NPAFC

Doc. No. 494

Rev. No. _____

A Summary of Genetic Stock Identification Research Conducted at the Pacific Biological Station, Canada

by

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submitted to the

NORTH PACIFIC ANADROMOUS FISH COMMISSION

by

CANADA

September 2000

This paper may be cited in the following manner:
Noakes, D.J. and T.D. Beacham. (NPAFC Doc. No. 494). 18 p. A summary of genetic stock identification research conducted at the Pacific Biological Station, Canada. Fisheries and Oceans Canada, Science Branch - Pacific Region, Pacific Biological Station, Nanaimo, B.C. Canada. V9R 5K6

Abstract

The genetics lab at the Pacific Biological Station has developed genetic tools and baseline information for Pacific salmon and other species to address stock identification problems and other issues. To date, 22,000 coho salmon *(Oncorhynchus kisutch)* representing 140 stocks from British Columbia, Washington, and Alaska have been evaluated at 8 microsatellite and 2 Mhc (major histocompatibility complex) loci. Twenty-three thousand (23,000) chinook salmon (O. tshawytscha) from 140 stocks from British Columbia, California, Washington, and Oregon have been analyzed using 13 microsatellite loci. In addition, 16,000 sockeye salmon (O. nerka) for 90 British Columbia stocks have been analyzed at 14 microsatellite loci, with variation at two Mhc loci currently being surveyed. Genetic markers and baseline information have also been developed for 4 other species of salmon and trout, 8 species of marine fish, and 3 species of marine invertebrates. Simulation studies have demonstrated that high levels of accuracy and precision are possible in the analysis of samples obtained from mixed-stock fisheries. Other applications of this technology include the definition of evolutionary significant units and the selection and improvement of broodstock for enhancement or aquaculture ventures.

Introduction

The genetics lab at the Pacific Biological Station has been a leader in developing and applying DNA – based tools for stock identification and other purposes. Applications have been developed for seven species of salmon and trout, eight marine fish species, and three species of marine invertebrates. Species currently under investigation include coho salmon *(Oncorhychus kisutch)*, chinook salmon *(O. tshawytscha)*, sockeye salmon *(O. nerka)*, chum salmon *(O. keta)*, Atlantic salmon *(Salmo salar)*, steelhead trout *(O. mykiss)*, cut-throat trout *(S. clarki)*, Atlantic cod *(Gadus morhua)*, Pacific herring *(Clupea harengus pallasi)*, Pacific ocean perch *(Sebastes alutus)*, yelloweye rockfish *(Sebastes ruberrimus)*, quillback rockfish *(Sebastes maliger)* lingcod *(Ophiodon elongatus)*, sablefish/blackcod *(Anoplopoma fimbria)*, eulachon *(Thaleichthys pacificus)* northern abalone *(Haliotis kamtschatkana)*, red sea urchin *(Strongylocentrous franciscanus)*, and geoduck clam *(Panope abrupta)*.

Areas of application of DNA technology include:

- determination of population structure;
- stock identification in mixed-stock fisheries
- escapement enumeration;
- evaluation of biodiversity and the identification of evolutionary significant units;
- evaluation, supplementation or replacement of coded-wire tagging;
- forensic identification of confiscated samples to species and/or stock;
- identification of escaped cultured salmon;
- pedigree analysis; and
- selection and improvement of broodstocks.

The DNA markers or probes employed are minisatellite or microsatellite loci depending on the length of the DNA markers employed. Typically, a number of genetic markers are required to provide sufficient accuracy and precision. Unique to the genetics lab at the Pacific Biological Station is the use of genetic markers associated with the major histocompatibility complex (Mhc). The Mhc genes have proved useful for stock identification purposes for some species including Pacific salmon. The Mhc genes have also been linked to disease resistance in fish and research into their function may result in improved fish health including the development of new vaccines.

Coho salmon

To date, approximately 22,000 coho salmon from 140 stocks from British Columbia, Washington, and Alaska have been analyzed and included in our baseline database. Eight microsatellite loci and 2 Mhc loci markers are used to identify salmon to the stock level. Approximately 15,000 coho have been surveyed in mixed-stock fishery samples. Current applications associated with coho salmon include mixed-stock fisheries analysis and escapement enumeration including the determination of Canadian versus US origin fish from ocean fisheries (Shaklee et al. 1999; Small et al. 1998). Specific applications are also being conducted on Fraser and Skeena river coho salmon in an effort to conserve stocks of particular concern such as Thompson River and upper

Skeena River coho (Small et al. 1998). This includes forensic analysis of illegally caught fish to determine the origin of these confiscated fish.

Populations from the same geographic region tend to be genetically similar (Small et al. 1998). For example, in one study DNA samples were collected and analyzed for 34 populations of coho salmon from British Columbia (Figure 1). Stocks from southern British Columbia tended to be genetically distinct from northern coho stocks (Figure 2). There were also genetic differences between Vancouver Island and Fraser River coho stocks. In particular, Thompson River coho stocks tended to be quite distinct (Figure 2). This has allowed fisheries managers to design special conservation measures to protect Thompson River coho salmon.

The degree of genetic difference among stocks determines the accuracy and precision that can be obtained in mixed stock fisheries analysis (Figure 3). For instance, in a problem involving the analysis of samples collected from a mixture containing both Canadian and US salmon, the mean error of estimation of the Canadian portion of mixtures was less than 1% (Shaklee et al. 1999). The mean error of estimation of stock composition for stocks within British Columbia was less than 2% (Shaklee et al. 1999). Small et al. (1998) have also found that genetically distinct (unique) stocks, such as Thompson River coho, can be detected in mixtures at very low frequencies (i.e. when Thompson River coho represent only 2-5% of the sample).

Sockeye Salmon

Approximately 16,000 sockeye salmon from 90 British Columbia stocks have been analysed using 14 microsatellite loci. Additional variation is being analyzed at two Mhc loci. Four thousand sockeye salmon have been analyzed in mixed-stock fishery samples. Samples from mixed stock fisheries have been used to estimate stock compositions for Nass River (Beacham and Wood 1999), Barkley Sound (Beacham et al. 2000), Skeena River (Beacham et al in press), and Fraser River fisheries. In each case, accurate and precise estimates of stock composition were obtained. Simulation modelling was employed to investigate the performance of this technology in there instances.

