield. 1997. The Geological, Glacial and Cultural History of Southern Southeast, Alaska Geographic, Vol. 24, No. 1, Anchorage: Alaska Geographical Society. 96 pp.

Cavalli-Sforza, L.L. & A.W.F. Edwards. 1967. Phylogenetic analysis: Models & estimation procedures. Evolution 21: 550–570.

Chakraborty, R. & O. Leimar. 1987. Variation within a subdivided population. pp. 89–120. *In*: N. Ryman & F.M. Utter (ed.) Population Genetics and Fishery Management, University of Washington Press, Seattle.

Clayton, J.W. & D.N. Tretiak. 1972. Amino citrate buffer for pH control of starch gel electrophoresis. J. Fish. Res. Board Can. 29: 1169–1172.

Gharrett, A.J., S.M. Shirley & G.R. Tromble. 1987. Genetic relationships among populations of Alaskan chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 44: 765–774.

Gharrett, A.J. & M.A. Thomason. 1987. Genetic changes in pink salmon (*Oncorhynchus gorbuscha*) following their introduction into the Great Lakes. Can. J. Fish. Aquat. Sci. 44: 787–792.

Hardy, G.H. 1908. Mendelian proportions in a mixed population. Science 28: 49–50.

Harris, H. & D.A. Hopkinson. 1976. Handbook of Enzyme Electrophoresis in Human Genetics, American Elsevier, New York. 120 pp.

Hawkins, S.L., N.V. Varnavskaya, E.A. Matzak, V.V. Efremov, C.M. Guthrie III, R.L. Wilmot, H. Mayama, F. Yamazaki &

A.J. Gharrett. 2002. Population structure of odd-broodline Asian pink salmon and its contrast to the even-broodline structure. J. Fish Biol. 60: 370–388.

Healey, M.C. 1991. Life history of chinook salmon (*Oncorhynchus tshawytscha*). pp. 311–394. *In*: C. Groot & L. Margolis (ed.) Pacific Salmon Life Histories, University of British Columbia Press, Vancouver.

Heard, W., R. Burkett, F. Thrower & S. McGee. 1995. A review of chinook salmon resources in Southeast Alaska and development of an enhancement program designed for minimal hatchery-wild stock interaction. pp. 21–37. *In*: H.L. Schramm, Jr. & R.G. Piper (ed.) 15th International Symposium and Workshop on Uses and Effects of Cultured Fishes in Aquatic Ecosystems, Albuquerque, NM (U.S.A.), 12–17 Mar 1994, American Fisheries Society, Bethesda.

Holmes, R.S. & C.J. Masters. 1970. Epigenetic interconversion of the multiple forms of mouse liver catalase. FEBS Lett. 11: 45–48.

IUBMBNC (International Union of Biochemistry and Molecular Biology, Nomenclature Committee). 1992. Enzyme Nomenclature 1992, Academic Press, Orlando, Florida. 864 pp.

Johnson, S.W., J.F. Thedinga & K.V. Koski. 1992. Life history ofjuvenile ocean-type chinook salmon(*Oncorhynchus tshawytscha*) in the Situk River, Alaska. Can. J. Fish. Aquat. Sci. 49: 2621–2629.

Kerr, F.A. 1948. Taku River map-area, British Columbia. Geol. Surv. Can. Mem. 248. 84 pp.

Lindsey, C.C., K. Patalas, R.A. Bodaly & C.P. Archibald. 1981. Glaciation and the physical, chemical, and biological limnology of Yukon lakes. Can. Tech. Rep. Fish. Aquat. Sci. 966: 37 pp.

Markert, C.L. & I. Faulhauber. 1965. Lactate dehydrogenase isozyme patterns of fish. J. Exp. Zool. 159: 319–332.

Rassmussen, C., C.O. Ostberg, D.R. Clifton, J.L. Holloway & R.J. Rodriguez. 2003. Identification of a genetic marker that discriminates ocean-type and stream-type chinook salmon in the Columbia River basin. Trans. Am. Fish. Soc. 132: 131–142.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43: 223–225.

Ridgway, G.J. & S.W. Sherburne & R.D. Lewis. 1970. Polymorphisms in the serum esterases of Atlantic herring. Trans. Am. Fish. Soc. 99: 147–151.

Roff, D.A. & P. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms: Π^2 and the problem of small samples. Mol. Biol. Evol. 6: 539–545.

Saitou, N. & M. Nei. 1987. The neighbor-joining method: A new method for constructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425.

Schaal, B.A. & W.W. Anderson. 1974. An outline of techniques for starch gel electrophoresis of enzymes from the American oyster (*Crassostrea virginica*) Gmelin. Georgia Mar. Sci. Cent. Tech. Rep. 74-3.

Shaklee, J., F. Allendorf, D. Morizot & G. Whitt. 1990. Genetic nomenclature for protein-coding loci in fish. Trans. Am. Fish. Soc. 119: 2–15.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47: 264–279.

Sokal, R.R. & F.J. Rohlf. 1981. Biometry, 2nd edition, W.H. Freeman, San Francisco. 859 pp.

Teel, D.J., G.B. Milner, G.A. Winans & W.S. Grant. 2000. Genetic population structure and origin of life-history types in chinook salmon in British Columbia, Canada. Trans. Am. Fish. Soc. 129: 194–209.

Unwin, M.J. & T.P. Quinn. 1993. Variation in life history patterns among New Zealand chinook salmon (*Oncorhynchus tshawytscha*) populations. Can. J. Fish. Aquat. Sci. 50: 1168–1175.

Waples, R.S. 1990. Temporal changes of allele frequency in Pacific salmon: Implications for mixed-stock fishery analysis. Can. J. Fish. Aquat. Sci. 47: 968–976.

Weir, B.S. & C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 28: 97–159.

Zhivotovsky, L.A., A.J. Gharrett, A.J. MacGregor, M.K. Glubokovsky & M.W. Feldman. 1994. Gene differentiation in Pacific salmon (*Oncorhynchus* sp.): Facts and models with respect to pink salmon (*O. gorbuscha*). Can. J. Fish. Aquat. Sci. 51(Suppl. 1): 223–232.