Exxon Valdez Oil Spill
Restoration Program Annual Report

Genetics of Populations of Pink Salmon Inhabiting Prince William Sound

Restoration Project 96196
Annual Report

This annual report was prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Study History: This study was submitted as a preproposal in FY 1991; it was deferred until funding was approved in FY 1994 as Restoration Project 94320D. The project continues in FY 1995 and beyond as Restoration Project 9x196.

Abstract: Allozyme and mtDNA data were collected from 16 putative populations of pink salmon spawning throughout Prince William Sound (PWS) in 1995. Sampling included nine upstream and seven tidal locations and ten early and six late collections. Seventy-one allozyme loci were screened in up to 100 fish per population. Forty loci met our stringency criteria and were used for population analyses. Forty fish per collection were screened for haplotype variation at the ND5/ND6 region using six restriction enzymes; ten haplotypes were detected. In order to maintain a balanced design, we used 14 of the collections in a statistical analysis comparing variation among streams, upstream vs tidal spawners, and early vs late spawners. Significant differences among streams were detected using both allozyme and mtDNA data. Significant differences between early and late collections were observed using allozymes in two of the three streams tested; however, mtDNA data did not detect such differences. No differences were detected between tidal and upstream collections in the four streams tested using either technique. These results support managing native populations of pink salmon in PWS on a temporal and possibly a regional level, considering local subpopulation structure, rather than as a single panmictic population.

Key Words: Allozymes, *Exxon Valdez* oil spill, mtDNA, *Oncorhynchus gorbuscha*, pink salmon, Prince William Sound, stock identification.

Project Data: (will be addressed in the final report)

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EXECUTIVE SUMMARY

- Allozyme and mtDNA data were collected from 16 aggregates of pink salmon spawning in 1995 from Prince William Sound (PWS). Collection location and timing selections were made emphasizing early-late and upstream-tidal comparisons and de-emphasizing regional comparisons. These collections were distributed throughout PWS and included locations within four of the major management regions (Southeast, East, Southwest, and Montague). Samples were collected from spawners from nine upstream and seven tidal locations which included ten early- and six late-spawning aggregations.

- We screened 71 allozyme loci from 90 to 100 fish per population; 1590 fish were analyzed. Of these loci, 40 had frequencies for alternate alleles ≥ 0.01 in at least one population and were retained for analysis.

- Haplotype data were collected from the ND5/ND6 region of mtDNA using six restriction enzymes on 40 fish per population for a total of 640 fish. Four of these enzymes yielded a total of ten haplotypes.

- We analyzed the data for genetic structure by organizing the 14 balanced collections hierarchically to test for homogeneity: among streams, within streams, between timing and between elevation within streams and between timing within elevation and between elevation within timing within streams.

- Significant differences among streams was detected using both allozyme and mtDNA data. Allozyme data detected differences between early- and late-run collections within two of the three streams tested (Koppen and Olsen); however, mtDNA data did not detect any such differences. No differences were detected between tidal and upstream samples using either allozyme of mtDNA data.

- These results support managing native populations of pink salmon in PWS on a temporal and possibly regional level, considering local subpopulation structure, rather than as a single panmictic population.

INTRODUCTION

On March 24, 1989, the supertanker *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound (PWS), Alaska, spilling 41 million liters of crude oil. The oil slick, pushed by winds and currents, moved through western PWS and the western Gulf of Alaska, contaminating approximately 2000 km of coastal habitat (see overview in Wells et al. 1995), killing thousands of sea otters *Enhydra lutris* (Garrott et al. 1993; Bodkin and Udevitz 1993) and hundreds of thousands of seabirds (Ford et al. 1991), and adversely affecting many other taxa (e.g., Barber et al. 1995; Bowman et al. 1995; Bowyer et al. 1994; Duffy et al. 1994). Sublethal effects, including reproductive impairment (Ford et al. 1991) and chromosome damage (Hose 1994), were documented. In controlled incubation, oiled substrate resulted in increased mortality of pink salmon to the eyed stage (Marty et al. *In press)*. Subsurface oil remains in some of the beaches in spite of the multi-billion dollar clean-up and restoration effort (Wolfe et al. 1994). Populations of some species including pink salmon *Oncorhynchus gorbuscha* may not be fully recovered (Craig et al. 1996).

Pink salmon is the most abundant North American species of Pacific salmon (Neave 1967; Heard 1991), making it an ecological cornerstone in biological communities of the Pacific Rim and an economic mainstay for many coastal communities. Pink salmon are both anadromous and semelparous: in their natural range, they make long oceanic migrations, home to their natal streams to spawn, and die at age two. Annual catches of pink salmon ranged from 46 to 128 million fish in Alaska alone during the period from 1985-1996.

Pink salmon, of both wild and hatchery origin, was also one of the most abundant vertebrate species inhabiting the spill area. Historically, wild populations produced approximately five-hundred million pink salmon fry which emerged from streams throughout PWS each year to migrate seaward. Adult returns from these juvenile migrations averaged over 10 million fish annually. These returning wild-stock adults play a critical role in the total PWS ecosystem, conveying essential nutrients and minerals from the marine ecosystem to estuaries, freshwater streams, and terrestrial ecosystems. Both juveniles and adults are important sources of food for many fishes, birds, and mammals. Wild pink salmon also play a major role in the economy of PWS because of their contribution to commercial, sport, and subsistence fisheries in the area.

As much as 75% of wild pink salmon spawning within PWS occurs in intertidal areas (Helle et al. 1964; Roys 1971). This extensive use of intertidal areas made pink salmon susceptible to adverse effects from the oil spill. Pink salmon embryos and alevins suffered increased mortality, diminished growth, and a high incidence of somatic cellular abnormalities as a result of spawning-ground contamination and rearing in oiled areas. Elevated mortality of embryos in the oiled streams continued through 1993, three generations after the oiling, implicating genetic damage (Bue et al. 1996). Also in 1989, the commercial harvest of pink salmon was shifted away from the hatchery and wild stocks in the oiled areas to target the wild stocks in eastern PWS (Geiger and Savikko 1990). This resulted in over-harvest and depletion of

these stocks evidenced by general run failures of eastern PWS populations of non-hatchery origin in 1991 (Geiger and Savikko 1992).

An array of conservation and restoration alternatives have been proposed for "species" impacted by the *Exxon Valdez* oil spill. However, species-based proposals often do not provide the resolution needed to sustain the conservation of genetically diverse aggregates of salmon populations; it is essential to manage and restore these damaged pink salmon resources on a population basis in order to conserve between-population diversity (e.g., Cuenco et al. 1993; Waples 1995). Between-population diversity provides optimal production for species inhabiting diverse ecosystems such as PWS; highly diverse population mixes also provide a biological buffer to environmental change (droughts, floods, major earthquakes, major shifts in oceanic conditions, and other routine catastrophic events that occur in Pacific Rim ecosystems). Our goal was to examine naturally occurring genetic markers to delineate the population structure of PWS pink salmon and to provide a genetic basis for fish management.

A number of life history characteristics of pink salmon in PWS and environmental factors suggest that between population genetic diversity could exist both temporally and spatially. Temporal differences in life history exist in both the timing of returns among regions within the Sound and within some streams. Fish return earliest in the Northeast portion of PWS and later in the Southwestern portion (Rugolo 1984). Within some streams, the numbers of fish entering over time is bimodally distributed (Helle et al. 1964; Wilcock, ADF&G Cordova per. com.) while the distribution is unimodal in others (Wilcock, ADF&G Cordova pers. com.). Spatial differences in the environment can be observed in the upstream and intertidal zones. Helle et al. (1964) found salt concentrations up to 9ppt at redd depths at the 11-foot tide level and temperature swings of 10°F within one hour at redd depths at the 8-foot tide level, well within the intertidal spawning areas of pink salmon. Upstream redds are not subject to these conditions. Lastly, pink salmon generally home to their natal streams. Selection for homing behavior can be explained by higher spawning success in natal streams than in non-natal streams. These differential success rates may be due to selection for individuals adapted to the conditions of their natal streams. For example, one condition that varies from stream to stream and region to region within the Sound is the temperature regime which is influenced by water source (glacier or rain) and stream length (Royce 1962; Sheridan 1962). This study was initiated because biological data raised questions about the genetic structure of pink salmon in PWS. Alternatively, these life history characteristics could also be the result of environmental factors, and pink salmon in PWS are actually composed of one panmictic population. For example, temporal differences in the timing of returns among regions could be due to factors such as differences in water temperature regimes between glacier- and rain-influenced watersheds.

Our objective was to test for both temporal and geographical genetic structuring among even- and odd-year classes by examining genetic differences between early- and late-season spawners, upstream and intertidal spawners, and stream-of-spawning. Additionally, genetic positioning of the local hatchery stocks within this structure was of interest because the extensive releases of pink salmon fry in PWS in recent decades may have affected the partitioning of naturally occurring genetic diversity. Some fear that hatchery production may pose as much or

more of a threat to native populations as the oil spill (see discussion in Gharrett and Smoker 1993).

Another important consideration is the fact that even- and odd-year classes have independent population structures because of the rigid two-year life cycle of pink salmon. For example, climactic, tectonic or other such events (such as the 1964 earthquake [Roys 1971] or the 1989 oil spill) may affect the population structure of one year class and cycle through subsequent generations, yet leave the alternate cycle of year-classes relatively unchanged (see data in Fetzner et al. *submitted*). Therefore, population structure and conservation strategies must be independently assessed for the even- and odd-year classes.

Two categories of molecular markers have been used extensively to define population structure of salmonids: allozymes and mitochondrial DNA (mtDNA). Allozyme analysis remains the preferred approach for study of population genetics of salmonids because of its power to resolve populations of many species in the tetraploid-derived family by assaying many nuclear loci rapidly and at low cost (Allendorf 1994). An additional advantage of allozymes is that many laboratories cooperate on inter-institutional examinations of pink salmon using this method, providing a support structure and a wealth of compatible data for potential comparisons among Pacific Rim populations (e.g., Seeb and Wishard 1977; Utter et al. 1980; Beacham et al. 1985, 1988; Gharrett et al. 1988; Shaklee et al. 1991; White and Shaklee 1991; Shaklee and Varnavskaya 1994).

The utility of mtDNA approaches to study genetic diversity of salmonid populations is controversial for reasons such as its relatively high cost and slow throughput (Allendorf 1994). Additionally, sometimes mtDNA data reveal less diversity than that detected through allozymes because mtDNA does not recombine and is maternally inherited as a single locus so that variation is absolutely linked (Smouse et al. 1994). However, haplotype data from PWS collections made in 1994 (Seeb et al. 1996) detected differences in one upstream-tidal comparison not detected with allozymes. We believed that the complementary use of the two techniques would provide optimal resolution of the population structure for this study.

In this paper we report the genetic structure of odd-year populations of wild pink salmon inhabiting four streams within PWS. After the assay of 1590 individuals from 16 collections for variation at 71 allozyme loci and assay of a subset of 40 individuals from each collection for variation at the ND5/ND6 region of mtDNA, we found genetic structuring among streams and between early and late collections.

OBJECTIVES

Our objective is to define the genetic structure of pink salmon stocks in the EVOS-affected area of PWS. In this multi-year project we will test for:

1. genetic differences between spawners from the five primary management regions within PWS (Southeast, East, North, Southwest, Montague).

2. genetic differences between spawners from different streams within PWS.

3. genetic differences between upstream and intertidal spawners within the same streams.

4. genetic relationships between hatcheries and native populations.

5. genetic differences between temporally isolated spawners within the same streams.

6. genetic differences between odd- and even-year pink lineages.

7. inheritance of newly detected isozyme variants and loci.

In this report, we review the results for the 1995 collections and address objectives 2, 3, and 5. Additionally, we report the parental genotypes from families produced to verify that putative allozyme variation is has a genetic basis. We addressed objectives 1, 2, 3, and 4 in even-year cohorts with the 1994 collections. Samples to address objective 5 in the even-year cohort were collected in 1996 and will be reported next year. Samples to address objectives 1 and 4 in odd-year cohorts were collected in 1997. The study is ongoing, and objectives 6 and 7 will be addressed in future years.

METHODS

Field Sampling

Tissues were collected from 90 - 100 individuals from each of 16 spawning aggregations from wild-stock streams 1995 (Table 1; Figure 1). Sampling incorporated intensive sampling of limited locations in order to investigate early-late and upstream-tidal differences within streams. Primary consideration was given to the sampling of tributaries that routinely support large runs of fish on both even and odd years. The limited number of streams sampled did not allow for regional comparisons (Figure 1), however these collections will be used in combination with 1997 collections to make regional comparisons within odd-year cohorts.

Within many streams in PWS, migration of pink salmon into streams has a bimodal distribution temporally. We chose three streams (Mink, Olsen, and Koppen) and sampled early in the spawning season (July 20 - August 6) and late in the spawning season (September 4 - 6) (Table 1) to test whether there is restricted gene flow between these two modes.

Finally, although a majority of pink salmon spawning in PWS occurs in areas of tidal influence, some larger tributaries also possess somewhat discrete aggregations that spawn in upstream areas, above the influence of tides. Samples were collected from both tidal and upstream sites from four of these creeks (Mink, Olsen, Koppen, and Constantine). Due to budget

restrictions, we chose to sample the late runs from three of these creeks (Mink, Olsen and Koppen) (Table 1; Figure 1). Rocky Creek was sampled in order to investigate regional comparisons and will be analyzed when we have a better representation of odd-year regional samples slated for collection in 1997. Lagoon Creek was sampled early in case we were unable, late in the season, to collect samples from the other creeks that were sampled early. The Lagoon Creek sample will also be analyzed for regional comparisons along with samples collected in 1997.

Tissue samples from heart, liver, muscle, and vitreous humor from each individual were immediately frozen on dry ice and returned to Anchorage for storage at -80°C. Subsamples were shipped to the Washington Department of Fisheries and Wildlife, Olympia, Washington, on dry ice where they were also stored at -80°C prior to allozyme analysis.

Allozyme Analysis

Genetic data were collected using the techniques of allozyme electrophoresis on all samples (Aebersold et al. 1987; Seeb et al. 1996). An extensive screening for resolution of allozyme phenotypes on 45 individuals collected in Erb Creek and Humpback Creek in 1991 and 1994, detected 78 putative loci in pink salmon within PWS (Seeb et al. 1996). Seven loci were not screened in 1995 samples due to poor resolution (*GAPDH-3*, sIDHP-1*, aMAN*, PEPB-2*, PEPD-1*, IDDH-1**) and because *mMDH-2,3** is now thought to be single locus (*mMDH-2**) rather than an isolocus (Shaklee pers. com.). The remaining 71 loci were screened for genetic variation in all 1995 collections (Table 2). Nomenclature followed the American Fisheries Society standard (Shaklee et al. 1990).

Alleles present at frequencies above 0.01 in one or more collections were retained for data analysis. Allele observations from alleles that did not meet this criterion were excluded to reduce statistical noise associated with low frequency alleles, thereby increasing our power to detect genetic structuring (see Shaklee and Varnavskaya 1994). This criteria reduced the number of loci further analyzed to 40 in the 1995 data set: *sAAT-3*; sAAT-4*; ADA-1*; ADA-2*; sAH*; mAH-3*; mAH-4*; AK*; ALAT*; CK-A1*; CK-A2*; CK-C2*; FDHG*; FH*; bGALA*, G3PDH-1*; G3PDH-2*; GDA*; GPI-A*; GR; sIDHP-2*; LDH-A1*; LGL*; sMDH-A1,2*; sMDHB-1,2*; mMEP-1*; mMEP-2*; MPI*; NTP*; PEPA*; PEPB-1*; PEPD-2*; PEPLT*; PGDH*; PGM-2*; mSOD*; sSOD-2*; TPI-2**. Loci dropped from the population analyses included: *sAAT-1,2*; mAAT-1*; mAAT-2*; mAH-1*; mAH-2*; CK-B*; CK-C1*; ESTD*; GAPDH-1*; GAPDH-2*; GAPDH-4*, GAPDH-5*; bHA*; G3PDH-3*; GPI-B1,2*; mIDHP-1*; mIDHP-2*; LDH-A2*; LDH-B1*; LDH-B2*; LDH-C*; mMDH-1*; mMDH-2*; PGK-1*; PGK-2*; sSOD-1*; TPI-1*; TPI-3*; TPI-4**. In contrast to data analysis of 1994 collections, in 1995 data analysis we excluded *sAAT-1,2*, mAAT-1*, bHA*, G3PDH-3*, GPI-B1,2*, mIDHP-1*, LDH-A2*, LDH-B2**, and *sSOD-1** and included *AK*, ALAT*, CK-A1*, CK-C2*, GPI-A*, LDH-A1*, LGL*, mMEP-2*, MPI**, and *sSOD-2** based on the same criteria.

Individual genotypic data were summarized into allelic frequencies, and tests for departure from Hardy-Weinberg were made using log-likelihood tests (modified from Weir

1990) with the experimentwise significance level set at 0.05 and adjusted for multiple tests (Rice 1989). For isoloci (*sMDH-A1,2*; sMDH-B1,2**), allele frequencies were calculated using a multinomial model assuming independence of alleles at both loci. Observed and expected heterozygosities were computed using the reduced set of loci. Paired *t*-tests were used to test for differences in heterozygosities between upstream and tidal collections and early and late collections. F_{st} values were calculated per Weir and Cockerham (1984) using the FSTAT analysis program (J. Goudet, Dorigny, Switzerland) to test for departures from zero.

S-plus analytical software (Mathsoft, Inc., Seattle WA) was used to calculate allele frequency estimates, to test for conformation of genotype frequencies to Hardy-Weinberg expected frequencies using log-likelihood ratios, and to calculate Cavalli-Sforza and Edwards (1967) genetic distance. *S-plus* was also used to perform hierarchical analyses using log-likelihood ratios to test for homogeneity within and among groups of pink salmon collections (modified from Smouse and Ward 1978). The collections were organized hierarchically to test for homogeneity: 1) among and within all streams, 2) among all collections within streams, 3a) between early and late collections within streams, 3b) between tidal and upstream collections within streams, 4a) between early and late collections within elevation (tidal-upstream) within streams, and 4b) between tidal and upstream collection within timing (early-late) within streams. For the hierarchical analysis, if an allele was observed in a collection, we assumed that it existed within all collections, potentially at an infinitely small frequency. Therefore, the degrees of freedom and log-likelihood statistics are summable, and differences among and within collection subdivisions can be examined.

For the hierarchical analysis, comparisonwise significance levels were adjusted for multiple tests using a sequential Bonferonni adjustment (modified from Miliken and Johnson 1984 and Rice 1989) with the overall experimentwise significance level set at 0.05. The first step in the analysis was a sequentially adjusted test for differences at the first hierarchical level, i.e., between streams and within streams. If a significant difference was found within streams, then a sequentially adjusted test was applied at the next level. Testing proceeded in this way through the hierarchy. If a test was not significant, then all remaining lower levels were combined, and a final sequentially adjusted multiple test of significance was performed.

Two gene diversity analyses (Nei 1973) were performed among the collections to partition variation into hierarchical levels. The first analysis partitioned variation within collections, then among collections within elevations, then between elevations within streams, then among streams. The second analysis partitioned variation within collections, then among collections within timing, then between timing within streams, then among streams. Isoloci were excluded.

We investigated genetic similarities by deriving a UPGMA tree (Sneath and Sokal 1973) with Cavalli-Sforza and Edwards (1967) genetic distance. Additionally we used multidimensional scaling (MDS, Lessa 1990) of Cavalli-Sforza and Edwards (1967) genetic distances. This ordination technique plots genetic relationships in multiple dimensions so that the plotted distances between collections closely match the observed distances in multidimensional

space. We then plotted the two most informative dimensions to examine how genetic structure separated by stream, run timing, and elevation. These calculations were performed using *S-Plus*.