DNA technology has also been employed to examine the genetic diversity of sockeye salmon within the Fraser River system (Withler et al. 2000). A landslide at Hell's Gate (Figure 4) in the Fraser River occurred in 1913 and many upper Fraser stocks were adversely affected (Ricker 1950, 1987). There have been concerns that genetic bottlenecks have been created as a result of the 1913 rock slides. Also, a number of attempts have been made to re-establish sockeye salmon runs by transplanting eggs or young fish from healthy stocks to stocks that were severely depressed by the landslide (Aro 1979; Williams 1987). The results are of interest from both a biological and fisheries management perspective.

The genetic composition of 29 Fraser River sockeye salmon stocks (Figure 4) were examined using six microsatellite loci (Withler et al. 2000). As with other species of Pacific salmon, stocks from the same geographic area tended to be genetically similar (Figure 5). This should allow for accurate and precise estimates of stock composition for mixed stock fisheries. It may also be possible to obtain estimates of escapement to the various Fraser tributaries by combining genetic analysis with in-river hydroacoustics.

There was some genetic evidence that transplants within the Fraser system (Figure 4) had in part been successful (Withler et al. 2000). In general, such transfers have not been successful in establishing self-sustaining anadromous populations (Withler 1982; Wood 1995). An examination of rare alleles did not indicate significant genetic bottlenecks as a result of the 1913 landslide

(Withler et al. 2000). Also, it appears that Fraser River sockeye are descendents of at least two postglacial races (Withler et al. 2000).

Steelhead Trout

Twenty-two (22) populations of steelhead trout from British Columbia, Washington State, and the Columbia River have been genetically catalogued using 8 microsatellite loci (Figure 6). As with other species of *Oncorhynchus,* there was a strong geographic grouping of genetically similar stocks (Figure 7). Bootstrap estimates of stock mixtures suggest that high levels of accuracy and precision may be achieved in practice (Beacham et al. 1999). Analysis of samples from mixed stock fisheries off the West coast of Vancouver Island found that Canadian and US origin fish could be identified successfully with a high degree of confidence (Beacham et al. 1999).

Chinook and Chum

Genetic stock identification work has been conducted using minisatellite DNA variation for both chinook (Beacham et al. 1998) and chum (Beacham 1996) salmon. Stock identifcation work using microsatellite markers is currently being conducted for both species. In particular, 23,000 chinook salmon from 140 BC stock have been analyzed using 13 microsatellite loci and have been added to our baseline data. An examination of minisatellite DNA markers for 28 stocks of chinook salmon (Figure 8) indicated that reasonably accurate and precise estimates of stock composition may be possible (Figure 9). The dicriminating power of the technique improved when larger geographic areas were considered as discreet classes (Beacham et al. 1996). A similar analysis was conducted using 42 stocks of chum salmon from Canada, United States, Russia, and Japan (Beacham 1996). Genetic differences were observed both within and between geographic regions (Table 1 and Figure 10). The results suggest that reasonably accurate and precise estimates of stock composition could be achieved using minisatellite technology (Beacham 1996). Preliminary results from our microsatellite DNA work suggest that this approach may result in more accurate and precise results for both chinook and chum salmon.

Summary

Analysis of genetic variation in fish populations has proven to be useful for stock identification and other applications. This technology is particularly useful for studying wild populations of salmon and other marine fish where conventional tagging is either impossible or impractical. Variations at microsatellite or Mhc loci show particular promise providing both increased accuracy and precision. Geographical distinct stocks also tend to be genetically distinct and this information can be used in management decisions. Also, using a restricted baseline sample such as examining only hatchery fish significantly improves the power of these techniques. Further work to expand our baseline information and to develop markers that allow a higher degree of accuracy and precision is progressing.

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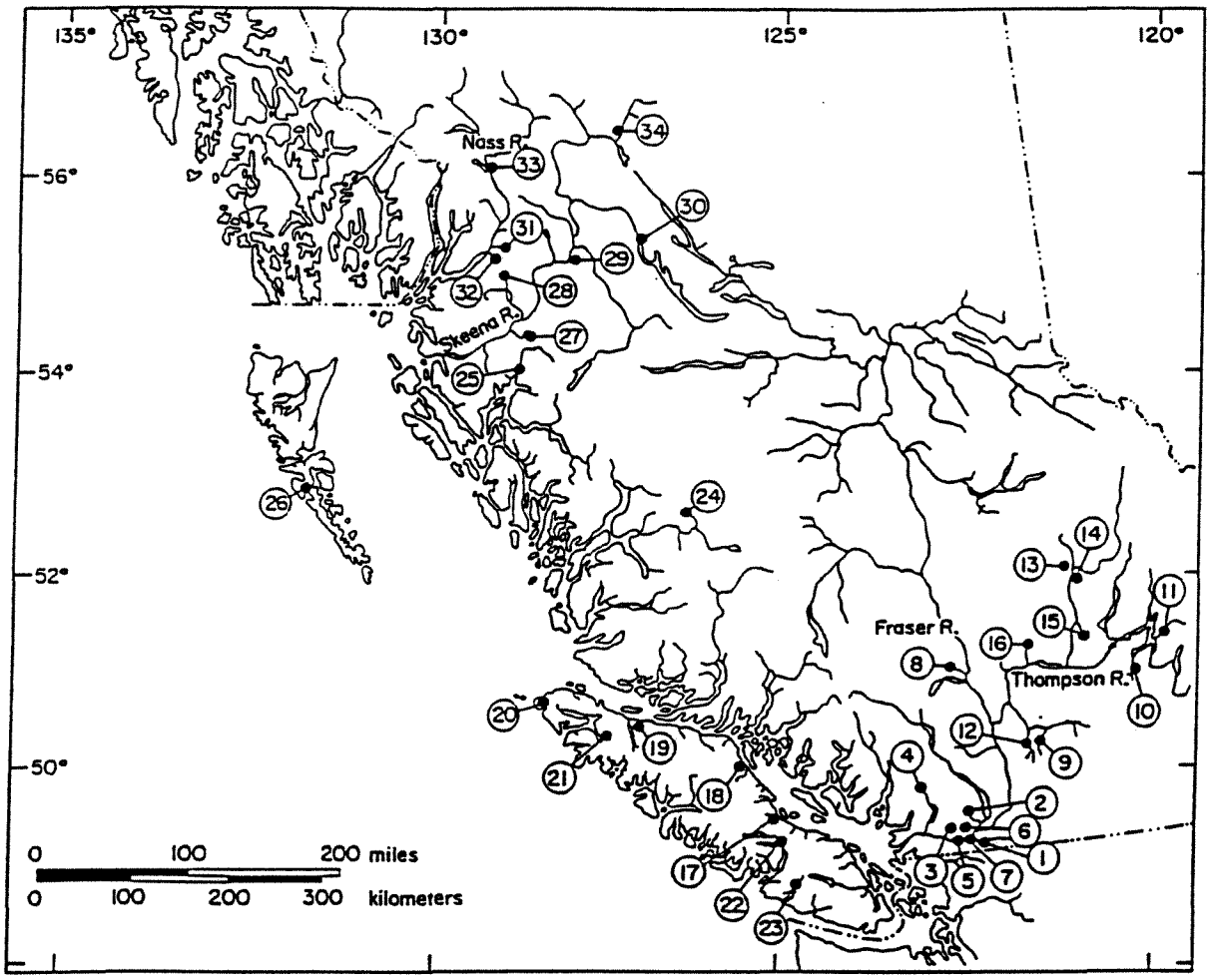

Figure 1: Map of British Columbia showing locations of coho salmon samples. Population numbers are placed near the waterway (region or river system name in bold type precedes population names): **lower Fraser River** 1) Chilliwack River; 2) Chehalis River; 3) Stave River; 4) Upper Pitt River; 5) Nicomen Slough; 6) Norrish Creek; 7) Inch Creek; **upper Fraser River** 8) Bridge River; **Thompson River** 9) Coldwater River; 10) Salmon River; 11) Eagle River; 12) Spius Creek; 13) Lemieux Creek; 14) Dunn Creek; 15) Louis River; 16) Deadman River; **East Coast Vancouver Island** 17) Big Qualicum River; 18) Quinsam River; **North Coast Vancouver Island** 19) Cluxewe River; 20) Stephens Creek; 21) Waukwaas Creek; **West Coast Vancouver Island** 22) Robertson Creek; 23) Nitinat River; **Central Coast** 24) Atnarko River; 25) Kitimat River; **Queen Charlotte Island** 26) Pallant Creek; Skeena River 27) Clearwater River; 28) Cedar River; 29) Toboggan Creek; 30) Babine River; 34) Sustut River; Nass River 31) Tseax River; 32) Zolzap River; 33) Meziadin River.

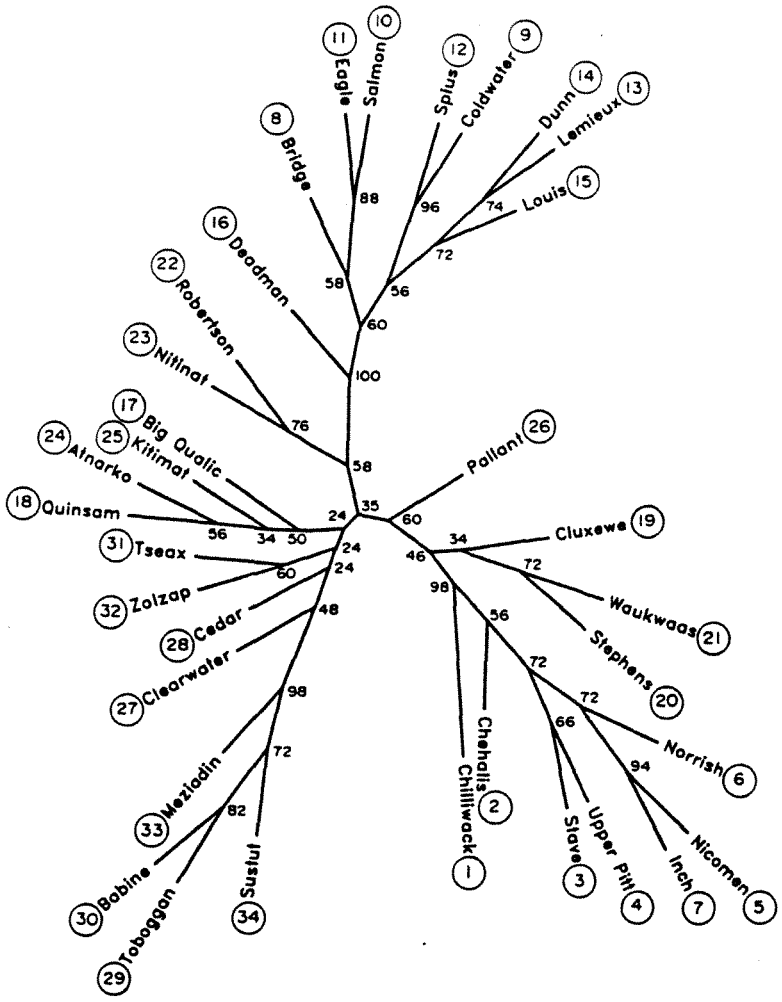

Figure 2: Unrooted neighbour-joining tree relating 34 British Columbia coho salmon populations. The tree was constructed from a consensus of Cavalli-Sforza and Edward's chord distances. Bootstrap values at the tree nodes were computed over 500 replications by resampling the allele frequencey matrix. Bootstrap values indicated the percentage of trees in which the populations beyond the node occurred together. All names correspond to population names given in **Figure 1** with the exception of Big Qualicum River which is shortened to Big Qualic. The number of the population (from **Figure 1**) is next to the name.