Mitochondrial DNA Analysis

A subset of 40 individuals from each of the 16 collections analyzed for allozyme variation was assayed for variation at sites previously identified in the ND5/ND6 region (Fetzner et al. *submitted*). Genomic DNA was extracted using Puregene DNA isolation kits for animal tissues (Gentra Systems, Inc. P.O. Box 13159, Research Triangle, NC 27709-13159). This process included: (1) a cell lysis solution to break down cell and nuclear membranes; (2) a Proteinase K digest to denature proteins; (3) an RNase treatment to digest RNA; (4) protein precipitation to remove Proteinase K, RNase, and denatured proteins; (5) isopropanol to precipitate DNA; (6) 70% ethanol to wash DNA; and finally (7) a hydration solution to rehydrate DNA.

After extraction, DNA was amplified using the polymerase chain reaction (PCR; Saiki et al. 1988; Kocher et al. 1989). Amplified DNA was cut with six restriction enzymes found to detect haplotype polymorphisms (of the 30 screened in Fetzner et al. [*submitted*]; *Apa I, BstU I, EcoR V, Hinf I, Rsa I, Xba I*) and electrophoresed on agarose gels. Fragments were visualized under UV light, and a photographic record was made of each gel. The restriction sites detected for each enzyme were pooled as composite haplotypes for the statistical analyses.

Nucleotide (π) and haplotype (h) diversity measures (Nei 1987) were calculated for all collections using the restriction enzyme analysis package (*REAP*; McElroy et al. 1992). These measures estimate the number of nucleotide substitutions per site between DNA sequences (i.e., sequence divergence) and the amount of DNA polymorphism within collections, respectively. We also used *REAP* to calculate nucleotide divergence among collections which were used to estimate genetic relationships by deriving UPGMA tree (Sneath and Sokal 1973) and an MDS plot.

To test for heterogeneity among populations, Monte Carlo simulations with 10,000 replicates were performed (Roff and Bentzen 1989) using the REAP analysis program. Independent tests were performed to test for heterogeneity in a hierarchical manner following the levels identified in the log-likelihood analysis of the allozyme data. However, unlike the log-likelihood analysis, the χ^2 values for individual tests are not summable. Significance levels were adjusted using sequential Bonferroni techniques (Rice 1989). In order to test whether a lack of statistical power may contribute to our inability to detect differences in tests, we chose the most significant of the insignificant tests and doubled the haplotype counts while maintaining the same ratios. We then tested these doubled counts for significance using the same Monte Carlo simulations.

An analysis of the distribution of molecular variance was made using AMOVA (Excoffier et al. 1992) and utilizing a matrix of Euclidean distances between haplotypes. Pairwise Euclidean distances were calculated as the total number of site changes between haplotypes. The AMOVA analysis incorporates distance between haplotypes in the calculation

of haplotypic diversity at different hierarchical levels. Haplotype correlation measures are expressed as Φ-statistics (Excoffier et al. 1992). Among elevations, Φ_{CT} is defined as the correlation of random haplotypes within a group of collections relative to that of random pairs of haplotypes drawn from the entire set of collections. For the analysis among collections within elevations, Φ_{SC} is the correlation of random haplotypes within collections relative to that of random pairs of haplotypes from the elevations. Finally for the within-collection analysis, Φ_{ST} is the correlation of random haplotypes within collections relative to that of random pairs of haplotypes drawn from the entire set of collections. The AMOVA analysis allows for only a two-level hierarchy, so we were unable to partition timings within elevations as in the preceding analyses. Rather, we performed two separate analyses, one based on elevation and one based on timing. The significance of the observed variance components and Φ-statistics were tested using a random permutation procedure in AMOVA. The permutation approach to significance testing avoids the parametric assumptions of normality and independence that are not met by molecular distance measures (Excoffier et al. 1992). The number of permutations was set at 1000 for each analysis.

Inheritance Study

Eggs and milt from pink salmon returning to Armin F. Koernig Hatchery (AFK) were taken in 1995 to confirm genetic basis for novel variation observed in putative allozyme alleles detected in the population study. Eggs from 100 females were placed in dry reclosable 4-L bags and milt from 50 males was placed into 50ml capped centrifuge tubes and placed on wet ice. Parental tissue samples from heart, liver, muscle, and vitreous humor from each parent were numbered so they could be cross-referenced to their corresponding gametes, immediately frozen on dry ice, and stored at -80°C.

We performed crosses and incubated eggs in two locations (AFK and Anchorage) to guard against catastrophic loss. At AFK we performed 12 single-pair matings and incubated them through egg yolk absorption in Heath trays separated by family. Fry from five of these families were individually packaged by family into 4-L reclosable bags and shipped to Anchorage for rearing.

We shipped the gametes to Anchorage on wet ice. Parents were assayed for variation at the following allozyme loci to identify the most useful single-pair matings to perform: *AK*; sAAT-3*; FH*; G3PDH1*; G3PDH2*; G3PDH3*; bGALA*; bGLUA*; GAPDH2*; GDA*; IDDH*; sMDH-A1,2*; sMDHB-1,2*; mMDH-1*; mSOD*; sSOD2**. Seventeen single-pair matings were performed, and embryos were incubated in Heath trays through egg yolk absorption.

Fry from families with more than 200 surviving individuals (including the families from AFK) were transferred to 20-L circular tanks (one per family) until they were on feed (approximately one month). Fry were then shipped to Fort Richardson Hatchery where they

were raised separately by family in 70-L to approximately 6mm. One hundred fish from each surviving family were sampled and stored at -80°C.

A complete allozyme screen was performed on parents from the surviving families.

RESULTS

Allozymes

Variation was detected at 73% of the allozyme loci (52/71), although twelve polymorphic loci were dropped because alleles were present at frequencies below 0.01 in all collections (Appendix A). The screening also yielded 38 rare alleles (<0.01 in each collection) which were excluded from analyses.

Observed heterozygosities based on 40 loci varied over a relatively narrow range (mean 0.097, range 0.091 to 0.104; Table 3). No significant differences in heterozygosities were observed in using paired *t*-tests between tidal and upstream (mean tidal =0.098, mean upstream = 0.097, $t = 0.464$, df = 12, $P = 0.651$) or early and late (mean early =0.096, mean late = 0.097, $t = 0.332$, df = 10, $P = 0.747$) collections.

The overall F_{st} was significantly larger than zero ($P < 0.001$), indicating that some barriers to gene flow are present among the collections. Loci most indicative of the lack of panmixia included *sAH**, *FH**, *bHA**, and *TPI2** (Table 4).

Genotypic frequencies were tested for departures from Hardy-Weinberg (H-W) expectations. No collection had an overall deviation from H-W. We made 445 tests, of which 11 were significant when comparisonwise significance level was set to 0.05, well within the range of positive results expected. The significant deviations were spread over eight loci, and no locus deviated from H-W in more than two collections. None of these deviations was significant when adjustmented for multiple tests.

The hierarchical analysis using log-likelihood ratios detected significant differences both among and within streams (Table 5; Appendix B). Within streams, significant differences were detected between early and late runs within two (Koppen and Olsen) of the three streams tested. Within Koppen Creek, significant differences between early and late runs were evident within each elevation. No differences were detected between tidal and upstream collections either within timing or pooled for both timings in any of the four streams tested.

The MDS analysis portrayed a result similar to that obtained in the hierarchical analysis (Figure 2). Some stream-to-stream and timing structuring is apparent from the plot. The Mink Creek collections (both early and late) tend to occupy the right portion of the plot along with late collections from Olsen and Koppen creeks, while the early collections from Olsen, Koppen and Constantine creeks occupy the left portion of the plot. Koppen Creek early collections are by themselves in the upper-left side of the plot.

The UPGMA tree shows structuring similar to the MDS analysis (Figure 3). All the late collections are on one branch while all the early collections from Olsen, Koppen, and

Constantine creeks are on the other two branches. Mink Creek early, upstream collection is in the branch with all the late collections. Finally, early collections from Koppen Creek are the most divergent.

We performed two hierarchical gene diversity analyses using 40 loci. The first hierarchical analysis was stratified by collection, elevation, and stream. The second hierarchical analysis was stratified by collection, timing, and stream. In both analyses, by far the majority of the variation (99.34%) occurred within collections (Tables 6 and 7) and was heavily weighted by variation at *sAAT-4**, *GDA-1**, *sIDHP-2**, and *PEPD-2**. In the first analysis the remaining heterogeneity was divided among collections within elevations (0.34%), between elevations within streams (0.14%), and among streams (0.18%). In the second analysis heterogeneity was divided among collections within timing (0.27%), between timing within streams (0.21%), and among streams (0.18%).

Mitochondrial DNA

Forty individuals from each of the 16 collections were examined for variation at ND5/ND6 using six restriction enzymes previously identified to reveal polymorphisms in pink salmon (Fetzner et al. *submitted*; Table 8). Ten unique haplotypes were defined from 640 individuals detected with the six restriction enzymes tested (Table 9). Six of the haplotypes (III, VI, VII, XVII, XVIII, and XIX) had overall frequencies less than 0.01 (six or fewer individuals observed within all populations combined). The two rarest haplotypes, III and XVIII, were observed only once each.

Haplotype and nucleotide diversity

Haplotype diversity (h) ranged from 0.406 in Mink Creek late, tidal to 0.662 in Olsen Creek late, tidal and averaged 0.581 (Table 9). Corresponding nucleotide diversity values (π) ranged from 0.0050 to 0.0118 in the same creeks, respectively, and averaged 0.0077. No stream, timing, or elevational patterns in diversities were observed. No significant differences in the nucleotide diversities between the paired early and late collections ($t = 0.159$, df = 10, P = 0.88) or between paired tidal and upstream collections ($t = 0.929$, df = 12P = 0.371) were detected.

Heterogeneity detected by Monte Carlo tests

A Monte Carlo test among all collections (all streams, both timings, both elevations) yielded a significant test statistic (Table 5). However, no within stream tests were significant (within stream tidal vs upstream collection or early vs late collections) indicating overall homogeneity in haplotype frequencies within streams.

The only Monte Carlo test that approached significance for within stream comparisons was between early and late runs in Mink Creek (P = 0.086; adjusted critical value = 0.008; Table 5). The haplotypes with the most divergent frequencies between early and late collections for

Mink Creek were haplotype I (early = 0.36, late = 0.21) and haplotype II (early = 0.56, late = 0.71; Table 9). In order to test for lack of power in the Mink Creek early-late comparison, we doubled the number of counts while maintaining the same ratios in allele frequencies and retested using Monte Carlo simulations. We did obtain a significant result (P = 0.0012), indicating that we may simply be lacking statistical power due to small sample size (n = 40 haplotypes in mtDNA compared with allozymes where n = 200 allele counts per population).

AMOVA analyses

An AMOVA analysis that partitioned the molecular variation by elevation and by timing was also performed. Again, the majority of the variation in both analyses (99.3% for analysis by elevation and 99.0% for analysis by timing) was within collections (Φ_{ST} = 0.007 for analysis by elevation and 0.010 for analysis by timing; Table 10). However, none of the molecular variation was partitioned significantly into collections, early or late runs, or upstream or tidal locations (Table 10).

Genetic similarities among collections

A UPGMA tree and an MDS plot were generated using nucleotide divergence among collections (Tables 5 and 6). Mink Creek collections are all on one branch of the UPGMA tree; however, no patterns for timing or elevation were evident in either of these analyses. In both analyses, the late, tidal collection from Olsen Creek and the early, tidal collection from Koppen Creek were most divergent, however, Cavalli-Sforza and Edward distances were small relative to those derived from the allozyme data.

Inheritance Study

All five families incubated at AFK and shipped to Anchorage and the 12 of the 17 families incubated in Anchorage had at least 100 fish surviving to approximately 6mm in length. Four Anchorage families did not develop due to either poor egg or milt quality. One family was discarded due to uncertain parentage.

Progeny from these crosses will be analyzed in FY 1998. Parental genotypes from progeny that were sampled will enable us to investigate genetic basis for variation observed in putative allozyme alleles in: *ADA2*; AK*; sAAT3*; sAAT4*; mAH3*; sAH*; CKC2*; FDHG*; G3PDH1*; G3PDH2*; GAPDH2*; GDA*; GPIB1,2*; bGALA*; IDDH*; sIDHP2*; sMDHA1,2*; sMDHB1,2*; mMEP1*; mMEP2*; PEPB1*; PEPD2*; PEPLT*; PGDH*; PGM2*; mSOD*; sSOD2*; TPI3*; TPI4** (Appendix C). Of these loci, alleles in *AK*, sAAT3*, bGALA*, GDA*, GAPDH2*, G3PDH2*, IDDH*, mSOD**, and *sSOD2** were identified in our proposal for this project as loci with variation that has not been tested for inheritance in pink salmon.

DISCUSSION

Understanding genetic structure of Pacific salmon populations is critical to their management and conservation. For example, managing on too fine a scale may adversely affect the fishing industry and waste management resources, while managing on too large a scale may result in loss of genetic adaptations and diversity (see Mundy et al. 1993). Here we report our initial findings in an examination of the odd-year lineage of commercially important populations of pink salmon that inhabit PWS, Alaska.

Inferences from studies showing genetic homogeneity for allozymes over vast geographic distances (e.g., Shaklee and Varnavskaya 1994) lead some to suggest that pink salmon populations within PWS, spanning only 100 kilometers, should be genetically homogenous. In contrast, implications from other allozyme studies (Lane 1990) suggest that pink salmon populations in PWS might be substantially heterogeneous. Our objective was to generate molecular genetic data to support or reject these alternatives.

Three recent and major factors have impacted these populations. The *Exxon Valdez* oil spill of 1989 adversely affected pink salmon through a combination of direct lethal effects, sublethal effects, and alterations in fishing pressure (Bue et al. 1996); study of the effects of the oil spill instigated our study. Further, the major tectonic upheaval of 1964 produced bottlenecks in some populations. However, arguably one of the most serious factors influencing population structure may be deleterious effects of hatchery/wild-stock interactions and the potential erosion of locally adapted genotypes (Gharrett and Smoker 1993). PWS is the center of one of the world's largest aquacultural industries. Six-hundred million pink salmon fry of hatchery origin are released annually. Alaska Department of Fish and Game has been grappling with management of the wild populations in face of intractable hatchery/wild-stock interactions for nearly a decade. The *Exxon Valdez* oil spill-related damages to wild populations, coupled with full-scale hatchery egg takes, exacerbated wild-stock conservation concerns.

Although the differences in allele frequencies were small relative to those found in other species (e.g. sockeye; Seeb 1996), they were significant. The chance that these results are due to Type II error is low. The P-values calculated in the hierarchical analysis, a conservative analysis because all alleles observed are assumed to exist in all collections thereby inflating the degrees of freedom, were much lower than the adjusted critical values. Further, genetic distances were within the range considered biologically significant for pink salmon (Shaklee and Varnavskaya 1994). Shaklee and Varnavskaya (1994) argued that pink salmon populations from the Pacific coast of Russia represent those that evolved *in situ* because of the lack of anthropomorphic activities except harvest (no hatcheries or stock transfers). Using identical methods to ours, the genetic distances they found among populations up to 3,000 ocean km apart (0.040 to 0.055) were similar to the distances we detected among the collections within PWS no more than 70 ocean km apart (0.035 to 0.065). Shaklee and Varnavskaya (1994) concluded that the Russian collections are not part of a single, panmictic stock based on slight, but significant heterogeneity in allelic composition among the eight Russian collections.

In addition, the two patterns of genetic relationships detected among our collections are consistent with biological observations. The number of fish entering individual streams over time is strongly bimodally distributed in Koppen Creek, somewhat bimodally distributed in Olsen Creek, and unimodally distributed in Mink Creek (John Wilcock ADF&G Cordova pers. com.). The hierarchical analysis (Table 4) found the largest differences between early and late collections at Koppen Creek, followed by those at Olsen Creek, and nonexistent at Mink Creek. Previous work has also implicated run timing in limiting gene flow among populations in other salmonids including chum salmon (Wilmot et al. 1994; Phelps et al. 1994; Kondzela et al. 1994), sockeye salmon (Seeb et al. 1997), and chinook salmon (Adams et al. 1994). Significant differences among streams revealed by both allozymes and mtDNA (Table 4) are consistent with biological data that indicate that pink salmon generally home to their natal streams (Helle et al. 1964).

Alternatively, the lack of differentiation between the early and late collections at Mink Creek could be due to timing of our sampling. However, although early Mink Creek collections (western side) were made two weeks after the early collections on the eastern side of the Sound (Table 1), it is unlikely that the early run was simply missed at Mink Creek. Pink salmon appear later in Mink Creek than they do in Koppen and Olsen Creeks (John Wilcock, ADF&G Cordova pers. com.), and we sampled Mink Creek soon after the first fish started to spawn. Timing might not be a barrier to gene flow in other creeks on the western side of the Sound based on distribution patterns of fish entering streams there. Additional streams from the western Sound will be collected early and late in 1997 to test for a regional basis in differences between timings.

These data do not demonstrate restrictions to gene flow between intertidal and upstream spawners for odd-year pink salmon within the streams we tested. The lack of differences between tidal and upstream collections contrasts somewhat with our results from pink salmon collected in 1994 from PWS (Seeb et al. 1996). In 1994 collections, differences were detected between tidal and upstream collections using both the allozyme (Lagoon Creek) and mtDNA (Koppen Creek) data sets. Where differences were detected, they were large (the upstream collections were the most dispersed in the MDS analyses). However, four of the five upstream-tidal comparisons made in 1994 were not significant within each data set. Therefore, it is possible that we simply missed streams that that have upstream-intertidal heterogeneity among the four streams sampled at both elevations in 1995. Additionally, in 1994 and 1995, upstream collections were made just above the high tide zone. In 1996 and 1997, upstream collections will be made as far upstream as fish are found. This change in methodology may reveal more heterogeneity between tidal and upstream spawners in future analyses.

It is also important to note that the genes that we are looking at are probably selectively neutral. They can only tell us whether there are barriers to gene flow, not how functionally genetically different two collections are. However, if barriers to gene flow are detected in these selectively neutral genes, then it is possible that genes under selection pressure will diverge much more quickly if the environments between populations differ. Therefore small, yet significant heterogeneity in non-selected genes may underestimate the magnitude of the adaptive genetic differentiation that may be present.

We did not to detect any significant differences in mtDNA haplotype frequencies between early and late spawning collections. This result was unexpected because mtDNA is maternally inherited and therefore has a smaller effective population size leading to higher genetic drift than allozymes (Avise and Vrijenhoek 1987); further, mtDNA lacks repair mechanisms (Wilson et al. 1985) present in nuclear DNA resulting in faster mutation rates than allozymes. In addition, mtDNA can detect barriers to gene flow in cases where only males stray which would be missed by nuclear markers (Melnick and Hoelzer 1992). Three hypotheses might explain our inability to detect differences with mtDNA when they were detectable using allozymes: higher straying rates in females than in males, bottlenecks or extinctions and recolonizations, or lack of statistical power. Higher straying rates in females could homogenize mtDNA allele frequencies because of strict maternal inheritance, while allozyme heterogeneity might be maintained if males stray little (Allendorf 1994). However, evidence from coded wire tag data indicates that straying rates of pink salmon in PWS is similar for males and females (Habicht, unpublished data). Other studies have observed low mtDNA variation in populations with high allozyme variation and have attributed these results to historical bottlenecks or extinction and subsequent recolonizations (reviewed in Allendorf 1994). MtDNA haplotypes in this study were variable; we found ten haplotypes of which three had frequencies greater than 5% (Table 9). Lastly, the lack of significant tests in the mtDNA data analysis could be due to reduced statistical power resulting from the lower allele counts observed per population using mtDNA, at this single locus. We analyzed 40 fish per population for mtDNA data which translates to 40 haplotype counts per population; conversely, we analyzed 100 fish per population using allozymes which translates to 200 allele counts per locus, with 40 different loci analyzed. Our power analysis using Monte Carlo simulations on double the haplotype counts for the Mink Creek early vs. late comparison indicated that small sample sizes may have been responsible for the insignificant tests. Furthermore, when we increased the number of samples analyzed from the 1994 samples from 40 to 100 in three collections, two of the three pair-wise comparisons that were previously insignificant became significant (P-values changed from 0.0490 to 0.0039, 0.0292 to 0.0135, and 0.0155 to 0.0033: critical values adjusted for multiple tests = 0.005).