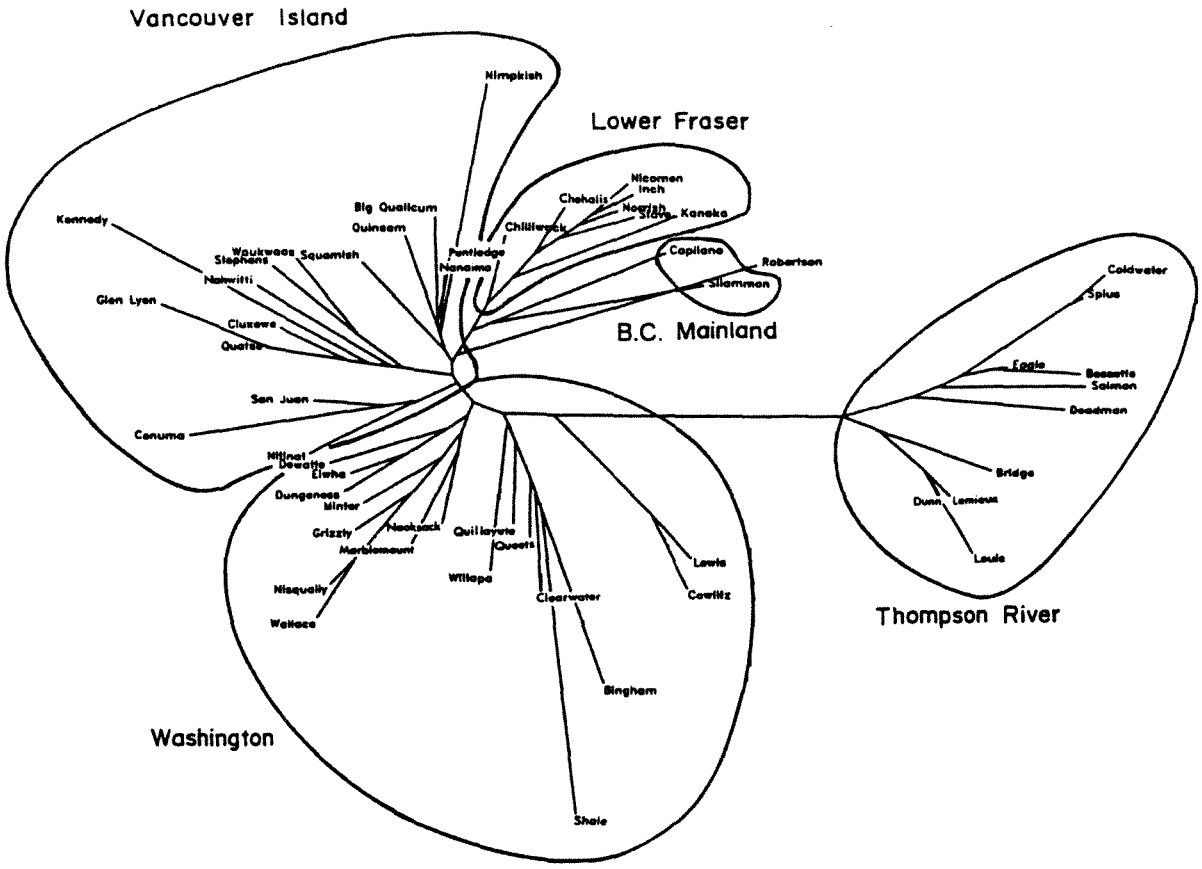

Figure 3: Neighbour-joining dendrogram based on Cavalli-Sforza and Edwards (1967) chord distance of 53 coho salmon populations from southern British Columbia and Washington State.

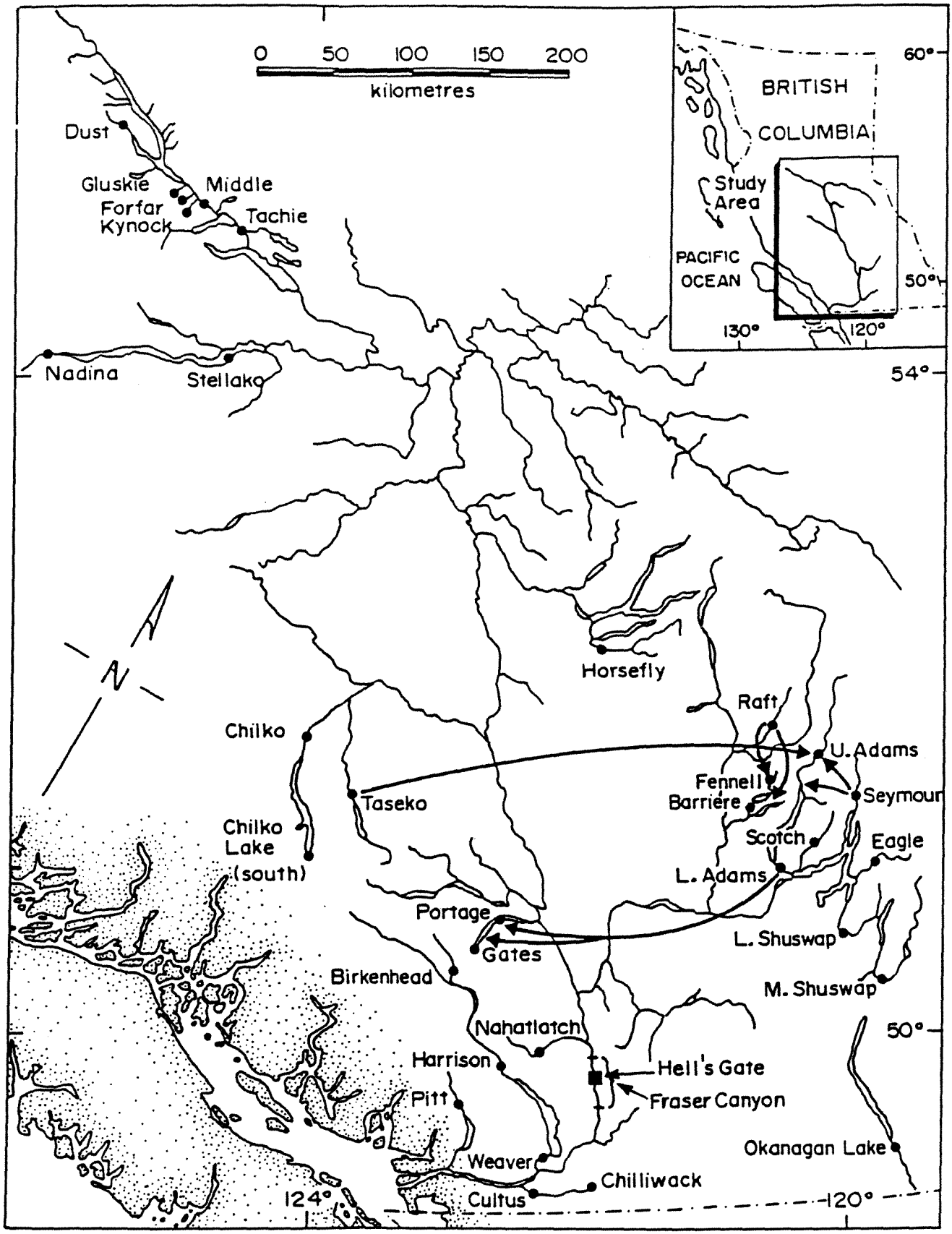

Figure 4: Location of sockeye salmon populations sampled within the Fraser River drainage. Donor and recipient populations involved in three apparently successful transplants of sockeye salmon within the Upper Fraser River drainage are also shown (arrows).

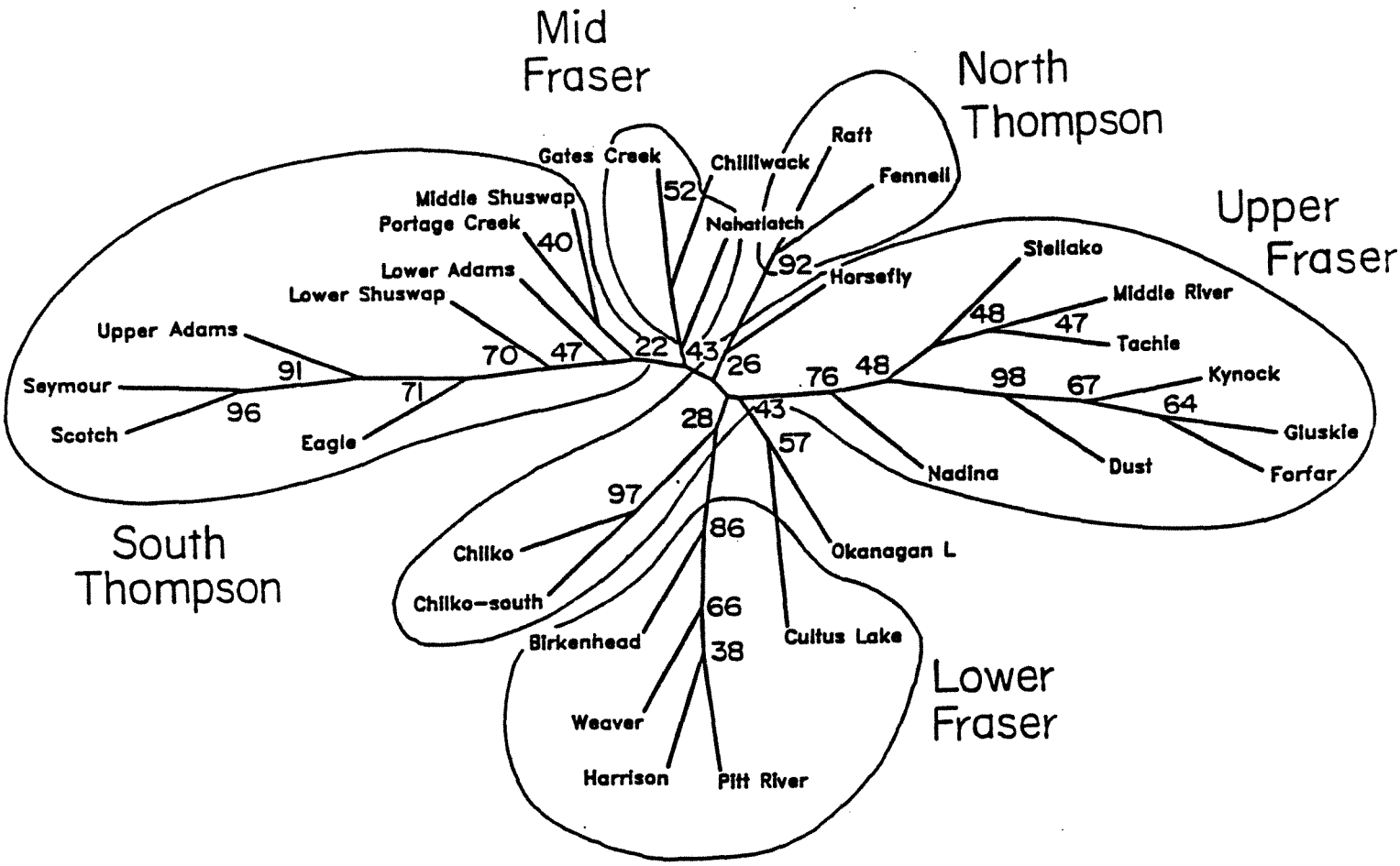

Figure 5: Unrooted neighbour-joining tree outlining relationships of 29 Fraser River and one Columbia River sockeye salmon populations. The values at the three nodes indicate the percentage of 500 trees (from bootstrap analyses) in which populations beyond the node occurred together.