Our objectives were to test for barriers to gene flow as a result of regional, elevational, or temporal isolation. Although our objectives did not include developing management strategies to preserve observed heterogeneity, our results can be and have been incorporated into the management of pink salmon within PWS to conserve some of the heterogeneity we have uncovered. Managers of the resource are eager to use information on population structure in guiding their management strategies (James Brady, Regional Manager, ADF&G Anchorage, pers. comm.). For example, these data provide managers with the evidence to discard the hypothesis that pink salmon in PWS are a single interbreeding population as has been suggested by hatchery operators. Based on our data, this fishery would best be managed on as fine a scale as possible. Given the financial constraints on the Department, our study upholds their current management strategy of trying to meet escapement goals throughout the season assessed on a region-by-region basis. It also validates concerns managers have regarding specific pink salmon runs within the Sound. For example, managers are concerned about pink salmon returns to the

small Coghill district in Northwestern PWS where fisheries targeting hatchery returns to Ester Hatchery are suspected of intercepting wild fish bound for the Coghill district.

In addition to fishery management actions, these data also have application in the assessment of fish transport permits. For example, these data can be used to support recommendations on fish transport requests such as changing hatchery broodstocks, transplanting stocks within the Sound, or supplementing streams.

Although these data show that the odd-year lineage of pink salmon in PWS has a shallow genetic structure relative to other salmonids (in contrast to the structure of sockeye salmon populations from a similar geographic range in Cook Inlet, Alaska, for example; Seeb et al. 1997), barriers to gene flow exist. The commercial harvest of pink salmon fluctuated dramatically between six and 44 million fish during the years since the oil spill. The instability of the run size is due to an array of anthropogenic and natural factors. Maintenance of genetic diversity will play a key role in ameliorating the affects of this instability. Our data confirm that harvest- and hatchery-management decisions made for conservation purposes should best be made on a population-specific rather than species-specific basis. Expansion of this study to include additional odd-year collections as well as comparisons to even-year collections is continuing; the analysis of data from multiple year classes will allow us to better test the appropriateness of current management and hatchery practices.

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Table 1. Pink salmon collected from Prince William Sound in 1995. Map numbers refer to Figure 1. All fish were screened for allozyme variation. Forty fish from each collection were screened for mtDNA variation.

Sample #	Map #	Location name	Elevation	Region	Sample Date	N
1	1	Rocky Creek	upstream	Montague	8/9	100
2	2	Mink Creek	tidal	North	8/6	100
3	2	Mink Creek	upstream	North	8/6	100
4	2	Mink Creek	tidal	North	9/6	90
5	2	Mink Creek	upstream	North	9/6	100
6	3	Lagoon Creek	upstream	East	7/23	100
7	4	Olsen Creek	tidal	East	7/20	100
8	4	Olsen Creek	upstream	East	7/20	100
9	4	Olsen Creek	tidal	East	9/5	100
10	4	Olsen Creek	upstream	East	9/5	100
11	5	Koppen Creek	tidal	East	7/21	100
12	5	Koppen Creek	upstream	East	7/21	100
13	5	Koppen Creek	tidal	East	9/4	100
14	5	Koppen Creek	upstream	East	9/4	100
15	6	Constantine Creek	tidal	Southeast	7/22	100
16	6	Constantine Creek	upstream	Southeast	7/22	100

Table 2. Enzymes, loci, and primary tissue-buffer combinations used to screen for allozyme variation in 1995. Enzyme nomenclature follows Shaklee et al. (1990), and locus abbreviations are given.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
Aspartate aminotransferase	2.6.1.1	*sAAT-1,2**	Heart	ACEN 6.8
		*sAAT-3**	Eye	TG
		*sAAT-4**	Liver	TG
		*mAAT-1**	Heart	ACEN 6.8
		*mAAT-2**	Muscle	ACE 6.5
Adenosine deaminase	3.5.4.4	*ADA-1**	Muscle	AC 6.1
		*ADA-2**	Muscle	AC 6.1
Aconitate hydratase	4.2.1.3	*mAH-1**	Heart	ACEN 6.8
		*mAH-2**	Heart	ACEN 6.8
		*mAH-3**	Muscle	ACE 6.8
		*mAH-4**	Muscle	ACE 6.8
		*sAH**	Liver	ACEN 6.8
Adenylate kinase	2.7.4.3	*AK**	Muscle	TG
Alanine aminotransferase	2.6.1.2	*ALAT**	Muscle	TG
Creatine kinase	2.7.3.2	*CK-A1**	Muscle	TG
		*CK-A2**	Muscle	TG
		*CK-B**	Eye	TG
		*CK-C1**	Eye	TG
		*CK-C2**	Eye	TG
Esterase-D	3.1.1.-	*ESTD**	Muscle	ACE 6.5
Formaldehyde dehydrogenase	1.2.1.1	*FDHG**	Heart	ACEN 6.8
Fumarate hydratase	4.2.1.2	*FH**	Muscle	ACE 6.8
B -N-Acetylgalactosaminidase	3.2.1.53	*BGALA**	Muscle	TG
Glyceraldehyde-3-phosphate	1.2.1.12	*GAPDH-1**	Muscle	AC 6.1
		*GAPDH-2**	Heart	ACEN 6.8
		*GAPDH-4**	Heart	ACEN 6.8
		*GAPDH-5**	Heart	ACEN 6.8
Guanine deaminase	3.5.4.3	*GDA**	Liver	TG
B-N-Acetyl- -hexosaminidase	3.2.1.53	*BHA**	Liver	ACE 6.8
Glycerol-3-phosphate	1.1.1.8	*G3PDH-1**	Muscle	TG
		*G3PDH-2**	Heart	ACEN 6.8
		*G3PDH-3**	Heart	ACEN 6.8
Glucose-6-phosphate isomerase	5.3.19	*GPI-B1,2**	Muscle	TG
		*GPI-A**	Muscle	TG
Glutathione reductase	1.6.4.2	*GR**	Heart	TC4

Table 2. Continued.

Enzyme	Enzyme Number	Locus	Tissue	Buffer[1]
Isocitrate dehydrogenase	1.1.1.42	*mIDHP-1**	Muscle	ACE 6.5
		*mIDHP-2**	Heart	ACEN 6.8
		*sIDHP-2**	Liver	ACE 6.8
L-Lactate dehydrogenase	1.1.1.27	*LDH-A1**	Muscle	TG
		*LDH-A2**	Muscle	TG
		*LDH-B1**	Heart	TG
		*LDH-B2**	Heart	TG
		*LDH-C**	Eye	TG
Lactoylglutathione lyase	4.4.1.5.	*aLGL*	Muscle	TG
Malate dehydrogenase	1.1.1.37	*sMDH-A1,2**	Heart	ACEN 6.5
		*sMDH-B1,2**	Heart	ACEN 6.5
		*mMDH-1**	Heart	ACEN 6.5
		*mMDH-2**	Heart	ACEN 6.5
Malic enzyme (NADP+)	1.1.1.40	*mMEP-1**	Muscle	ACE 6.8
		*mMEP-2**	Muscle	ACE 6.8
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	Heart	TG
Nucleoside-triphosphate	3.6.1.19	*NTP**	Muscle	ACE 6.5
Cytosol non-specific Dipeptidase	3.4.13.18	*PEPA**	Muscle	TG
Tripeptide aminopeptidase	3.4.11.4	*PEPB-1**	Heart	TG
X-pro-dipeptidase	3.4.13.9	*PEPD-2**	Heart	ACEN 6.5
Peptidase-LT	3.4.-.-	*PEPLT**	Muscle	TG
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	Muscle	ACE 6.5
Phosphoglycerate kinase	2.7.2.3	*PGK-1**	Muscle	ACE 6.8
		*PGK-2**	Muscle	ACE 6.8
Phosphoglucomutase	5.4.2.2	*PGM-2**	Heart	TG
Superoxide dismutase	1.15.1.1	*sSOD-1**	Heart	ACEN 6.8
		*sSOD-2**	Heart	ACEN 6.8
		*mSOD**	Heart	ACEN 6.8
Triose-phosphate isomerase	5.3.1.1	*TPI-1**	Muscle	TG
		*TPI-2**	Muscle	TG
		*TPI-3**	Muscle	TG
		*TPI-4**	Muscle	TG

Buffers: AC: amine-citric acid buffer, pH 6.8 (Clayton and Tretiak 1972) modified with EDTA (E), NAD (N), or both (Harris and Hopkinson 1976); TBCL: Tris-citric acid gel, pH 8.7 and lithium hydroxide-boric acid electrode buffer, pH 8.0 (Ridgway et al. 1970); TC4: Tris-citric acid buffer pH 5.8 (Schaal and Anderson 1974); TG: Tris-glycine buffer, pH 8.5 (Holmes and Masters 1970).

Table 3. Observed and expected heterozygosities calculated from 40 polymorphic loci from pink salmon collected in 1995 from PWS.

Stream	Timing	Elevation	Observed Heterozygosity		Expected Heterozygosity	
			H	Std. Dev.	*H*	Std. Dev.
Constantine	Early	Tidal	0.0984	0.0351	0.0988	0.0003
	Early	Upstream	0.1037	0.0380	0.1025	0.0004
Koppen	Early	Tidal	0.0995	0.0363	0.0962	0.0004
	Early	Upstream	0.0936	0.0306	0.0966	0.0003
	Late	Tidal	0.0983	0.0373	0.0964	0.0003
	Late	Upstream	0.0995	0.0362	0.0990	0.0004
Lagoon	Early	Upstream	0.0968	0.0347	0.0973	0.0003
Mink	Early	Tidal	0.0952	0.0331	0.0978	0.0004
	Early	Upstream	0.0910	0.0384	0.0976	0.0004
	Late	Tidal	0.0959	0.0377	0.0965	0.0004
	Late	Upstream	0.0979	0.0352	0.0968	0.0003
Olsen	Early	Tidal	0.0998	0.0363	0.1006	0.0003
	Early	Upstream	0.0949	0.0341	0.0997	0.0003
	Late	Tidal	0.0973	0.0338	0.0981	0.0004
	Late	Upstream	0.0952	0.0346	0.0956	0.0003
Rocky	Early	Upstream	0.1023	0.0407	0.0981	0.0003

Table 4. The 95% confidence intervals of the probability that F_{st} is not greater than zero for each locus and for all loci except isoloci analyzed from pink salmon collections made in 1995 from PWS.

Locus	High interval	Low interval
*SAAT3**	0.218	0.218
*SAAT4**	0.143	0.143
*ADA1**	0.341	0.335
*ADA2**	0.470	0.469
*mAH3**	0.285	0.284
*SAH**	0.001	0.001
*mAH4**	0.204	0.204
*AK**	0.066	0.064
*ALAT**	0.156	0.156
*CKA1**	0.076	0.076
*CKC2**	0.063	0.063
*FH**	0.003	0.003
*FDHG**	0.136	0.135
*BHA**	0.001	0.001
*GDA1**	0.033	0.033
*PEPA**	0.217	0.217
*G3PDH1**	0.322	0.322
*G3PDH2**	0.394	0.394
*GPIA**	0.259	0.243
*GR**	0.295	0.251
*SIDHP2**	0.026	0.026
*LGL**	0.211	0.211
*PEPB1**	0.094	0.094
*PEPLT**	0.741	0.741
*mMEP1**	0.334	0.333
*mMEP2**	0.639	0.416
*MPI**	0.231	0.177
*NTP**	0.358	0.340
*PGDH**	0.022	0.022
*PGM2**	0.032	0.032
*PEPD2**	0.017	0.017
*mSOD**	0.013	0.013
*SSOD2**	0.461	0.461
*TPI2**	0.001	0.001
All Loci	0.001	0.001

Table 5. Hierarchical analysis of 1995 pink salmon collections in PWS using log-likelihood ratios for allozyme data and Monte Carlo simulation probabilities for mtDNA data. Comparisonwise significance levels ($?_c$) were adjusted for multiple tests done within the same test groups (Test) using sequential Bonferonni adjustments (modified from Miliken and Johnson 1984 and Rice 1989). Experimentwise significance level was set to 0.05. Complete allozyme table with all loci is in Appendix B.

	Allozyme data					mtDNA data		
Source of Variation	DF	Overall	*P* - value	$?_c$	Test	*P* - value	$?_c$	Test
Between streams	177	307.06	0.000	* 0.025	1	0.029	* 0.05	1
Within Streams	590	732.34	0.000	* 0.050	1			
Constantine Ck. (upstream vs tidal)	59	59.03	0.474	0.050	2	0.104	0.01	2
Koppen Ck.	177	260.96	0.000	* 0.013	2	0.852	0.05	2
Between Timing	59	131.20	0.000	* 0.013	3	0.288	0.00	3
Within Timing	118	129.76	0.216	0.025	3			
Early (upstream vs tidal)	59	60.36	0.426	0.050	4	0.738	0.01	3
Late (upstream vs tidal)	59	69.40	0.167	0.025	4	0.936	0.02	3
Between Elevation	59	64.03	0.305	0.050	3	0.978	0.05	3
Within Elevation	118	196.87	0.000	* 0.017	3			
Tidal (early vs late)	59	106.20	0.000	* 0.025	5	0.596	0.01	3
Upstream (early vs late)	59	90.67	0.005	* 0.050	5	0.370	0.01	3
Mink Ck.	177	205.88	0.068	0.025	2	0.339	0.02	2
Between Timing	59	82.55	0.023	0.006	6	0.086	0.00	4
Within Timing	118	123.33	0.350	0.017	6			
Early (upstream vs tidal)	59	69.55	0.164	0.008	6	0.892	0.05	4
Late (upstream vs tidal)	59	53.78	0.668	0.050	6	0.388	0.01	4
Between Elevation	59	79.64	0.038	0.007	6	0.854	0.02	4
Within Elevation	118	126.23	0.285	0.013	6			
Tidal (early vs late)	59	57.85	0.518	0.025	6	0.312	0.01	4
Upstream (early vs late)	59	68.38	0.189	0.010	6	0.127	0.01	4
Olsen Ck.	177	206.47	0.064	0.017	2	0.169	0.01	2
Between Timing	59	106.20	0.000	* 0.006	7	0.111	0.00	5
Within Timing	118	100.27	0.880	0.025	7			
Early (upstream vs tidal)	59	58.04	0.511	0.013	7	0.397	0.05	5
Late (upstream vs tidal)	59	42.23	0.951	0.050	7	0.223	0.01	5
Between Elevation	59	54.98	0.624	0.017	7	0.350	0.02	5
Within Elevation	118	151.44	0.021	0.008	7			
Tidal (early vs late)	59	85.54	0.014	0.007	7	0.246	0.01	5
Upstream (early vs late)	59	65.90	0.251	0.010	7	0.178	0.01	5

* Significant at experimentwise ? = 0.05.

Table 6. Gene diversity analysis (Nei 1973) by locus of 1995 data among streams, between elevations within streams, among collections within elevations and within collections.

Locus	Absolute Gene Diversity		Relative Gene Diversity			
	Total	Within Collections	Within Collections	Among Collections Within Elevations	Between Elevations Within Streams	Among Streams
*sAAT3**	0.38855	0.38641	0.9945	0.0015	0.0013	0.0027
*sAAT4**	0.50448	0.50104	0.9932	0.0042	0.0014	0.0013
*ADA1**	0.00517	0.00515	0.9956	0.0019	0.0010	0.0014
*ADA2**	0.14681	0.14609	0.9951	0.0022	0.0009	0.0019
*mAH3**	0.05221	0.05187	0.9934	0.0032	0.0018	0.0016
*sAH**	0.01507	0.01500	0.9952	0.0026	0.0006	0.0016
*mAH4**	0.08007	0.07957	0.9937	0.0030	0.0018	0.0015
*AK**	0.00293	0.00291	0.9939	0.0014	0.0024	0.0023
*ALAT**	0.04951	0.04920	0.9938	0.0029	0.0024	0.0009
*CKA1**	0.01924	0.01907	0.9913	0.0023	0.0028	0.0035
*CKA2**	0.00143	0.00142	0.9907	0.0050	0.0025	0.0018
*CKC2**	0.01146	0.01136	0.9911	0.0066	0.0008	0.0015
*FH**	0.00428	0.00423	0.9871	0.0071	0.0023	0.0035
*FDHG**	0.08142	0.08088	0.9934	0.0020	0.0030	0.0016
*bHA**	0.13031	0.12890	0.9892	0.0058	0.0037	0.0013
*GDA1**	0.61588	0.61111	0.9922	0.0042	0.0014	0.0021
*PEPA**	0.01643	0.01633	0.9941	0.0011	0.0030	0.0019
*G3PDH1**	0.17234	0.17139	0.9945	0.0042	0.0002	0.0011
*G3PDH2**	0.28898	0.28788	0.9962	0.0019	0.0009	0.0010
*GPIA**	0.00293	0.00291	0.9939	0.0014	0.0011	0.0037
*GR**	0.00215	0.00213	0.9927	0.0042	0.0021	0.0010
*sIDHP2**	0.47417	0.47057	0.9924	0.0037	0.0006	0.0033
*LDHA1**	0.00217	0.00215	0.9926	0.0043	0.0021	0.0010
*LGL**	0.00581	0.00577	0.9941	0.0012	0.0013	0.0034
*PEPB1**	0.02290	0.02276	0.9939	0.0018	0.0019	0.0024
*PEPLT**	0.40196	0.40041	0.9961	0.0019	0.0017	0.0003
*sMDHA1,2**	0.00250	0.00248	0.9916	0.0030	0.0033	0.0021
*sMDHB1,2**	0.06714	0.06696	0.9973	0.0009	0.0011	0.0007
*mMEP1**	0.08691	0.08643	0.9945	0.0030	0.0024	0.0001
*mMEP2**	0.00357	0.00355	0.9948	0.0035	0.0013	0.0005
*MPI**	0.00428	0.00425	0.9938	0.0038	0.0015	0.0010
*NTP**	0.00437	0.00435	0.9954	0.0017	0.0020	0.0008
*PGDH**	0.24816	0.24581	0.9905	0.0047	0.0022	0.0026
*PGM2**	0.15205	0.15113	0.9940	0.0015	0.0021	0.0024
*PEPD2**	0.46436	0.46073	0.9922	0.0048	0.0011	0.0020
*mSOD**	0.00799	0.00796	0.9961	0.0027	0.0007	0.0004
*sSOD2**	0.02736	0.02724	0.9955	0.0029	0.0011	0.0005
*TPI2**	0.02967	0.02919	0.9838	0.0061	0.0022	0.0080
Overall	4.59703	4.56662	0.9934	0.0034	0.0014	0.0018

Table 7. Gene diversity analysis (Nei 1973) by locus of 1995 data among streams, between timing within streams, among collections within timing and within collections.