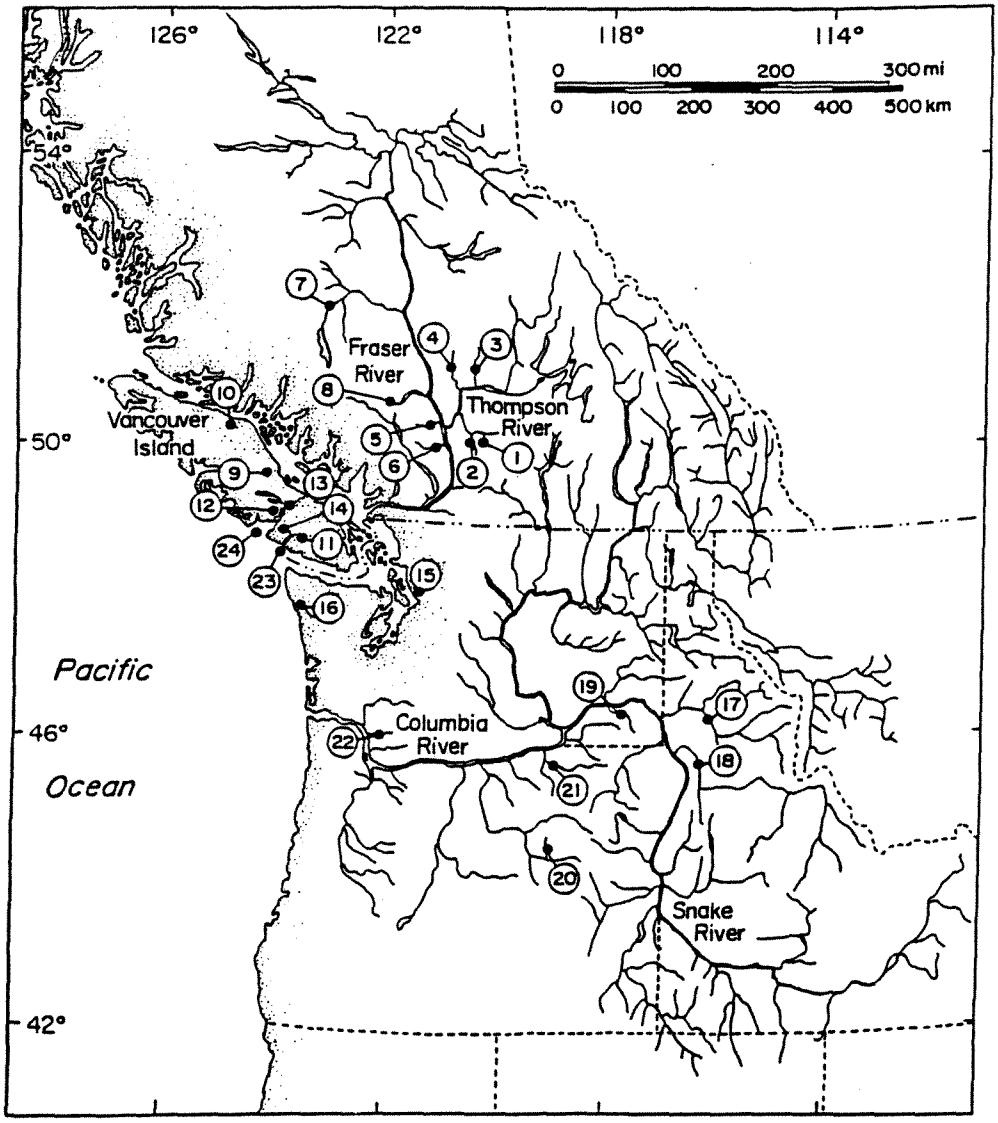

Figure 6: Location of steelhead populations and commercial salmon fisheries sampled in the survey: 1) Spius Creek, 2) Coldwater River, 3) Deadman River, 4) Bonaporte River, 5) Stein River, 6) Nahatlutch River, 7) Chilko River, 8) Bridge River, 9) Puntledge River, 10) Salmon River, 11) Caycuse River, 12) Nahmint River, 13) Robertson Creek, 14) China Creek, 15) Deer Creek, 16) Boyachiel River, 17) Clearwater River, 18) Lower Salmon River, 19) Upper Tucannon River, 20) Beech River, 21) Umatilla River, 22) Kalma River, 23) Nitinat River, and 24) Barkley Sound.

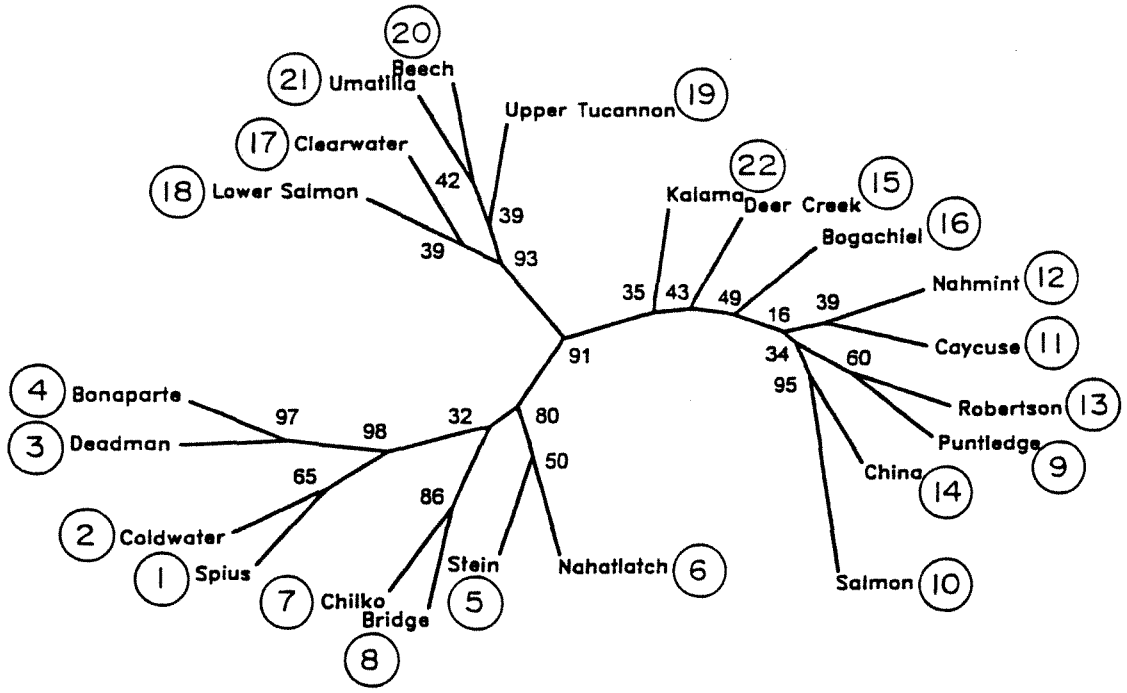

Figure 7: Unrooted neighbour-joining tree outlining relationships of 22 steelhead populations. Bootstrap values at the tree nodes indicate the percentage of 500 trees where populations beyond the node occurred together. Numbers next to the population names correspond with the locations identified in Figure 6.