Locus	Absolute Gene Diversity		Relative Gene Diversity			
	Total	Within Collections	Within Collections	Among Collections Within Timing	Between Timing Within Streams	Among Streams
*sAAT3**	0.38855	0.38641	0.9945	0.0014	0.0014	0.0027
*sAAT4**	0.50448	0.50104	0.9932	0.0031	0.0024	0.0013
*ADA1**	0.00517	0.00515	0.9956	0.0021	0.0009	0.0014
*ADA2**	0.14681	0.14609	0.9951	0.0024	0.0007	0.0019
*mAH3**	0.05221	0.05187	0.9934	0.0037	0.0013	0.0016
*sAH**	0.01507	0.01500	0.9952	0.0013	0.0019	0.0016
*mAH4**	0.08007	0.07957	0.9937	0.0027	0.0021	0.0015
*AK**	0.00293	0.00291	0.9939	0.0024	0.0014	0.0023
*ALAT**	0.04951	0.04920	0.9938	0.0040	0.0013	0.0009
*CKA1**	0.01924	0.01907	0.9913	0.0032	0.0019	0.0035
*CKA2**	0.00143	0.00142	0.9907	0.0050	0.0025	0.0018
*CKC2**	0.01146	0.01136	0.9911	0.0025	0.0048	0.0015
*FH**	0.00428	0.00423	0.9871	0.0041	0.0052	0.0035
*FDHG**	0.08142	0.08088	0.9934	0.0039	0.0012	0.0016
*bHA**	0.13031	0.12890	0.9892	0.0067	0.0027	0.0013
*GDA1**	0.61588	0.61111	0.9922	0.0020	0.0036	0.0021
*PEPA**	0.01643	0.01633	0.9941	0.0036	0.0004	0.0019
*G3PDH1**	0.17234	0.17139	0.9945	0.0027	0.0017	0.0011
*G3PDH2**	0.28898	0.28788	0.9962	0.0018	0.0010	0.0010
*GPIA**	0.00293	0.00291	0.9939	0.0024	0.0000	0.0037
*GR**	0.00215	0.00213	0.9927	0.0042	0.0021	0.0010
*sIDHP2**	0.47417	0.47057	0.9924	0.0029	0.0014	0.0033
*LDHA1**	0.00217	0.00215	0.9926	0.0043	0.0021	0.0010
*LGL**	0.00581	0.00577	0.9941	0.0020	0.0006	0.0034
*PEPB1**	0.02290	0.02276	0.9939	0.0027	0.0011	0.0024
*PEPLT**	0.40196	0.40041	0.9961	0.0019	0.0017	0.0003
*sMDHA1,2**	0.00250	0.00248	0.9916	0.0048	0.0015	0.0021
*sMDHB1,2**	0.06714	0.06696	0.9973	0.0012	0.0007	0.0007
*mMEP1**	0.08691	0.08643	0.9945	0.0050	0.0004	0.0001
*mMEP2**	0.00357	0.00355	0.9948	0.0035	0.0013	0.0005
*MPI**	0.00428	0.00425	0.9938	0.0042	0.0010	0.0010
*NTP**	0.00437	0.00435	0.9954	0.0025	0.0013	0.0008
*PGDH**	0.24816	0.24581	0.9905	0.0030	0.0039	0.0026
*PGM2**	0.15205	0.15113	0.9940	0.0026	0.0010	0.0024
*PEPD2**	0.46436	0.46073	0.9922	0.0030	0.0029	0.0020
*mSOD**	0.00799	0.00796	0.9961	0.0007	0.0027	0.0004
*sSOD2**	0.02736	0.02724	0.9955	0.0029	0.0011	0.0005
*TPI2**	0.02967	0.02919	0.9838	0.0040	0.0043	0.0080
Overall	4.59703	4.56662	0.9934	0.0027	0.0021	0.0018

Table 8. Restriction enzymes, length of recognition sequence (r), and approximate fragment sizes detected in ND5/ND6 haplotypes in 1995 collections.

Restriction Enzyme	r	Haplotype	Fragment sizes (bp)
Apa I	6	A	1300, 1100
		B	1300, 650, 450
		C	1100, 950, 350
BstU I	4	A	1650, 750
		C	1200, 750, 450
		D	1150, 750, 450, 50
EcoR V	6	A	2400
		B	1500, 900
		C	1250, 1150
Hinf I	4	A	800, 500, 350, 300, 225[a]
		B	1025, 500, 350, 300, 225
Rsa I	4	A	1605, 265[b]
		B	1100, 405, 265[b]
Xba I	6	A	2400
		B	1700, 700

[a] There are two fragments of the indicated size in these patterns.
[b] There are three fragments of the indicated size in these patterns.

Table 9. Haplotype counts for 1995 collections from Prince William Sound (E = early, L = late; T = tidally spawning, U = upstream spawning). Haplotype designations after Fetzner et al. (*submitted*): I = AAAAAA, II = ACAAAA, III = AAABAA, V = AABAAA, VI = BAAAAA, VIII = BCAAAA, XII = ACAABA, XVII = CCAAAA, XVIII = ADAAAA, XIX = AAAAAB. Order of restriction enzymes is *Apa I, BstU I, EcoR V, Hinf I, Rsa I, Xba I.* Haplotype diversity (h) and nucleotide diversity (π) are given.

			ND5/ND6 Haplotypes											
	Sampling Site		I	II	III	V	VI	VIII	XII	XVII	XVIII	XIX	*h*	π
1	Rocky Creek	E U	14	22	0	2	0	1	0	0	0	1	0.5859	0.0076
2	Mink Creek	E T	14	23	0	1	0	1	0	0	0	1	0.5590	0.0070
3	Mink Creek	E U	15	22	0	0	0	2	0	0	0	1	0.5679	0.0070
4	Mink Creek	L T	8	30	1	0	0	1	0	0	0	0	0.4064	0.0050
5	Mink Creek	L U	9	27	0	3	0	1	0	0	0	0	0.5000	0.0068
6	Lagoon Creek	E U	18	16	0	0	0	6	0	0	0	0	0.6308	0.0084
7	Olsen Creek	E T	18	16	0	0	0	6	0	0	0	0	0.6308	0.0084
8	Olsen Creek	E U	19	18	0	1	0	2	0	0	0	0	0.5833	0.0072
9	Olsen Creek	L T	19	14	0	1	1	2	2	0	0	1	0.6615	0.0118
10	Olsen Creek	L U	11	25	0	0	1	1	1	0	0	0	0.5209	0.0076
11	Koppen Creek	E T	17	17	0	1	0	4	0	0	0	1	0.6436	0.0088
12	Koppen Creek	E U	15	20	0	0	0	5	0	0	0	0	0.6090	0.0077
13	Koppen Creek	L T	14	21	0	2	0	2	0	0	1	0	0.6115	0.0081
14	Koppen Creek	L U	16	19	0	2	0	2	0	1	0	0	0.6244	0.0083
15	Constantine Creek	E T	21	18	0	0	0	0	0	1	0	0	0.5346	0.0061
16	Constantine Creek	E U	16	19	0	2	0	3	0	0	0	0	0.6218	0.0082

Table 10. Hierarchical analysis of molecular variation (AMOVA) observed in Prince William Sound pink salmon collections from 1995.

a. Elevation

Variance Component	Observed Partition		*P*[a]	?-statistic
	Variance	% Total		
Among elevation	0.001	0.28	0.655	$?_{CT} = 0.003$
Among collections within elevation	0.003	0.94	0.130	$?_{SC} = 0.009$
Within collections	0.340	99.34	0.116	$?_{ST} = 0.007$

b. Timing

Variance Component	Observed Partition		*P*[a]	?-statistic
	Variance	% Total		
Among timing	0.001	0.40	0.157	$?_{CT} = 0.004$
Among collections within timing	0.002	0.57	0.234	$?_{SC} = 0.006$
Within collections	0.342	99.03	0.158	$?_{ST} = 0.010$

[a] Probability of having a more extreme variance component than the observed value by chance alone (1,000 permutations).

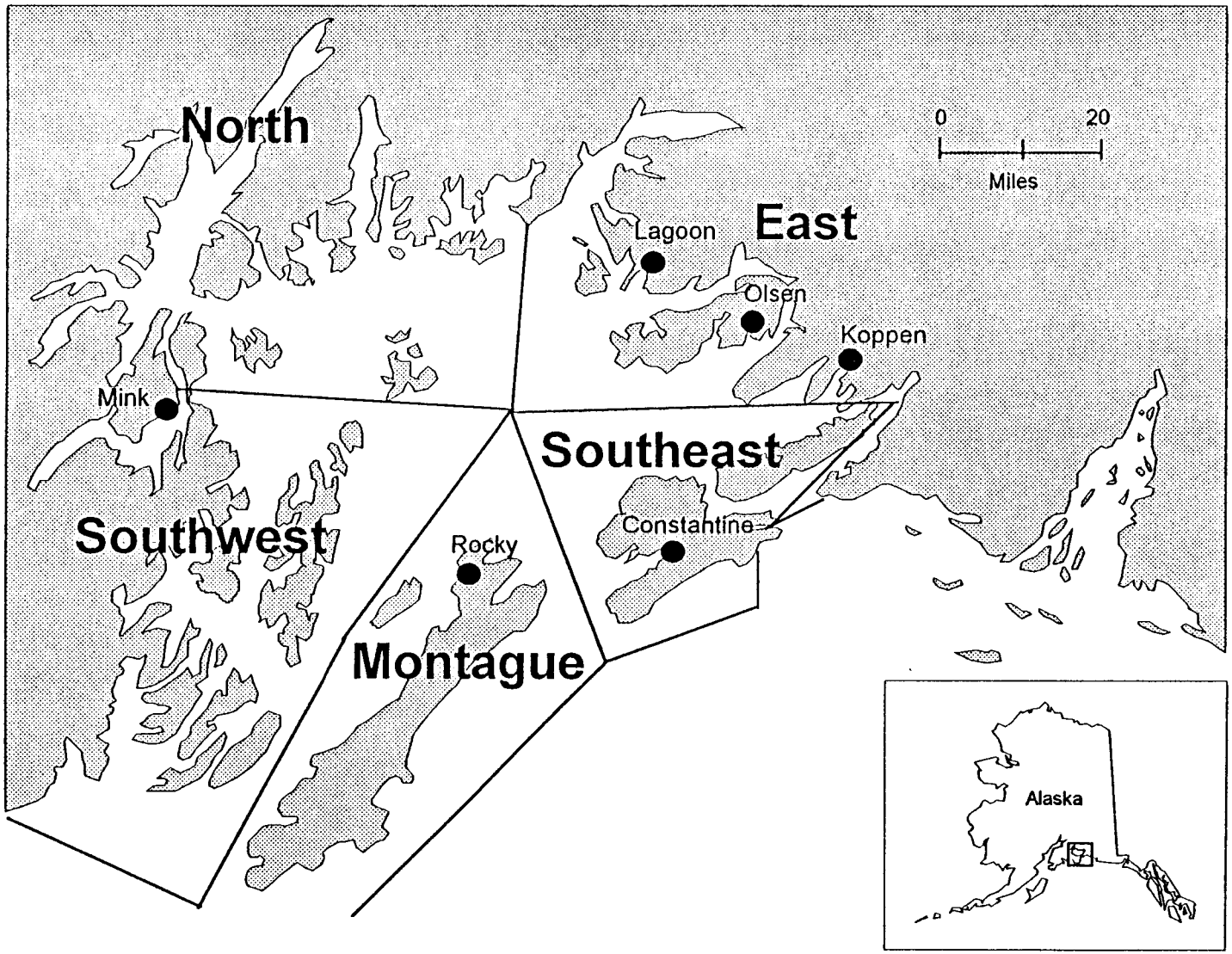

Figure 1. Location of sample collection sites within the major management regions of Prince William Sound.

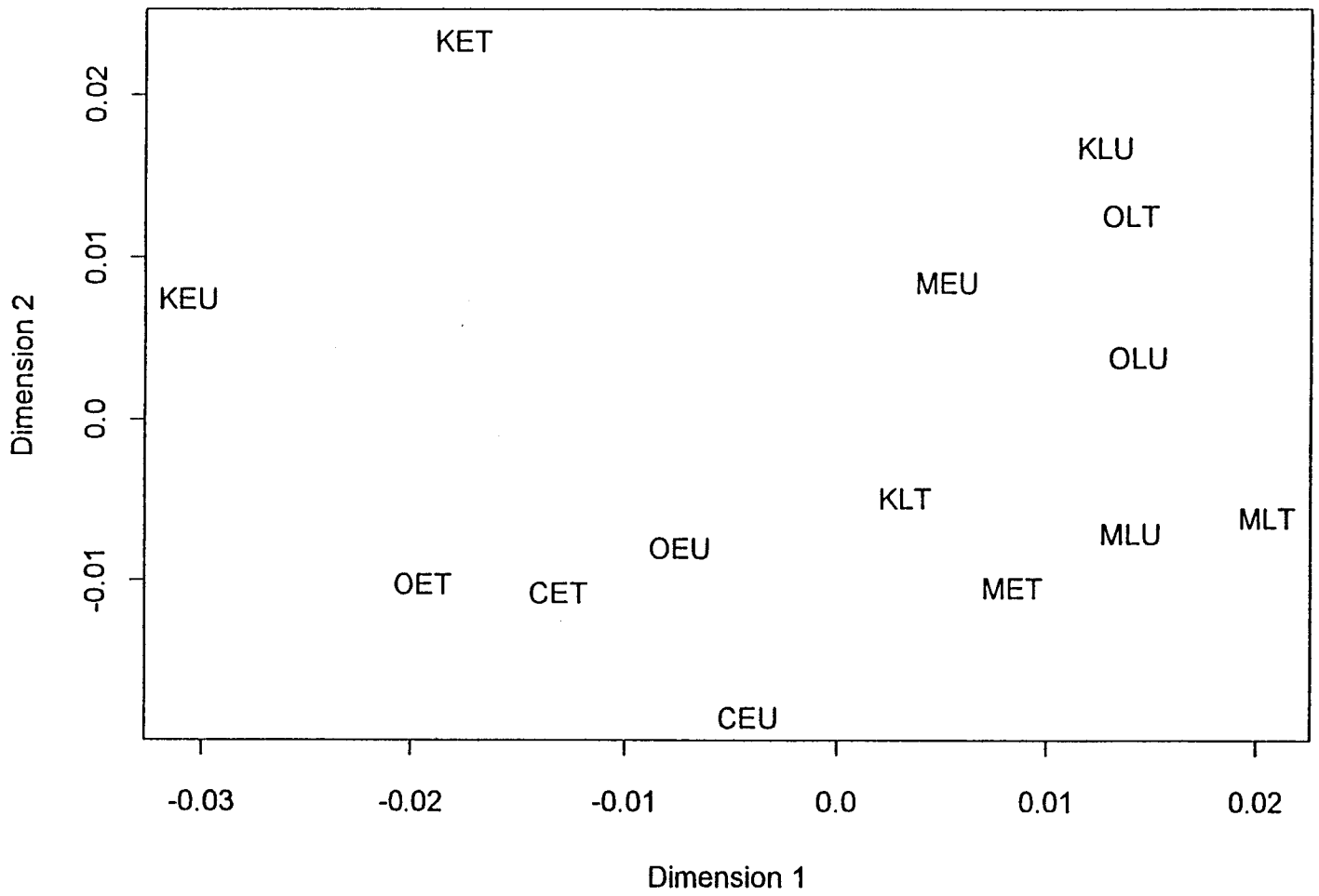

Figure 2. Multidimensional scaling analysis using Cavalli-Sforza and Edwards chord distances, calculated from 40 allozyme loci. In the three letter abreviations for collections, the first letter represents stream (K - Koppen, O - Olsen, C - Constantine, and M - Mink), the second letter represents timing (E - Early, L - Late), and the last letter represents elevation (U - upstream, T - Tidal). Eastern collections (K, O, and C) appear to segregate by timing - early on the right and late on the left of this MDS. Mink collections cluster without timing separation.

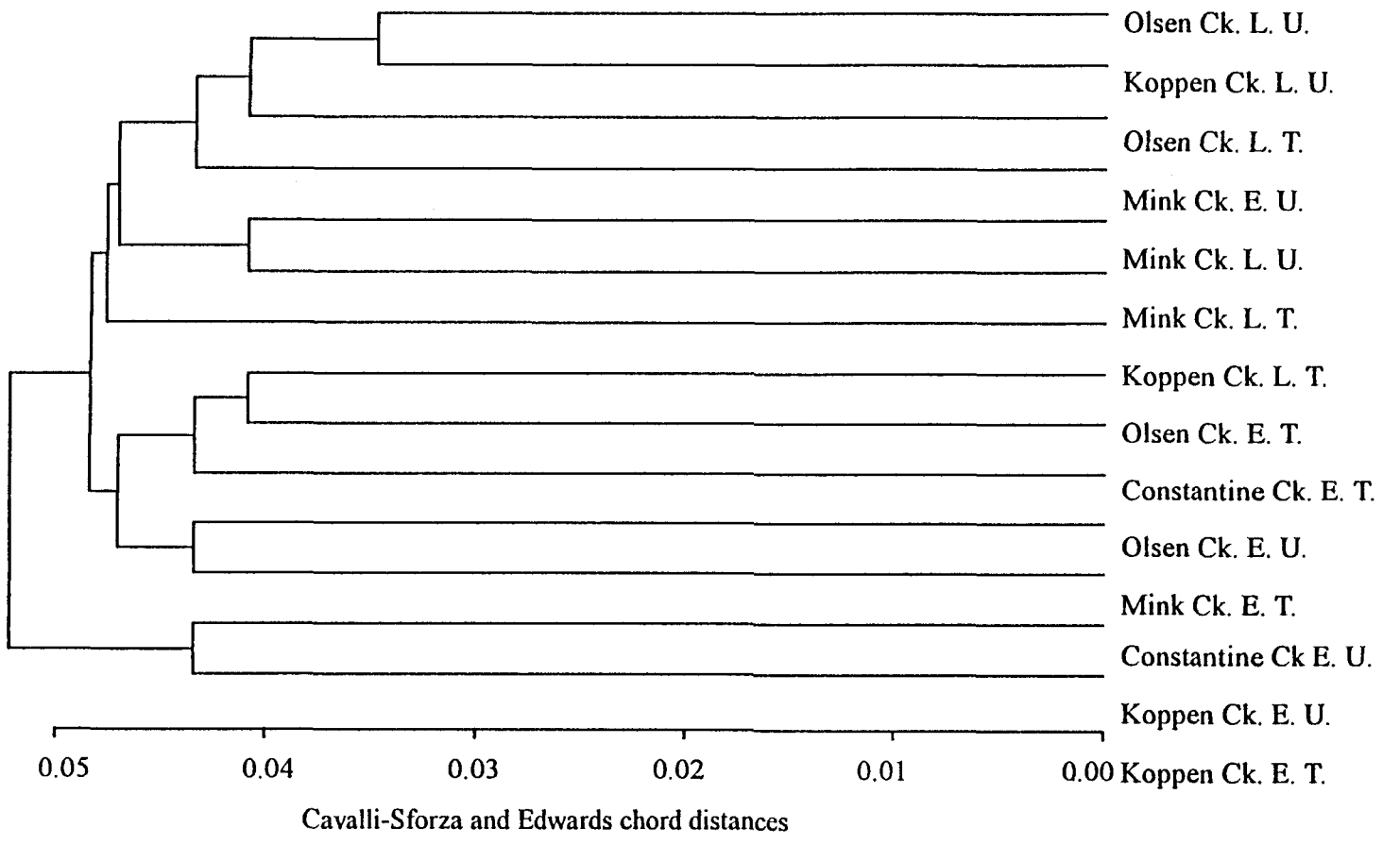

Figure 3. UPGMA tree using Cavalli-Sforza and Edwards chord distances, calculated from 40 allozyme loci. Eastern collections (Koppen, Olsen, and Constantine) segregate by timing - late collections cluster together (upper cluster). However, the early, upstream Mink collection clusters with all the late collections.

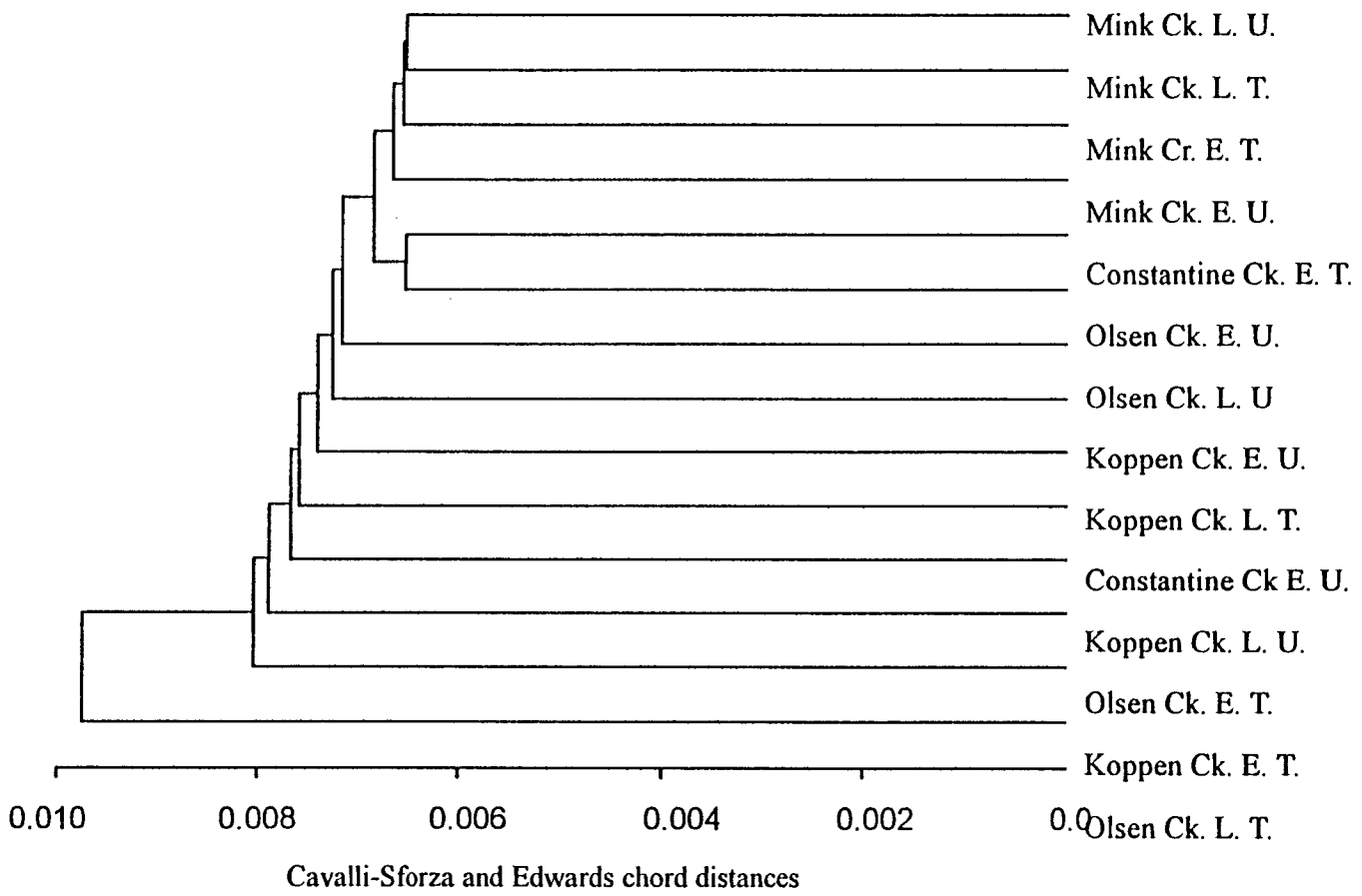

Figure 4. UPGMA tree using nucleotide divergence among collections, calculated from mtDNA restriction site data. No apparent clustering by timing or elevation are apparent, however Mink Cr. collections are on one branch indicating some possible structuring by stream.

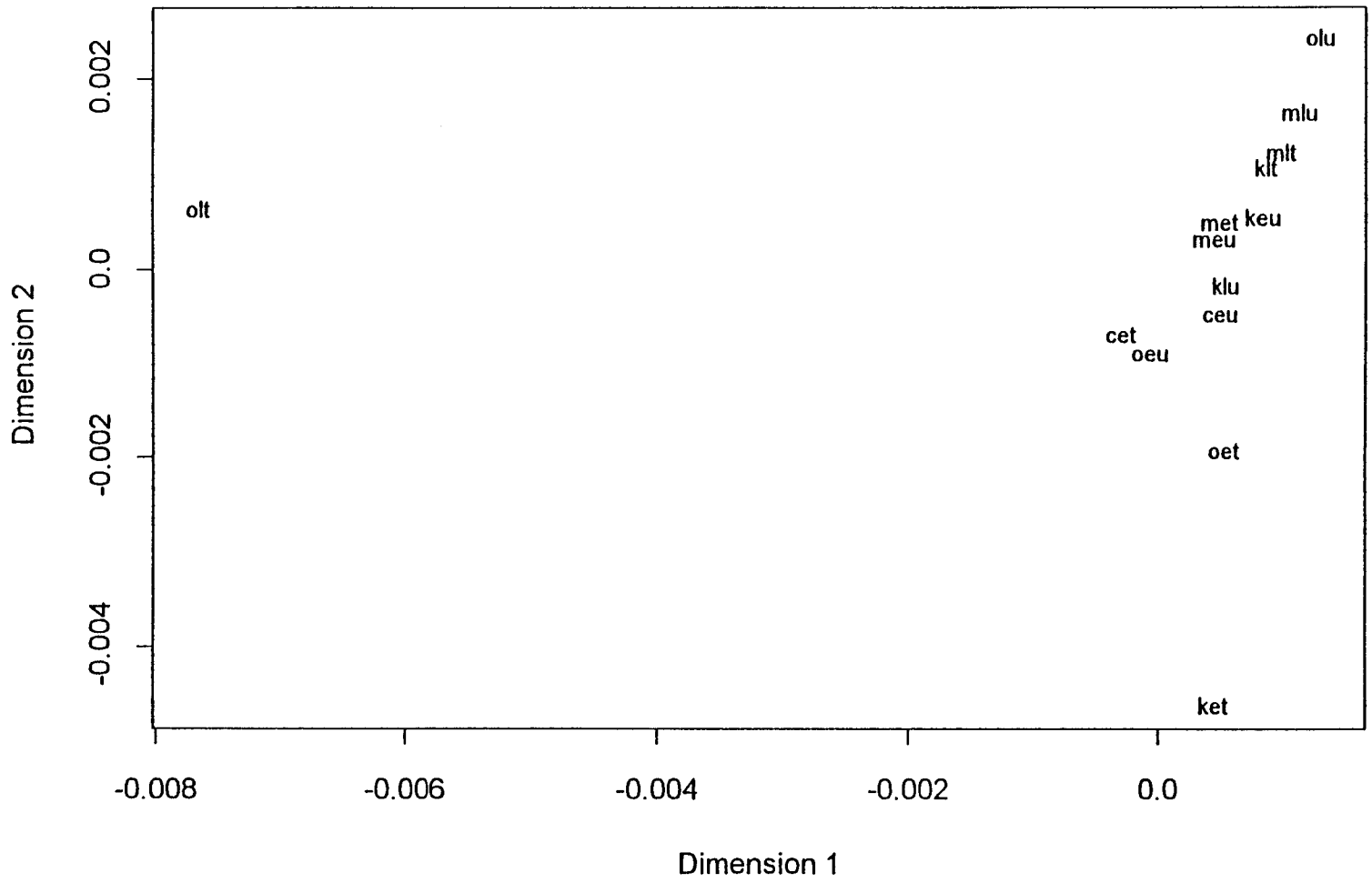

Figure 5. Multidimensional scaling analysis using nucleotide divergence among collections, calculated from mtDNA restriction site data. In the three letter abreviations for collections, the first letter represents stream (K - Koppen, O - Olsen, C - Constantine, and M - Mink), the second letter represents timing (E - Early, L - Late), and the last letter represents elevation (U - upstream, T - Tidal).

Appendix A. Allele frequency estimates of polymorphic allozyme loci for pink salmon collected from Prince William Sound, Alaska in 1995. Within the population names, "E" designates collections made early, and "L" designates collections made late in the spawning cycle; "T" designates collections made in tidal zones and "U" designates collections made in upstream zones. Mobilities are based on buffers in Table 2.

	*sAAT-1,2**				*sAAT-3**		
Population	N	*100*	*110*	*83*	N	*100*	*91*
Constantine Ck. E. T.	100	1.0000	0.0000	0.0000	100	0.6700	0.3300
Constantine Ck. E. U.	100	1.0000	0.0000	0.0000	100	0.7200	0.2800
Koppen Ck. E. T.	100	1.0000	0.0000	0.0000	100	0.7350	0.2650
Koppen Ck. E. U.	100	1.0000	0.0000	0.0000	99	0.6919	0.3081
Koppen Ck. L. T.	100	1.0000	0.0000	0.0000	99	0.7475	0.2525
Koppen Ck. L. U.	100	0.9975	0.0000	0.0025	100	0.7050	0.2950
Lagoon Ck. E. U.	100	1.0000	0.0000	0.0000	99	0.6717	0.3283
Mink Ck. E. T.	100	0.9950	0.0000	0.0050	100	0.7350	0.2650
Mink Ck. E. U.	100	1.0000	0.0000	0.0000	100	0.7350	0.2650
Mink Ck. L. T.	90	0.9972	0.0000	0.0028	88	0.7727	0.2273
Mink Ck. L. U.	100	1.0000	0.0000	0.0000	99	0.7929	0.2071
Olsen Ck. E. T.	100	1.0000	0.0000	0.0000	99	0.7323	0.2677
Olsen Ck. E. U.	100	1.0000	0.0000	0.0000	100	0.7300	0.2700
Olsen Ck. L. T.	100	0.9975	0.0025	0.0000	100	0.7850	0.2150
Olsen Ck. L. U.	100	1.0000	0.0000	0.0000	99	0.7525	0.2475
Rocky Ck. E. U.	100	1.0000	0.0000	0.0000	100	0.7250	0.2750

Population	sAAT-4*				ADA-1*			ADA-2*		
	N	100	210	290	-10	N	100	86	N	100	90
Constantine Ck. E. T.	99	0.4747	0.5202	0.0051	0.0000	100	1.0000	0.0000	100	0.9250	0.0750
Constantine Ck. E. U.	99	0.3889	0.6111	0.0000	0.0000	100	1.0000	0.0000	99	0.8990	0.1010
Koppen Ck. E. T.	98	0.4847	0.5000	0.0051	0.0102	100	1.0000	0.0000	100	0.9000	0.1000
Koppen Ck. E. U.	98	0.5102	0.4694	0.0000	0.0204	100	1.0000	0.0000	99	0.8990	0.1010
Koppen Ck. L. T.	100	0.4500	0.5350	0.0050	0.0100	100	1.0000	0.0000	100	0.9050	0.0950
Koppen Ck. L. U.	100	0.4700	0.5150	0.0000	0.0150	99	0.9949	0.0051	99	0.9141	0.0859
Lagoon Ck. E. U.	98	0.4184	0.5714	0.0000	0.0102	100	1.0000	0.0000	100	0.9100	0.0900
Mink Ck. E. T.	100	0.4000	0.5900	0.0000	0.0100	100	0.9950	0.0050	100	0.9150	0.0850
Mink Ck. E. U.	99	0.4949	0.5051	0.0000	0.0000	100	0.9950	0.0050	99	0.9192	0.0808
Mink Ck. L. T.	90	0.4944	0.4944	0.0056	0.0056	89	0.9888	0.0112	90	0.9278	0.0722
Mink Ck. L. U.	98	0.4337	0.5612	0.0051	0.0000	100	1.0000	0.0000	100	0.9550	0.0450
Olsen Ck. E. T.	100	0.4100	0.5850	0.0000	0.0050	100	1.0000	0.0000	100	0.9050	0.0950
Olsen Ck. E. U.	99	0.4192	0.5707	0.0000	0.0101	98	1.0000	0.0000	98	0.9592	0.0408
Olsen Ck. L. T.	99	0.5101	0.4848	0.0000	0.0051	100	0.9950	0.0050	100	0.9400	0.0600
Olsen Ck. L. U.	100	0.4800	0.5050	0.0050	0.0100	100	0.9950	0.0050	100	0.9200	0.0800
Rocky Ck. E. U.	99	0.4899	0.5051	0.0000	0.0051	100	1.0000	0.0000	100	0.9050	0.0950

Population	mAH-3*					sAH*			
	N	100	74	90	58	N	100	115	76
Constantine Ck. E. T.	100	0.9700	0.0250	0.0050	0.0000	100	0.9950	0.0000	0.0050
Constantine Ck. E. U.	100	0.9800	0.0200	0.0000	0.0000	100	1.0000	0.0000	0.0000
Koppen Ck. E. T.	100	0.9600	0.0300	0.0100	0.0000	99	0.9848	0.0152	0.0000
Koppen Ck. E. U.	100	0.9650	0.0300	0.0050	0.0000	99	0.9798	0.0152	0.0051
Koppen Ck. L. T.	100	0.9750	0.0200	0.0050	0.0000	100	0.9950	0.0000	0.0050
Koppen Ck. L. U.	100	0.9650	0.0300	0.0050	0.0000	100	0.9900	0.0100	0.0000
Lagoon Ck. E. U.	100	0.9850	0.0150	0.0000	0.0000	98	0.9490	0.0408	0.0102
Mink Ck. E. T.	100	0.9850	0.0150	0.0000	0.0000	98	0.9898	0.0051	0.0051
Mink Ck. E. U.	100	0.9850	0.0100	0.0000	0.0050	100	0.9950	0.0050	0.0000
Mink Ck. L. T.	90	0.9944	0.0000	0.0056	0.0000	90	0.9944	0.0056	0.0000
Mink Ck. L. U.	100	0.9650	0.0350	0.0000	0.0000	99	1.0000	0.0000	0.0000
Olsen Ck. E. T.	100	0.9400	0.0400	0.0050	0.0150	100	0.9900	0.0050	0.0050
Olsen Ck. E. U.	100	0.9800	0.0200	0.0000	0.0000	100	0.9850	0.0150	0.0000
Olsen Ck. L. T.	100	0.9800	0.0100	0.0100	0.0000	99	0.9949	0.0051	0.0000
Olsen Ck. L. U.	100	0.9800	0.0200	0.0000	0.0000	100	1.0000	0.0000	0.0000
Rocky Ck. E. U.	100	0.9700	0.0300	0.0000	0.0000	99	1.0000	0.0000	0.0000

Population	mAH-4*				mAAT-1*			
	N	100	116	76	81	N	-100	-83	-70
Constantine Ck. E. T.	99	0.9495	0.0000	0.0101	0.0404	100	1.0000	0.0000	0.0000
Constantine Ck. E. U.	100	0.9400	0.0000	0.0200	0.0400	100	1.0000	0.0000	0.0000
Koppen Ck. E. T.	100	0.9600	0.0000	0.0050	0.0350	100	1.0000	0.0000	0.0000
Koppen Ck. E. U.	100	0.9550	0.0000	0.0200	0.0250	100	1.0000	0.0000	0.0000
Koppen Ck. L. T.	100	0.9650	0.0100	0.0200	0.0050	100	1.0000	0.0000	0.0000
Koppen Ck. L. U.	100	0.9550	0.0000	0.0300	0.0150	100	1.0000	0.0000	0.0000
Lagoon Ck. E. U.	100	0.9450	0.0000	0.0200	0.0350	100	1.0000	0.0000	0.0000
Mink Ck. E. T.	100	0.9650	0.0000	0.0250	0.0100	100	0.9950	0.0000	0.0050
Mink Ck. E. U.	99	0.9495	0.0000	0.0202	0.0303	100	0.9950	0.0050	0.0000
Mink Ck. L. T.	90	0.9778	0.0000	0.0167	0.0056	90	1.0000	0.0000	0.0000
Mink Ck. L. U.	100	0.9650	0.0000	0.0300	0.0050	100	1.0000	0.0000	0.0000
Olsen Ck. E. T.	100	0.9450	0.0000	0.0050	0.0500	100	1.0000	0.0000	0.0000
Olsen Ck. E. U.	100	0.9850	0.0000	0.0050	0.0100	100	1.0000	0.0000	0.0000
Olsen Ck. L. T.	100	0.9450	0.0000	0.0450	0.0100	100	1.0000	0.0000	0.0000
Olsen Ck. L. U.	100	0.9650	0.0000	0.0250	0.0100	100	1.0000	0.0000	0.0000
Rocky Ck. E. U.	100	0.9500	0.0000	0.0300	0.0200	100	1.0000	0.0000	0.0000

Population	AK*				ALAT*				
	N	-100	-145	-420	N	100	111	108	88	106
Constantine Ck. E. T.	99	1.0000	0.0000	0.0000	100	0.9800	0.0100	0.0000	0.0050	0.0050
Constantine Ck. E. U.	100	0.9900	0.0100	0.0000	100	0.9700	0.0250	0.0000	0.0000	0.0050
Koppen Ck. E. T.	100	0.9900	0.0000	0.0100	100	0.9850	0.0000	0.0000	0.0050	0.0100
Koppen Ck. E. U.	100	1.0000	0.0000	0.0000	100	0.9850	0.0000	0.0000	0.0050	0.0100
Koppen Ck. L. T.	100	1.0000	0.0000	0.0000	100	0.9900	0.0050	0.0000	0.0050	0.0000
Koppen Ck. L. U.	99	1.0000	0.0000	0.0000	99	0.9495	0.0303	0.0000	0.0051	0.0152
Lagoon Ck. E. U.	100	1.0000	0.0000	0.0000	100	0.9900	0.0050	0.0000	0.0050	0.0000
Mink Ck. E. T.	100	0.9950	0.0000	0.0050	99	0.9848	0.0152	0.0000	0.0000	0.0000
Mink Ck. E. U.	100	1.0000	0.0000	0.0000	100	0.9600	0.0300	0.0000	0.0000	0.0100
Mink Ck. L. T.	90	0.9944	0.0056	0.0000	90	0.9833	0.0111	0.0000	0.0056	0.0000
Mink Ck. L. U.	100	0.9850	0.0050	0.0100	100	0.9800	0.0200	0.0000	0.0000	0.0000
Olsen Ck. E. T.	100	1.0000	0.0000	0.0000	100	0.9900	0.0050	0.0000	0.0050	0.0000
Olsen Ck. E. U.	100	1.0000	0.0000	0.0000	98	0.9592	0.0255	0.0051	0.0102	0.0000
Olsen Ck. L. T.	100	1.0000	0.0000	0.0000	100	0.9650	0.0200	0.0050	0.0100	0.0000
Olsen Ck. L. U.	100	1.0000	0.0000	0.0000	100	0.9650	0.0300	0.0000	0.0050	0.0000
Rocky Ck. E. U.	100	0.9950	0.0050	0.0000	100	0.9700	0.0150	0.0000	0.0150	0.0000