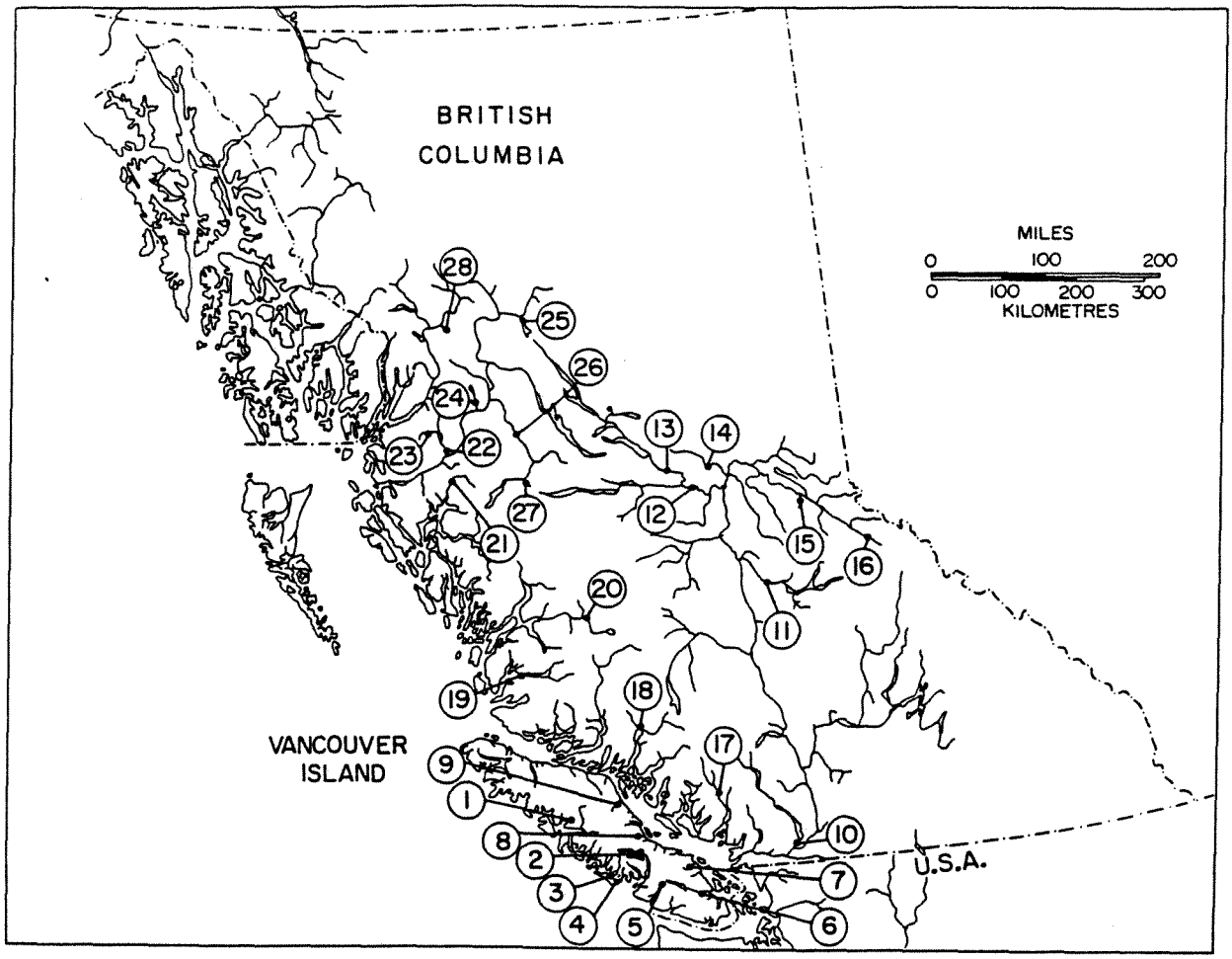

Figure 8: Locations in British Columbia where chinook salmon were sampled during 1987-1993. *West coast of Vancouver Island:* Conuma River (1), Robertson Creek (2), Kennedy River (3), Thornton Creek (4), Nitinat River (5). *East Coast of Vancouver Island:* Cowichan River (6), Nanaimo River (7), Big Qualicum River (8), Quinsam River (9). *Fraser River:* Harrison River (10), Quesnel River (11), Nechako River (12), Stuart River (13), Salmon River (14), Dome Creek (15), Fraser River at Tete Jaune (16). *Southern mainland:* Squamish River (17), Bute Inlet (18), Wannock river (19). Northern mainland: Atnarko River (20), Kitimat River (21), Skeena River: lower Kitsumkalum River (22), upper Kitsumkalum River (23), Kitwanga River (24), Bear River (25), Bulkley River (26), Morice River (27). *Nass River:* Kwinageese River (28)