Population	CK-A1*				CK-A2*				CK-C1*		
	N	100	66	110	N	100	108	135	N	100	92
Constantine Ck. E. T.	100	0.9950	0.0000	0.0050	100	1.0000	0.0000	0.0000	91	0.9945	0.0055
Constantine Ck. E. U.	100	1.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000	90	0.9944	0.0056
Koppen Ck. E. T.	100	0.9850	0.0150	0.0000	100	1.0000	0.0000	0.0000	90	0.9944	0.0056
Koppen Ck. E. U.	100	0.9700	0.0300	0.0000	100	1.0000	0.0000	0.0000	95	1.0000	0.0000
Koppen Ck. L. T.	100	0.9850	0.0100	0.0050	100	1.0000	0.0000	0.0000	99	1.0000	0.0000
Koppen Ck. L. U.	99	0.9848	0.0152	0.0000	99	1.0000	0.0000	0.0000	97	1.0000	0.0000
Lagoon Ck. E. U.	100	0.9950	0.0050	0.0000	100	1.0000	0.0000	0.0000	94	1.0000	0.0000
Mink Ck. E. T.	100	1.0000	0.0000	0.0000	100	0.9850	0.0100	0.0050	96	1.0000	0.0000
Mink Ck. E. U.	100	0.9950	0.0050	0.0000	100	1.0000	0.0000	0.0000	93	0.9946	0.0054
Mink Ck. L. T.	90	0.9944	0.0056	0.0000	90	1.0000	0.0000	0.0000	75	0.9933	0.0067
Mink Ck. L. U.	99	0.9848	0.0152	0.0000	99	1.0000	0.0000	0.0000	88	0.9943	0.0057
Olsen Ck. E. T.	100	0.9850	0.0100	0.0050	100	1.0000	0.0000	0.0000	98	0.9949	0.0051
Olsen Ck. E. U.	98	1.0000	0.0000	0.0000	98	1.0000	0.0000	0.0000	94	0.9947	0.0053
Olsen Ck. L. T.	100	0.9750	0.0250	0.0000	100	1.0000	0.0000	0.0000	92	0.9946	0.0054
Olsen Ck. L. U.	100	0.9900	0.0050	0.0050	100	1.0000	0.0000	0.0000	92	1.0000	0.0000
Rocky Ck. E. U.	100	0.9850	0.0150	0.0000	100	1.0000	0.0000	0.0000	97	0.9948	0.0052

Population	CK-C2*				CK-B*			FH*				
	N	100	105	82	N	100	106	N	100	136	84	45
Constantine Ck. E. T.	93	0.9892	0.0054	0.0054	100	1.0000	0.0000	100	0.9950	0.0000	0.0000	0.0050
Constantine Ck. E. U.	92	0.9946	0.0054	0.0000	99	1.0000	0.0000	100	0.9950	0.0000	0.0050	0.0000
Koppen Ck. E. T.	91	1.0000	0.0000	0.0000	100	1.0000	0.0000	100	0.9800	0.0000	0.0200	0.0000
Koppen Ck. E. U.	96	0.9948	0.0052	0.0000	98	1.0000	0.0000	99	0.9949	0.0000	0.0051	0.0000
Koppen Ck. L. T.	99	1.0000	0.0000	0.0000	99	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Koppen Ck. L. U.	94	1.0000	0.0000	0.0000	100	0.9950	0.0050	99	1.0000	0.0000	0.0000	0.0000
Lagoon Ck. E. U.	92	1.0000	0.0000	0.0000	100	1.0000	0.0000	99	1.0000	0.0000	0.0000	0.0000
Mink Ck. E. T.	98	0.9949	0.0051	0.0000	100	0.9950	0.0050	100	1.0000	0.0000	0.0000	0.0000
Mink Ck. E. U.	92	1.0000	0.0000	0.0000	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Mink Ck. L. T.	79	0.9873	0.0063	0.0063	89	1.0000	0.0000	90	0.9944	0.0056	0.0000	0.0000
Mink Ck. L. U.	89	0.9775	0.0225	0.0000	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Olsen Ck. E. T.	99	0.9798	0.0202	0.0000	99	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Olsen Ck. E. U.	95	0.9895	0.0105	0.0000	99	1.0000	0.0000	98	1.0000	0.0000	0.0000	0.0000
Olsen Ck. L. T.	91	1.0000	0.0000	0.0000	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Olsen Ck. L. U.	91	0.9945	0.0000	0.0055	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Rocky Ck. E. U.	98	0.9949	0.0051	0.0000	98	1.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000

Population	N	FDHG* 100	132	143	108	52	128
Constantine Ck. E. T.	100	0.9600	0.0400	0.0000	0.0000	0.0000	0.0000
Constantine Ck. E. U.	100	0.9700	0.0200	0.0000	0.0000	0.0000	0.0100
Koppen Ck. E. T.	100	0.9650	0.0350	0.0000	0.0000	0.0000	0.0000
Koppen Ck. E. U.	100	0.9550	0.0400	0.0000	0.0050	0.0000	0.0000
Koppen Ck. L. T.	100	0.9600	0.0250	0.0000	0.0150	0.0000	0.0000
Koppen Ck. L. U.	100	0.9350	0.0500	0.0000	0.0050	0.0100	0.0000
Lagoon Ck. E. U.	100	0.9650	0.0300	0.0000	0.0050	0.0000	0.0000
Mink Ck. E. T.	100	0.9750	0.0250	0.0000	0.0000	0.0000	0.0000
Mink Ck. E. U.	100	0.9350	0.0400	0.0000	0.0250	0.0000	0.0000
Mink Ck. L. T.	90	0.9944	0.0056	0.0000	0.0000	0.0000	0.0000
Mink Ck. L. U.	100	0.9650	0.0350	0.0000	0.0000	0.0000	0.0000
Olsen Ck. E. T.	99	0.9394	0.0505	0.0051	0.0051	0.0000	0.0000
Olsen Ck. E. U.	100	0.9450	0.0450	0.0000	0.0100	0.0000	0.0000
Olsen Ck. L. T.	100	0.9350	0.0600	0.0000	0.0000	0.0000	0.0050
Olsen Ck. L. U.	100	0.9700	0.0250	0.0000	0.0000	0.0050	0.0000
Rocky Ck. E. U.	100	0.9700	0.0150	0.0000	0.0100	0.0050	0.0000

Population	N	bGALA* 100	111	91	105	N	GDA* 100	108	113	118	115	123
Constantine Ck. E. T.	81	0.9568	0.0062	0.0370	0.0000	99	0.4596	0.4091	0.0707	0.0202	0.0354	0.0051
Constantine Ck. E. U.	94	0.8883	0.0053	0.0851	0.0213	100	0.3650	0.4350	0.0700	0.0600	0.0350	0.0350
Koppen Ck. E. T.	97	0.9124	0.0309	0.0515	0.0052	98	0.5612	0.3367	0.0408	0.0255	0.0153	0.0204
Koppen Ck. E. U.	97	0.9588	0.0000	0.0361	0.0052	99	0.5202	0.3535	0.0505	0.0253	0.0354	0.0152
Koppen Ck. L. T.	100	0.9450	0.0000	0.0350	0.0200	100	0.3950	0.4200	0.0750	0.0200	0.0900	0.0000
Koppen Ck. L. U.	98	0.9235	0.0153	0.0510	0.0102	100	0.4350	0.3950	0.0700	0.0350	0.0650	0.0000
Lagoon Ck. E. U.	93	0.9785	0.0054	0.0161	0.0000	98	0.4694	0.3929	0.0612	0.0459	0.0306	0.0000
Mink Ck. E. T.	98	0.8827	0.0051	0.1122	0.0000	97	0.4639	0.4021	0.0464	0.0309	0.0464	0.0103
Mink Ck. E. U.	91	0.9560	0.0110	0.0330	0.0000	95	0.4158	0.4421	0.1000	0.0053	0.0368	0.0000
Mink Ck. L. T.	83	0.9277	0.0181	0.0422	0.0120	89	0.3820	0.4270	0.0843	0.0281	0.0562	0.0225
Mink Ck. L. U.	100	0.9150	0.0250	0.0450	0.0150	99	0.3939	0.4545	0.0808	0.0152	0.0404	0.0152
Olsen Ck. E. T.	86	0.9709	0.0000	0.0233	0.0058	99	0.4495	0.3687	0.0455	0.0758	0.0253	0.0354
Olsen Ck. E. U.	89	0.9607	0.0056	0.0225	0.0112	98	0.4388	0.4286	0.0510	0.0204	0.0255	0.0357
Olsen Ck. L. T.	98	0.9286	0.0204	0.0510	0.0000	96	0.4115	0.4427	0.0521	0.0417	0.0469	0.0052
Olsen Ck. L. U.	100	0.9100	0.0150	0.0650	0.0100	99	0.4444	0.4495	0.0556	0.0253	0.0253	0.0000
Rocky Ck. E. U.	81	0.9630	0.0123	0.0123	0.0123	99	0.3990	0.4141	0.0808	0.0202	0.0657	0.0202

Population	PEPA*				G3PDH-1*		
	N	*100*	*109*	*93*	N	*-100*	*60*
Constantine Ck. E. T.	100	0.9700	0.0300	0.0000	100	0.8900	0.1100
Constantine Ck. E. U.	100	0.9950	0.0050	0.0000	100	0.8800	0.1200
Koppen Ck. E. T.	100	0.9900	0.0100	0.0000	100	0.9350	0.0650
Koppen Ck. E. U.	100	0.9950	0.0050	0.0000	100	0.8700	0.1300
Koppen Ck. L. T.	100	1.0000	0.0000	0.0000	100	0.9000	0.1000
Koppen Ck. L. U.	99	0.9949	0.0051	0.0000	100	0.9450	0.0550
Lagoon Ck. E. U.	100	0.9900	0.0100	0.0000	100	0.8750	0.1250
Mink Ck. E. T.	100	0.9800	0.0150	0.0050	100	0.9150	0.0850
Mink Ck. E. U.	100	0.9950	0.0050	0.0000	100	0.9050	0.0950
Mink Ck. L. T.	89	0.9944	0.0056	0.0000	90	0.9167	0.0833
Mink Ck. L. U.	100	0.9900	0.0050	0.0050	100	0.9100	0.0900
Olsen Ck. E. T.	100	0.9950	0.0050	0.0000	100	0.8750	0.1250
Olsen Ck. E. U.	98	0.9898	0.0102	0.0000	100	0.8850	0.1150
Olsen Ck. L. T.	100	0.9900	0.0100	0.0000	100	0.9200	0.0800
Olsen Ck. L. U.	100	0.9950	0.0050	0.0000	100	0.9200	0.0800
Rocky Ck. E. U.	100	0.9950	0.0000	0.0050	100	0.8950	0.1050

Population	G3PDH-2*						GPIB-1,2*				
	N	*100*	*120*	*90*	*vf*	*110*	N	*100*	*200*	*25*	*180*
Constantine Ck. E. T.	98	0.8367	0.1276	0.0306	0.0051	0.0000	100	1.0000	0.0000	0.0000	0.0000
Constantine Ck. E. U.	90	0.8556	0.1222	0.0222	0.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Koppen Ck. E. T.	97	0.8144	0.1649	0.0155	0.0000	0.0052	100	1.0000	0.0000	0.0000	0.0000
Koppen Ck. E. U.	96	0.8906	0.0990	0.0104	0.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Koppen Ck. L. T.	99	0.8333	0.1111	0.0556	0.0000	0.0000	100	0.9975	0.0000	0.0025	0.0000
Koppen Ck. L. U.	96	0.8281	0.1094	0.0625	0.0000	0.0000	99	0.9975	0.0000	0.0025	0.0000
Lagoon Ck. E. U.	94	0.8670	0.1064	0.0266	0.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Mink Ck. E. T.	93	0.8280	0.1290	0.0430	0.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Mink Ck. E. U.	94	0.8404	0.1170	0.0426	0.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Mink Ck. L. T.	89	0.8258	0.1011	0.0730	0.0000	0.0000	90	0.9972	0.0028	0.0000	0.0000
Mink Ck. L. U.	94	0.8351	0.1170	0.0479	0.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Olsen Ck. E. T.	99	0.8131	0.1414	0.0455	0.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000
Olsen Ck. E. U.	97	0.8093	0.1495	0.0361	0.0000	0.0052	98	1.0000	0.0000	0.0000	0.0000
Olsen Ck. L. T.	98	0.8316	0.1071	0.0612	0.0000	0.0000	100	0.9975	0.0000	0.0025	0.0000
Olsen Ck. L. U.	98	0.8010	0.1480	0.0459	0.0051	0.0000	100	0.9950	0.0000	0.0025	0.0025
Rocky Ck. E. U.	96	0.8958	0.0781	0.0260	0.0000	0.0000	100	1.0000	0.0000	0.0000	0.0000

Population	GPI-A*					GR*			
	N	*100*	*108*	*91*	*120*	*80*	N	*100*	*114*	*78*
Constantine Ck. E. T.	100	0.9950	0.0000	0.0050	0.0000	0.0000	100	1.0000	0.0000	0.0000
Constantine Ck. E. U.	100	1.0000	0.0000	0.0000	0.0000	0.0000	100	0.9950	0.0000	0.0050
Koppen Ck. E. T.	100	1.0000	0.0000	0.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Koppen Ck. E. U.	100	1.0000	0.0000	0.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Koppen Ck. L. T.	100	1.0000	0.0000	0.0000	0.0000	0.0000	99	1.0000	0.0000	0.0000
Koppen Ck. L. U.	100	1.0000	0.0000	0.0000	0.0000	0.0000	97	1.0000	0.0000	0.0000
Lagoon Ck. E. U.	100	1.0000	0.0000	0.0000	0.0000	0.0000	94	1.0000	0.0000	0.0000
Mink Ck. E. T.	100	0.9950	0.0000	0.0000	0.0000	0.0050	100	1.0000	0.0000	0.0000
Mink Ck. E. U.	100	0.9900	0.0050	0.0000	0.0050	0.0000	100	0.9900	0.0100	0.0000
Mink Ck. L. T.	90	0.9944	0.0000	0.0000	0.0056	0.0000	89	1.0000	0.0000	0.0000
Mink Ck. L. U.	100	0.9950	0.0000	0.0000	0.0050	0.0000	99	1.0000	0.0000	0.0000
Olsen Ck. E. T.	100	1.0000	0.0000	0.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Olsen Ck. E. U.	100	1.0000	0.0000	0.0000	0.0000	0.0000	100	1.0000	0.0000	0.0000
Olsen Ck. L. T.	100	1.0000	0.0000	0.0000	0.0000	0.0000	99	0.9949	0.0051	0.0000
Olsen Ck. L. U.	100	1.0000	0.0000	0.0000	0.0000	0.0000	99	1.0000	0.0000	0.0000
Rocky Ck. E. U.	100	0.9900	0.0000	0.0000	0.0100	0.0000	99	1.0000	0.0000	0.0000

Population	mIDHP-1*			mIDHP-2*			sIDHP-2*					
	N	*100*	*165*	N	*100*	*118*	N	*100*	*125*	*134*	*76*	*124*
Constantine Ck. E. T.	100	1.0000	0.0000	100	1.0000	0.0000	99	0.6717	0.2929	0.0303	0.0000	0.0051
Constantine Ck. E. U.	100	1.0000	0.0000	100	1.0000	0.0000	99	0.6566	0.3131	0.0303	0.0000	0.0000
Koppen Ck. E. T.	100	1.0000	0.0000	100	0.9950	0.0050	99	0.6919	0.2576	0.0455	0.0000	0.0051
Koppen Ck. E. U.	100	1.0000	0.0000	100	1.0000	0.0000	99	0.6717	0.3131	0.0101	0.0000	0.0051
Koppen Ck. L. T.	100	1.0000	0.0000	100	1.0000	0.0000	100	0.6500	0.3150	0.0300	0.0000	0.0050
Koppen Ck. L. U.	100	1.0000	0.0000	100	1.0000	0.0000	100	0.7500	0.2050	0.0450	0.0000	0.0000
Lagoon Ck. E. U.	100	1.0000	0.0000	100	1.0000	0.0000	98	0.7194	0.2092	0.0612	0.0000	0.0102
Mink Ck. E. T.	100	1.0000	0.0000	100	1.0000	0.0000	100	0.7050	0.2500	0.0450	0.0000	0.0000
Mink Ck. E. U.	100	0.9950	0.0050	100	1.0000	0.0000	100	0.6550	0.2600	0.0850	0.0000	0.0000
Mink Ck. L. T.	90	1.0000	0.0000	90	1.0000	0.0000	90	0.6333	0.3056	0.0611	0.0000	0.0000
Mink Ck. L. U.	100	1.0000	0.0000	100	1.0000	0.0000	99	0.6616	0.2677	0.0657	0.0051	0.0000
Olsen Ck. E. T.	100	1.0000	0.0000	100	1.0000	0.0000	99	0.5960	0.3737	0.0202	0.0101	0.0000
Olsen Ck. E. U.	100	1.0000	0.0000	100	1.0000	0.0000	99	0.6111	0.3434	0.0303	0.0000	0.0152
Olsen Ck. L. T.	100	1.0000	0.0000	100	1.0000	0.0000	99	0.6616	0.2828	0.0505	0.0051	0.0000
Olsen Ck. L. U.	100	0.9950	0.0050	100	1.0000	0.0000	100	0.6350	0.3250	0.0400	0.0000	0.0000
Rocky Ck. E. U.	100	1.0000	0.0000	100	1.0000	0.0000	99	0.6465	0.2980	0.0556	0.0000	0.0000

Population	LDH-A1*			LDH-B1*				LDH-B2*		
	N	-100	-250	N	100	153	48	N	100	151
Constantine Ck. E. T.	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	100	1.0000	0.0000
Constantine Ck. E. U.	100	1.0000	0.0000	100	0.9950	0.0000	0.0050	100	1.0000	0.0000
Koppen Ck. E. T.	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	100	1.0000	0.0000
Koppen Ck. E. U.	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	100	1.0000	0.0000
Koppen Ck. L. T.	100	1.0000	0.0000	100	0.9950	0.0000	0.0050	100	1.0000	0.0000
Koppen Ck. L. U.	99	1.0000	0.0000	100	1.0000	0.0000	0.0000	100	1.0000	0.0000
Lagoon Ck. E. U.	99	1.0000	0.0000	100	1.0000	0.0000	0.0000	100	1.0000	0.0000
Mink Ck. E. T.	100	1.0000	0.0000	100	0.9950	0.0000	0.0050	100	0.9950	0.0050
Mink Ck. E. U.	100	0.9950	0.0050	100	1.0000	0.0000	0.0000	100	1.0000	0.0000
Mink Ck. L. T.	90	1.0000	0.0000	90	1.0000	0.0000	0.0000	90	1.0000	0.0000
Mink Ck. L. U.	99	1.0000	0.0000	100	1.0000	0.0000	0.0000	100	1.0000	0.0000
Olsen Ck. E. T.	100	1.0000	0.0000	99	0.9949	0.0000	0.0051	100	1.0000	0.0000
Olsen Ck. E. U.	98	0.9898	0.0102	100	1.0000	0.0000	0.0000	100	1.0000	0.0000
Olsen Ck. L. T.	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	100	1.0000	0.0000
Olsen Ck. L. U.	100	1.0000	0.0000	100	1.0000	0.0000	0.0000	100	1.0000	0.0000
Rocky Ck. E. U.	100	1.0000	0.0000	100	0.9950	0.0050	0.0000	100	1.0000	0.0000

Population	LGL*			PEPB-1*				PEPLT*				
	N	100	80	N	100	138	50	N	100	108	90	80
Constantine Ck. E. T.	87	1.0000	0.0000	98	0.9847	0.0153	0.0000	100	0.7550	0.0000	0.2350	0.0100
Constantine Ck. E. U.	100	1.0000	0.0000	100	0.9850	0.0150	0.0000	100	0.7050	0.0150	0.2700	0.0100
Koppen Ck. E. T.	100	1.0000	0.0000	98	0.9949	0.0051	0.0000	100	0.7350	0.0050	0.2600	0.0000
Koppen Ck. E. U.	100	1.0000	0.0000	85	1.0000	0.0000	0.0000	100	0.7200	0.0050	0.2700	0.0050
Koppen Ck. L. T.	100	1.0000	0.0000	97	0.9948	0.0052	0.0000	100	0.7500	0.0100	0.2350	0.0050
Koppen Ck. L. U.	100	1.0000	0.0000	95	0.9789	0.0211	0.0000	99	0.7374	0.0051	0.2525	0.0051
Lagoon Ck. E. U.	95	1.0000	0.0000	96	0.9948	0.0000	0.0052	100	0.7000	0.0050	0.2950	0.0000
Mink Ck. E. T.	100	0.9950	0.0050	99	0.9747	0.0253	0.0000	100	0.6800	0.0150	0.2900	0.0150
Mink Ck. E. U.	92	1.0000	0.0000	96	0.9896	0.0104	0.0000	100	0.7400	0.0100	0.2450	0.0050
Mink Ck. L. T.	90	0.9944	0.0056	88	0.9716	0.0284	0.0000	90	0.6944	0.0000	0.3056	0.0000
Mink Ck. L. U.	100	1.0000	0.0000	97	0.9897	0.0103	0.0000	100	0.7600	0.0050	0.2350	0.0000
Olsen Ck. E. T.	100	1.0000	0.0000	95	0.9947	0.0053	0.0000	100	0.7700	0.0100	0.2100	0.0100
Olsen Ck. E. U.	98	0.9898	0.0102	100	1.0000	0.0000	0.0000	98	0.7602	0.0051	0.2296	0.0051
Olsen Ck. L. T.	100	0.9900	0.0100	95	0.9895	0.0105	0.0000	100	0.6900	0.0100	0.2900	0.0100
Olsen Ck. L. U.	100	0.9900	0.0100	96	0.9896	0.0104	0.0000	100	0.7100	0.0000	0.2800	0.0100
Rocky Ck. E. U.	100	0.9900	0.0100	97	0.9691	0.0309	0.0000	100	0.7050	0.0150	0.2600	0.0200

Population	sMDH-A1,2*				sMDH-B1,2*				
	N	*100*	*-32*	*50*	N	*100*	*124*	*66*	*69*
Constantine Ck. E. T.	100	1.0000	0.0000	0.0000	100	0.9700	0.0075	0.0225	0.0000
Constantine Ck. E. U.	100	0.9975	0.0000	0.0025	100	0.9500	0.0200	0.0275	0.0025
Koppen Ck. E. T.	100	0.9950	0.0025	0.0025	100	0.9775	0.0100	0.0125	0.0000
Koppen Ck. E. U.	100	0.9950	0.0000	0.0050	100	0.9600	0.0125	0.0250	0.0025
Koppen Ck. L. T.	100	0.9875	0.0125	0.0000	100	0.9650	0.0225	0.0125	0.0000
Koppen Ck. L. U.	100	1.0000	0.0000	0.0000	100	0.9525	0.0325	0.0150	0.0000
Lagoon Ck. E. U.	100	1.0000	0.0000	0.0000	100	0.9650	0.0100	0.0250	0.0000
Mink Ck. E. T.	100	1.0000	0.0000	0.0000	100	0.9725	0.0125	0.0150	0.0000
Mink Ck. E. U.	100	1.0000	0.0000	0.0000	100	0.9750	0.0125	0.0125	0.0000
Mink Ck. L. T.	90	0.9972	0.0000	0.0028	90	0.9750	0.0083	0.0167	0.0000
Mink Ck. L. U.	99	0.9975	0.0025	0.0000	100	0.9700	0.0150	0.0150	0.0000
Olsen Ck. E. T.	100	0.9950	0.0000	0.0050	100	0.9700	0.0100	0.0175	0.0025
Olsen Ck. E. U.	100	0.9975	0.0000	0.0025	100	0.9500	0.0150	0.0350	0.0000
Olsen Ck. L. T.	100	1.0000	0.0000	0.0000	100	0.9700	0.0200	0.0100	0.0000
Olsen Ck. L. U.	100	0.9950	0.0000	0.0050	100	0.9600	0.0225	0.0175	0.0000
Rocky Ck. E. U.	100	1.0000	0.0000	0.0000	99	0.9621	0.0152	0.0227	0.0000

Population	mMEP-1*				mMEP-2*			mMDH-2*		
	N	*100*	*123*	*84*	N	*100*	*70*	N	*100*	*s*
Constantine Ck. E. T.	100	0.9650	0.0300	0.0050	100	1.0000	0.0000	100	1.0000	0.0000
Constantine Ck. E. U.	100	0.9300	0.0700	0.0000	100	1.0000	0.0000	100	1.0000	0.0000
Koppen Ck. E. T.	100	0.9500	0.0500	0.0000	100	1.0000	0.0000	100	1.0000	0.0000
Koppen Ck. E. U.	100	0.9750	0.0250	0.0000	100	1.0000	0.0000	100	1.0000	0.0000
Koppen Ck. L. T.	100	0.9400	0.0600	0.0000	100	1.0000	0.0000	99	1.0000	0.0000
Koppen Ck. L. U.	100	0.9550	0.0450	0.0000	100	0.9950	0.0050	97	1.0000	0.0000
Lagoon Ck. E. U.	100	0.9300	0.0700	0.0000	100	0.9950	0.0050	100	1.0000	0.0000
Mink Ck. E. T.	100	0.9450	0.0550	0.0000	100	1.0000	0.0000	100	1.0000	0.0000
Mink Ck. E. U.	100	0.9650	0.0350	0.0000	100	0.9900	0.0100	96	1.0000	0.0000
Mink Ck. L. T.	90	0.9778	0.0222	0.0000	90	1.0000	0.0000	89	1.0000	0.0000
Mink Ck. L. U.	100	0.9250	0.0750	0.0000	100	1.0000	0.0000	99	1.0000	0.0000
Olsen Ck. E. T.	100	0.9650	0.0350	0.0000	100	1.0000	0.0000	99	1.0000	0.0000
Olsen Ck. E. U.	100	0.9500	0.0500	0.0000	100	0.9950	0.0050	100	1.0000	0.0000
Olsen Ck. L. T.	100	0.9500	0.0500	0.0000	100	0.9950	0.0050	99	1.0000	0.0000
Olsen Ck. L. U.	100	0.9650	0.0350	0.0000	100	1.0000	0.0000	99	1.0000	0.0000
Rocky Ck. E. U.	100	0.9500	0.0500	0.0000	100	1.0000	0.0000	99	0.9949	0.0051

Population		*MPI**			*NTP**			*PGDH**		
	N	*100*	*94*	N	*100*	*53*	N	*100*	*96*	*86*
Constantine Ck. E. T.	100	1.0000	0.0000	100	1.0000	0.0000	100	0.8450	0.0950	0.0600
Constantine Ck. E. U.	100	0.9950	0.0050	100	1.0000	0.0000	100	0.8800	0.0950	0.0250
Koppen Ck. E. T.	100	0.9900	0.0100	100	1.0000	0.0000	100	0.8300	0.1450	0.0250
Koppen Ck. E. U.	100	1.0000	0.0000	100	1.0000	0.0000	100	0.8450	0.1400	0.0150
Koppen Ck. L. T.	100	1.0000	0.0000	100	1.0000	0.0000	100	0.9200	0.0500	0.0300
Koppen Ck. L. U.	100	1.0000	0.0000	100	0.9950	0.0050	100	0.8700	0.1050	0.0250
Lagoon Ck. E. U.	100	0.9900	0.0100	100	1.0000	0.0000	100	0.8600	0.1000	0.0400
Mink Ck. E. T.	100	1.0000	0.0000	99	1.0000	0.0000	100	0.8700	0.1050	0.0250
Mink Ck. E. U.	100	1.0000	0.0000	94	1.0000	0.0000	100	0.8350	0.1450	0.0200
Mink Ck. L. T.	89	1.0000	0.0000	90	0.9944	0.0056	90	0.8444	0.1167	0.0389
Mink Ck. L. U.	100	1.0000	0.0000	98	0.9949	0.0051	100	0.7800	0.1850	0.0350
Olsen Ck. E. T.	100	1.0000	0.0000	100	0.9950	0.0050	100	0.8450	0.1200	0.0350
Olsen Ck. E. U.	100	0.9900	0.0100	98	1.0000	0.0000	100	0.8650	0.1150	0.0200
Olsen Ck. L. T.	100	0.9950	0.0050	100	0.9900	0.0100	100	0.9050	0.0850	0.0100
Olsen Ck. L. U.	100	1.0000	0.0000	100	1.0000	0.0000	100	0.8950	0.0800	0.0250
Rocky Ck. E. U.	100	1.0000	0.0000	97	1.0000	0.0000	100	0.8750	0.1000	0.0250

Population		*PGM-2**			*PEPD-2**				
	N	*100*	*155*	N	*100*	*120*	*80*	*110*	*84*
Constantine Ck. E. T.	100	0.9200	0.0800	100	0.6800	0.2050	0.1100	0.0050	0.0000
Constantine Ck. E. U.	100	0.9250	0.0750	100	0.7100	0.1750	0.1150	0.0000	0.0000
Koppen Ck. E. T.	100	0.8900	0.1100	100	0.7750	0.1150	0.1050	0.0000	0.0050
Koppen Ck. E. U.	100	0.9100	0.0900	100	0.7550	0.1600	0.0800	0.0050	0.0000
Koppen Ck. L. T.	100	0.8750	0.1250	100	0.6950	0.2200	0.0800	0.0050	0.0000
Koppen Ck. L. U.	100	0.9250	0.0750	100	0.6900	0.1700	0.1350	0.0050	0.0000
Lagoon Ck. E. U.	100	0.9050	0.0950	100	0.6950	0.2400	0.0650	0.0000	0.0000
Mink Ck. E. T.	100	0.9550	0.0450	100	0.6850	0.2450	0.0700	0.0000	0.0000
Mink Ck. E. U.	100	0.9100	0.0900	100	0.7300	0.1550	0.1150	0.0000	0.0000
Mink Ck. L. T.	90	0.9444	0.0556	90	0.6278	0.2500	0.1222	0.0000	0.0000
Mink Ck. L. U.	100	0.9300	0.0700	100	0.6850	0.2300	0.0750	0.0100	0.0000
Olsen Ck. E. T.	100	0.8950	0.1050	100	0.6900	0.2050	0.1050	0.0000	0.0000
Olsen Ck. E. U.	100	0.9000	0.1000	100	0.6300	0.2450	0.1100	0.0150	0.0000
Olsen Ck. L. T.	100	0.9200	0.0800	100	0.6850	0.2000	0.1150	0.0000	0.0000
Olsen Ck. L. U.	100	0.9400	0.0600	100	0.7150	0.1500	0.1300	0.0050	0.0000
Rocky Ck. E. U.	100	0.9750	0.0250	100	0.6350	0.2500	0.1050	0.0100	0.0000

Population	mSOD*					sSOD-2*		
	N	*100*	*32*	*200*	*118*	N	*100*	*121*
Constantine Ck. E. T.	100	1.0000	0.0000	0.0000	0.0000	100	0.9850	0.0150
Constantine Ck. E. U.	100	0.9900	0.0050	0.0000	0.0050	100	0.9950	0.0050
Koppen Ck. E. T.	100	1.0000	0.0000	0.0000	0.0000	99	0.9949	0.0051
Koppen Ck. E. U.	100	1.0000	0.0000	0.0000	0.0000	100	0.9950	0.0050
Koppen Ck. L. T.	99	0.9899	0.0101	0.0000	0.0000	99	0.9747	0.0253
Koppen Ck. L. U.	97	0.9897	0.0103	0.0000	0.0000	97	0.9897	0.0103
Lagoon Ck. E. U.	100	1.0000	0.0000	0.0000	0.0000	100	1.0000	0.0000
Mink Ck. E. T.	100	0.9900	0.0100	0.0000	0.0000	100	0.9900	0.0100
Mink Ck. E. U.	100	0.9950	0.0050	0.0000	0.0000	100	0.9850	0.0150
Mink Ck. L. T.	89	0.9944	0.0056	0.0000	0.0000	89	0.9719	0.0281
Mink Ck. L. U.	99	1.0000	0.0000	0.0000	0.0000	99	0.9899	0.0101
Olsen Ck. E. T.	100	1.0000	0.0000	0.0000	0.0000	99	0.9949	0.0051
Olsen Ck. E. U.	100	1.0000	0.0000	0.0000	0.0000	100	0.9750	0.0250
Olsen Ck. L. T.	99	0.9949	0.0051	0.0000	0.0000	99	0.9798	0.0202
Olsen Ck. L. U.	99	0.9949	0.0051	0.0000	0.0000	99	0.9848	0.0152
Rocky Ck. E. U.	99	0.9697	0.0253	0.0051	0.0000	99	0.9798	0.0202

Population	TPI-2*			
	N	*-100*	*110*	*174*
Constantine Ck. E. T.	100	0.9550	0.0100	0.0350
Constantine Ck. E. U.	100	0.9600	0.0000	0.0400
Koppen Ck. E. T.	100	0.9900	0.0050	0.0050
Koppen Ck. E. U.	100	0.9550	0.0000	0.0450
Koppen Ck. L. T.	100	1.0000	0.0000	0.0000
Koppen Ck. L. U.	100	1.0000	0.0000	0.0000
Lagoon Ck. E. U.	100	0.9700	0.0050	0.0250
Mink Ck. E. T.	100	0.9850	0.0000	0.0150
Mink Ck. E. U.	100	0.9900	0.0000	0.0100
Mink Ck. L. T.	90	0.9944	0.0000	0.0056
Mink Ck. L. U.	99	1.0000	0.0000	0.0000
Olsen Ck. E. T.	100	0.9850	0.0050	0.0100
Olsen Ck. E. U.	100	0.9850	0.0000	0.0150
Olsen Ck. L. T.	100	0.9900	0.0050	0.0050
Olsen Ck. L. U.	100	1.0000	0.0000	0.0000
Rocky Ck. E. U.	100	0.9950	0.0000	0.0050

Appendix B. Hierarchical analysis using likelihood ratios for pink salmon collected in 1995 from Prince William Sound. Within the "Overall" columns, one asterisk indicates significance at the 0.05 level after adjusting for multiple comparisons (modified from Miliken and Johnson 1984 and Rice 1989).

Source of Variation	DF	*sAAT3**	DF	*sAAT4**	DF	*ADA1**	DF	*ADA2**	DF	*mAH3**	DF	*sAH**	DF	*mAH4**	DF	*AK**
Between streams	3	7.26	6	14.38	3	4.28	3	5.08	3	4.37	6	7.86	9	14.46	3	7.31
Within Streams	10	7.92	20	21.76	10	8.55	10	9.65	10	13.23	20	20.73	30	43.96	10	5.64
Constantine Ck.	1	1.18	2	3.13	1	0.00	1	0.84	1	0.41	2	1.38	3	0.67	1	2.76
Between Elevation	1	1.18	2	3.13	1	0.00	1	0.84	1	0.41	2	1.38	3	0.67	1	2.76
Koppen Ck.	3	1.95	6	2.68	3	2.78	3	0.33	3	0.74	6	7.69	9	15.14	3	0.00
Between Timing	1	0.15	2	1.33	1	1.39	1	0.23	1	0.34	2	2.15	3	8.65	1	0.00
Within Timing	2	1.80	4	1.35	2	1.39	2	0.10	2	0.40	4	5.54	6	6.49	2	0.00
Early	1	0.90	2	0.99	1	0.00	1	0.00	1	0.06	2	1.38	3	2.26	1	0.00
Late	1	0.90	2	0.36	1	1.39	1	0.10	1	0.34	2	4.16	3	4.23	1	0.00
Between Elevation	1	1.80	2	1.28	1	1.39	1	0.03	1	0.03	2	0.51	3	4.48	1	0.00
Within Elevation	2	0.16	4	1.39	2	1.39	2	0.28	2	0.72	4	7.18	6	10.66	2	0.00
Tidal	1	0.08	2	0.49	1	0.00	1	0.02	1	0.72	2	5.57	3	9.76	1	0.00
Upstream	1	0.08	2	0.90	1	1.39	1	0.26	1	0.00	2	1.61	3	0.90	1	0.00
Mink Ck.	3	2.70	6	9.58	3	3.02	3	3.16	3	4.93	6	4.53	9	6.64	3	2.88
Between Timing	1	2.48	2	0.50	1	0.00	1	1.86	1	0.40	2	1.64	3	3.63	1	2.88
Within Timing	2	0.22	4	9.08	2	3.02	2	1.30	2	4.53	4	2.89	6	3.01	2	0.00
Early	1	0.00	2	6.07	1	0.00	1	0.02	1	0.00	2	1.41	3	2.26	1	0.00
Late	1	0.22	2	3.01	1	3.02	1	1.28	1	4.53	2	1.48	3	0.75	1	0.00
Between Elevation	1	0.13	2	4.48	1	1.16	1	0.77	1	2.40	2	1.84	3	1.64	1	0.00
Within Elevation	2	2.56	4	5.10	2	1.85	2	2.40	2	2.53	4	2.67	6	4.99	2	2.87
Tidal	1	0.71	2	3.73	1	0.47	1	0.21	1	0.85	2	1.30	3	0.57	1	1.49
Upstream	1	1.85	2	1.37	1	1.38	1	2.19	1	1.68	2	1.37	3	4.42	1	1.38
Olsen Ck.	3	2.09	6	6.37	3	2.75	3	5.32	3	7.15	6	7.13	9	21.51	3	0.00
Between Timing	1	1.50	2	5.38	1	2.75	1	0.01	1	2.80	2	3.31	3	14.33	1	0.00
Within Timing	2	0.59	4	0.99	2	0.00	2	5.31	2	4.35	4	3.82	6	7.18	2	0.00
Early	1	0.00	2	0.40	1	0.00	1	4.70	1	4.35	2	2.43	3	5.98	1	0.00
Late	1	0.59	2	0.59	1	0.00	1	0.61	1	0.00	2	1.39	3	1.20	1	0.00
Between Elevation	1	0.32	2	0.72	1	0.00	1	0.88	1	2.80	2	1.58	3	5.38	1	0.00
Within Elevation	2	1.77	4	5.65	2	2.74	2	4.44	2	4.35	4	5.55	6	16.13	2	0.00
Tidal	1	1.51	2	4.04	1	1.38	1	1.72	1	4.35	2	1.37	3	13.18	1	0.00
Upstream	1	0.26	2	1.61	1	1.36	1	2.72	1	0.00	2	4.18	3	2.95	1	0.00

Appendix B, continued

Source of Variation	DF	*ALAT**	DF	*CKA1**	DF	*CKA2**	DF	*CKC2**	DF	*FH**	DF	*FDHG**	DF	*bGALA**	DF	*GDA1**
Between streams	9	12.88	3	12.91	3	5.09	3	5.16	3	10.96	12	19.12	9	7.44	6	8.99
Within Streams	30	39.35	10	14.49	10	5.46	10	17.68	10	10.26	40	49.31	30	64.71	20	31.95
Constantine Ck.	3	2.72	1	0.00	1	0.00	1	0.00	1	1.38	4	4.14	3	8.77	2	5.17
Between Elevation	3	2.72	1	0.00	1	0.00	1	0.00	1	1.38	4	4.14	3	8.77	2	5.17
Koppen Ck.	9	18.29	3	2.38	3	0.00	3	2.74	3	8.88	12	11.77	9	17.66	6	15.67
Between Timing	3	9.92	1	1.14	1	0.00	1	1.41	1	6.96	4	4.73	3	3.09	2	14.12
Within Timing	6	8.37	2	1.24	2	0.00	2	1.33	2	1.92	8	7.04	6	14.57	4	1.55
Early	3	0.00	1	1.04	1	0.00	1	1.33	1	1.92	4	1.46	3	9.07	2	0.89
Late	3	8.37	1	0.20	1	0.00	1	0.00	1	0.00	4	5.58	3	5.50	2	0.66
Between Elevation	3	5.41	1	1.19	1	0.00	1	1.38	1	1.90	4	4.24	3	1.50	2	0.03
Within Elevation	6	12.87	2	1.20	2	0.00	2	1.36	2	6.96	8	7.52	6	16.16	4	15.64
Tidal	3	4.16	1	0.19	1	0.00	1	0.00	1	5.58	4	4.49	3	11.08	2	12.38
Upstream	3	8.71	1	1.01	1	0.00	1	1.36	1	1.38	4	3.03	3	5.08	2	3.26
Mink Ck.	9	10.38	3	4.36	3	5.46	3	6.37	3	0.00	12	20.07	9	22.39	6	4.64
Between Timing	3	4.60	1	2.11	1	2.67	1	3.46	1	0.00	4	7.75	3	12.56	2	2.68
Within Timing	6	5.78	2	2.25	2	2.79	2	2.91	2	0.00	8	12.32	6	9.83	4	1.96
Early	3	3.80	1	1.38	1	2.79	1	1.32	1	0.00	4	7.79	3	9.54	2	0.91
Late	3	1.98	1	0.87	1	0.00	1	1.59	1	0.00	4	4.53	3	0.29	2	1.05
Between Elevation	3	5.57	1	1.80	1	2.87	1	0.63	1	0.00	4	10.45	3	6.34	2	0.96
Within Elevation	6	4.81	2	2.56	2	2.58	2	5.74	2	0.00	8	9.63	6	16.05	4	3.68
Tidal	3	1.60	1	1.49	1	2.58	1	0.02	1	0.00	4	2.54	3	10.58	2	3.48
Upstream	3	3.21	1	1.07	1	0.00	1	5.72	1	0.00	4	7.09	3	5.47	2	0.20
Olsen Ck.	9	7.96	3	7.75	3	0.00	3	8.57	3	0.00	12	13.33	9	15.89	6	6.47
Between Timing	3	0.99	1	2.07	1	0.00	1	7.97	1	0.00	4	7.07	3	10.96	2	2.56
Within Timing	6	6.97	2	5.68	2	0.00	2	0.60	2	0.00	8	6.26	6	4.93	4	3.91
Early	3	4.85	1	2.75	1	0.00	1	0.60	1	0.00	4	0.39	3	1.67	2	2.43
Late	3	2.12	1	2.93	1	0.00	1	0.00	1	0.00	4	5.87	3	3.26	2	1.48
Between Elevation	3	2.41	1	5.04	1	0.00	1	0.63	1	0.00	4	5.02	3	2.10	2	3.37
Within Elevation	6	5.55	2	2.70	2	0.00	2	7.93	2	0.00	8	8.31	6	13.78	4	3.10
Tidal	3	3.71	1	1.33	1	0.00	1	5.25	1	0.00	4	2.93	3	8.66	2	2.41
Upstream	3	1.84	1	1.37	1	0.00	1	2.68	1	0.00	4	5.38	3	5.12	2	0.69