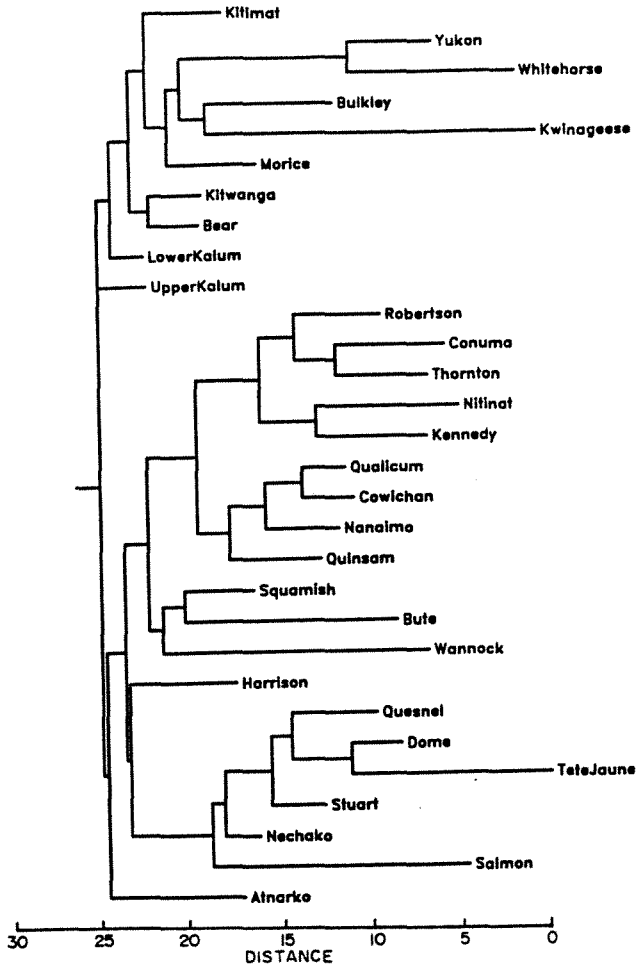

Figure 9: Neighbour-joining dendrogram of relationships among 30 chinook salmon stocks based upon a generalized chord distance (Mahalanobis) calculated from allele frequencies and band counts for the restriction enzymes and probes indicated in Fig. 2. See Fig. 1 for the geographic locations of the individual stocks.

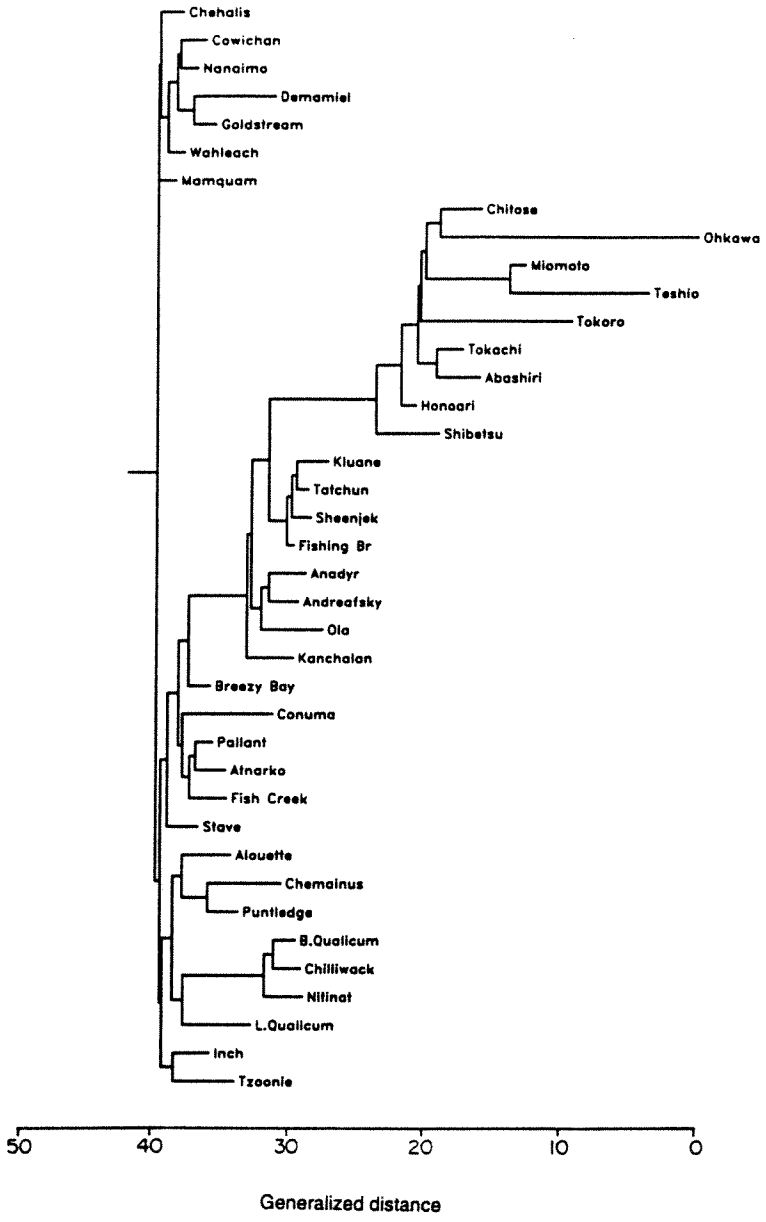

Figure 10: A neighbour-joining dendrogram of relationships among 39 stocks of chum salmon based on allele frequencies at the *Ssa*-A33 and *Ssa*-A34 loci and on band counts of occurrence for the probe Ssa1 (Beacham 1996).

Table 1: Chum salmon stocks used to investigate the utility of minisatellite DNA markers for stock identification.

Japan	Russia	Yukon River	BC/Alaska	British Columbia
Chitose	Anadyr	Andreafsky	Fish Creek	Conuma
Tokachi	Kanchalan	Kluane	Breezy Bay	Nitinat
Miomoto	Ola	Fishing Branch	Atnarko	Demamiel
Ohkawa	Kamchatka	Sheenjek	Pallant	Alouette
Shibetsu		Tatchun		Chehalis
Teshio				Chilliwack
Tokoro				Inch
Honoari				Stave
Abashiri				Wahleach
				Cowichan
				Big Qualicum
				Chemainus
				Goldstream
				Little Qualicum
				Nanaimo
				Puntledge
				Tzoonie
				Mamquam
				Sliammon
				Cheakamus