Appendix B, continued

Source of Variation	DF	*PEPA**	DF	*G3PDH1**	DF	*G3PDH2**	DF	*GPIA**	DF	*GR**	DF	*sIDHP2**	DF	*LDHA1**	DF	*LGL**
Between streams	3	4.43	3	2.91	6	6.89	3	10.19	3	3.75	9	27.06	3	3.72	3	10.94
Within Streams	10	9.12	10	12.69	20	23.56	10	2.77	10	8.20	30	32.74	10	8.32	10	6.27
Constantine Ck.	1	4.02	1	0.09	2	0.30	1	0.00	1	0.00	3	0.10	1	0.00	1	0.00
Between Elevation	1	4.02	1	0.09	2	0.30	1	0.00	1	0.00	3	0.10	1	0.00	1	0.00
Koppen Ck.	3	2.77	3	8.75	6	17.37	3	0.00	3	0.00	9	14.27	3	0.00	3	0.00
Between Timing	1	1.04	1	1.00	2	13.23	1	0.00	1	0.00	3	1.23	1	0.00	1	0.00
Within Timing	2	1.73	2	7.75	4	4.14	2	0.00	2	0.00	6	13.04	2	0.00	2	0.00
Early	1	0.34	1	4.88	2	4.06	1	0.00	1	0.00	3	5.93	1	0.00	1	0.00
Late	1	1.39	1	2.87	2	0.08	1	0.00	1	0.00	3	7.11	1	0.00	1	0.00
Between Elevation	1	0.00	1	0.25	2	2.10	1	0.00	1	0.00	3	1.73	1	0.00	1	0.00
Within Elevation	2	2.78	2	8.51	4	15.27	2	0.00	2	0.00	6	12.54	2	0.00	2	0.00
Tidal	1	2.78	1	1.63	2	6.81	1	0.00	1	0.00	3	2.02	1	0.00	1	0.00
Upstream	1	0.00	1	6.88	2	8.46	1	0.00	1	0.00	3	10.52	1	0.00	1	0.00
Mink Ck.	3	1.63	3	0.19	6	2.68	3	2.77	3	5.43	9	7.23	3	2.71	3	2.79
Between Timing	1	0.57	1	0.02	2	1.38	1	0.00	1	2.65	3	2.42	1	1.33	1	0.00
Within Timing	2	1.06	2	0.17	4	1.30	2	2.77	2	2.78	6	4.81	2	1.38	2	2.79
Early	1	1.06	1	0.12	2	0.12	1	2.77	1	2.78	3	2.90	1	1.38	1	1.30
Late	1	0.00	1	0.05	2	1.18	1	0.00	1	0.00	3	1.91	1	0.00	1	1.49
Between Elevation	1	0.80	1	0.16	2	0.59	1	0.94	1	2.67	3	3.07	1	1.34	1	2.79
Within Elevation	2	0.84	2	0.03	4	2.10	2	1.83	2	2.76	6	4.17	2	1.37	2	0.00
Tidal	1	0.84	1	0.00	2	2.04	1	1.49	1	0.00	3	2.25	1	0.00	1	0.00
Upstream	1	0.00	1	0.03	2	0.06	1	0.34	1	2.76	3	1.92	1	1.37	1	0.00
Olsen Ck.	3	0.70	3	3.66	6	3.21	3	0.00	3	2.77	9	11.14	3	5.61	3	3.48
Between Timing	1	0.00	1	3.57	2	1.14	1	0.00	1	1.39	3	5.54	1	2.79	1	0.66
Within Timing	2	0.70	2	0.09	4	2.07	2	0.00	2	1.38	6	5.60	2	2.82	2	2.82
Early	1	0.36	1	0.09	2	0.25	1	0.00	1	0.00	3	3.27	1	2.82	1	2.82
Late	1	0.34	1	0.00	2	1.82	1	0.00	1	1.38	3	2.33	1	0.00	1	0.00
Between Elevation	1	0.00	1	0.05	2	1.54	1	0.00	1	1.38	3	4.29	1	2.79	1	0.70
Within Elevation	2	0.70	2	3.60	4	1.67	2	0.00	2	1.39	6	6.85	2	2.82	2	2.78
Tidal	1	0.34	1	2.21	2	1.43	1	0.00	1	1.39	3	6.17	1	0.00	1	2.78
Upstream	1	0.36	1	1.39	2	0.24	1	0.00	1	0.00	3	0.68	1	2.82	1	0.00

Appendix B, continued

Source of Variation	DF	*PEPB1**	DF	*PEPLT**	DF	*sMDHA1,2**	DF	*sMDHB1,2**	DF	*mMEP1**	DF	*mMEP2**	DF	*MPI**	DF	*NTP**
Between streams	3	6.07	9	3.38	3	11.76	6	7.26	3	0.43	3	2.01	3	4.19	3	2.94
Within Streams	10	12.36	30	30.67	10	13.97	20	22.38	10	14.75	10	10.97	10	11.43	10	10.06
Constantine Ck.	1	0.00	3	4.98	1	0.00	2	2.87	1	3.41	1	0.00	1	1.38	1	0.00
Between Elevation	1	0.00	3	4.98	1	0.00	2	2.87	1	3.41	1	0.00	1	1.38	1	0.00
Koppen Ck.	3	6.00	9	2.89	3	11.24	6	9.92	3	3.26	3	2.76	3	5.55	3	2.76
Between Timing	1	2.74	3	0.97	1	2.90	2	6.59	1	1.05	1	1.38	1	2.77	1	1.38
Within Timing	2	3.26	6	1.92	2	8.34	4	3.33	2	2.21	2	1.38	2	2.78	2	1.38
Early	1	1.25	3	1.45	1	1.38	2	2.48	1	1.76	1	0.00	1	2.78	1	0.00
Late	1	2.01	3	0.47	1	6.96	2	0.85	1	0.45	1	1.38	1	0.00	1	1.38
Between Elevation	1	0.85	3	0.74	1	8.33	2	2.73	1	1.87	1	1.38	1	2.77	1	1.38
Within Elevation	2	5.15	6	2.15	2	2.92	4	7.18	2	1.39	2	1.38	2	2.78	2	1.38
Tidal	1	0.00	3	2.00	1	2.92	2	2.00	1	0.19	1	0.00	1	2.78	1	0.00
Upstream	1	5.15	3	0.15	1	0.00	2	5.18	1	1.20	1	1.38	1	0.00	1	1.38
Mink Ck.	3	2.92	9	14.25	3	2.73	6	0.97	3	6.98	3	5.45	3	0.00	3	2.83
Between Timing	1	0.01	3	8.15	1	1.44	2	0.12	1	0.10	1	2.67	1	0.00	1	2.83
Within Timing	2	2.91	6	6.10	2	1.29	4	0.85	2	6.88	2	2.78	2	0.00	2	0.00
Early	1	1.25	3	2.51	1	0.00	2	0.09	1	0.93	1	2.78	1	0.00	1	0.00
Late	1	1.66	3	3.59	1	1.29	2	0.76	1	5.95	1	0.00	1	0.00	1	0.00
Between Elevation	1	2.90	3	4.63	1	1.34	2	0.44	1	1.04	1	2.67	1	0.00	1	0.00
Within Elevation	2	0.03	6	9.62	2	1.39	4	0.53	2	5.94	2	2.78	2	0.00	2	2.82
Tidal	1	0.03	3	7.80	1	0.00	2	0.35	1	2.80	1	0.00	1	0.00	1	1.48
Upstream	1	0.00	3	1.82	1	1.39	2	0.18	1	3.14	1	2.78	1	0.00	1	1.34
Olsen Ck.	3	3.44	9	8.55	3	0.00	6	8.62	3	1.10	3	2.76	3	4.50	3	4.47
Between Timing	1	2.00	3	4.87	1	0.00	2	5.58	1	0.00	1	0.00	1	0.34	1	0.33
Within Timing	2	1.44	6	3.68	2	0.00	4	3.04	2	1.10	2	2.76	2	4.16	2	4.14
Early	1	1.44	3	0.82	1	0.00	2	2.14	1	0.55	1	1.38	1	2.78	1	1.36
Late	1	0.00	3	2.86	1	0.00	2	0.90	1	0.55	1	1.38	1	1.38	1	2.78
Between Elevation	1	0.23	3	2.05	1	0.00	2	2.91	1	0.00	1	0.00	1	0.34	1	4.14
Within Elevation	2	3.20	6	6.49	2	0.00	4	5.71	2	1.10	2	2.76	2	4.16	2	0.34
Tidal	1	0.34	3	3.44	1	0.00	2	2.71	1	0.55	1	1.38	1	1.38	1	0.34
Upstream	1	2.86	3	3.05	1	0.00	2	3.00	1	0.55	1	1.38	1	2.78	1	0.00

Appendix B, continued

Source of Variation	DF	*PGDH**	DF	*PGM2**	DF	*PEPD2**	DF	*mSOD**	DF	*sSOD2**	DF	*TPI2**	DF	Overall	*P* - value
Between streams	6	11.84	3	6.55	6	9.26	3	1.21	3	1.34	6	21.38	177	307.06 *	0.0000
Within Streams	20	30.99	10	10.25	20	32.58	10	12.58	10	10.88	20	38.78	590	732.34 *	0.0001
Constantine Ck.	2	3.11	1	0.03	2	0.78	1	1.39	1	1.05	2	2.84	59	59.03	0.4744
Between Elevation	2	3.11	1	0.03	2	0.78	1	1.39	1	1.05	2	2.84	59	59.03	0.4744
Koppen Ck.	6	13.94	3	3.24	6	12.90	3	5.64	3	4.34	6	24.27	177	260.96 *	0.0000
Between Timing	2	9.03	1	0.00	2	6.01	1	5.64	1	3.05	2	15.40	59	131.20 *	0.0000
Within Timing	4	4.91	2	3.24	4	6.89	2	0.00	2	1.29	4	8.87	118	129.76	0.2164
Early	2	0.55	1	0.44	2	2.79	1	0.00	1	0.00	2	8.87	59	60.36	0.4264
Late	2	4.36	1	2.80	2	4.10	1	0.00	1	1.29	2	0.00	59	69.40	0.1668
Between Elevation	2	1.68	1	2.73	2	0.35	1	0.00	1	1.01	2	8.81	59	64.03	0.3045
Within Elevation	4	12.26	2	0.50	4	12.55	2	5.64	2	3.32	4	15.46	118	196.87 *	0.0000
Tidal	2	10.68	1	0.21	2	9.07	1	2.80	1	2.95	2	2.78	59	106.20 *	0.0002
Upstream	2	1.58	1	0.29	2	3.48	1	2.84	1	0.37	2	12.68	59	90.67 *	0.0050
Mink Ck.	6	7.86	3	3.66	6	11.76	3	2.76	3	2.36	6	4.38	177	205.88	0.0676
Between Timing	2	2.85	1	0.06	2	2.50	1	0.93	1	0.47	2	2.70	59	82.55	0.0232
Within Timing	4	5.01	2	3.60	4	9.26	2	1.83	2	1.89	4	1.68	118	123.33	0.3501
Early	2	1.54	1	3.27	2	6.58	1	0.34	1	0.20	2	0.20	59	69.55	0.1637
Late	2	3.47	1	0.33	2	2.68	1	1.49	1	1.69	2	1.48	59	53.78	0.6677
Between Elevation	2	4.92	1	2.90	2	2.88	1	1.15	1	0.45	2	0.78	59	79.64	0.0380
Within Elevation	4	2.93	2	0.76	4	8.88	2	1.60	2	1.92	4	3.61	118	126.23	0.2854
Tidal	2	0.76	1	0.22	2	3.21	1	0.23	1	1.73	2	0.85	59	57.85	0.5180
Upstream	2	2.17	1	0.54	2	5.67	1	1.37	1	0.19	2	2.76	59	68.38	0.1888
Olsen Ck.	6	6.08	3	3.32	6	7.14	3	2.79	3	3.13	6	7.29	177	206.47	0.0640
Between Timing	2	3.82	1	2.69	2	3.82	1	2.79	1	0.08	2	2.93	59	106.20 *	0.0002
Within Timing	4	2.26	2	0.63	4	3.32	2	0.00	2	3.05	4	4.36	118	100.27	0.8797
Early	2	0.89	1	0.02	2	1.87	1	0.00	1	2.91	2	1.58	59	58.04	0.5109
Late	2	1.37	1	0.61	2	1.45	1	0.00	1	0.14	2	2.78	59	42.23	0.9513
Between Elevation	2	0.05	1	0.39	2	0.26	1	0.00	1	0.69	2	2.77	59	54.98	0.6244
Within Elevation	4	6.02	2	2.93	4	6.88	2	2.78	2	2.44	4	4.52	118	151.44	0.0206
Tidal	2	4.55	1	0.74	2	0.10	1	1.39	1	1.95	2	0.34	59	85.54	0.0136
Upstream	2	1.47	1	2.19	2	6.78	1	1.39	1	0.49	2	4.18	59	65.90	0.2505

Appendix C. Allele socres for polymorphic allozyme loci from parents of 17 pink salmon matings done in 1995 from Armin F. Koernig Hatchery broodstock in 1995. Within each cross, the first line is the dam and the second is the sire. Progeny from these parents will be analyzed for inheritance. Highlighted alleles have not been tested for inheritance in pink salmon.

Cross	Fish #	*ADA2**	*AK**	*sAAT3**	*sAAT4**	*mAH3**	*sAH**	*CKC2**	*FDHG**	*G3PDH1**	*G3PDH2**	*GAPDH2**	*GDA**	*GPIB1,2**	*bGALA**	*IDDH**
1	103	13		12	22			12					12		12	
	53				12								27			
2	104				12								15			
	54			12	22											
3	114		0	12							12		23			
	64												22			
4	115		0	12	22											
	65				12						23		15			
5	120		0		12								25			
	66	13			12								12			
6	2			12	22								12			
	57				12											
7	7			12	12				12				27			
	51			12									12			
8	8				12		12									
	62	13			12								12			
9	9															
	93			12									12			
10	14			12	12	12							23			
	94			22	22				12				22			
11	20									13	12					
	78			12	12						22		12			
12	21			12	22					13						12
	63				12								12			
13	28			12	12				12			12	12			
	80		13	12	12									14		
14	29										13					
	92			12	12								17			
15	42			12	12	12							23			
	77				12								25			
16	43	13			12				12			13	13			
	53				12								27			
17	50				12								16			
	85			12												

Appendix C continued.

Cross	Fish #	*sIDHP2**	*sMDHA1,2*	*sMDHB1,2*	*mMEP1**	*mMEP2**	*PEPB1**	*PEPD2**	*PEPLT**	*PGDH**	*PGM2**	*mSOD**	*sSOD2**	*TPI3**	*TPI4**
1	103							12							
	53						12								13?
2	104	12						12		13		13			
	54														
3	114	15						12	0						
	64	13						12			12				
4	115	13						12	0	14					
	65							12	13						
5	120	12						12					12		
	66			1311					33	13				13?	
6	2								13	14					
	57	12							13	33					
7	7	12						12	13						
	51	12							0						
8	8	12			12				13						
	62			2211				13							
9	9	15						13	13						
	93	13						23	0	13					
10	14	12		1311				13	13						
	94	22													
11	20	12							33						
	78	12	1411					13	12						
12	21					12		22		14	12				
	63	12		1311					13						
13	28							13	33						
	80	13													
14	29			1211				12		13		13			
	92	12						13							
15	42	12						23	23						
	77	13			12					33	12				
16	43	22						23							
	53						12								13?
17	50	23						12	13	13					
	85	12		1211						13					

Appendix D: Genetic Interpretation of Broad-Scale Microsatellite Polymorphism in Odd-Year Pink Salmon

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Abstract

We examined genetic variation at five microsatellite loci in 12 odd-year populations and one even-year population of North American pink salmon *Oncorhynchus gorbuscha* from six geographic regions. The degree of polymorphism varied widely among loci. The total number of alleles in the odd-year samples varied from four (*One*μ3) to 53 (*Ssa*85). A probability test revealed significant heterogeneity in allele frequencies among all odd-year samples, and among pooled odd-year samples from six regions. We compared estimates of a standard index of population structure (R) based on variance in allele frequency with a new index suggested for microsatellites (R_{ST}) based on variance in allele size. Our results suggest $\hat{\theta}$ is a better estimator of intra-lineage (odd-year x odd-year) population structure while $\hat{\rho}_{ST}$ is best suited for estimating inter-lineage (odd-year x even-year) population structure. The difference in performance of $\hat{\theta}$ and $\hat{\rho}_{ST}$ for estimating intra- and inter-lineage population structure suggests high migration rates and possibly low divergence times are dominant influences on genetic population structure in odd-year pink salmon. We showed statistical support for genetic isolation by distance and geographically correlated allele frequency clines, suggesting broad-scale gene flow is best described by a linear stepping stone model. An analysis of molecular variation showed weak but significant regional structuring under two different population grouping schemes. Our results suggest broad-scale population aggregations of odd-year pink salmon are temporally stable but that differentiation is weak presumably due to migration.

Introduction

The pink salmon *Oncorhynchus gorbuscha* is the most abundant species of Pacific salmon and is of significant economic, ecological, and cultural importance to coastal communities of the Pacific rim (Heard 1991). Spawning populations are present in freshwater drainages on both east and west shores of the Pacific Ocean north of about 40°N (Heard 1991). Like most *Oncorhynchus*, pink salmon are anadromous and semelparous. They are philopatric and often exhibit temporal separation of spawning aggregations within drainages (Heard 1991; Bue et al. 1996).

Pink salmon in their native range exhibit a rigid two-year life cycle that has resulted in two reproductively isolated odd- and even-year lineages (Davidson 1934, Aspinwall 1974). Genetic studies using allozyme loci show divergence of the two lineages is the most significant genetic subdivision in pink salmon (Beacham et al. 1985; Beacham et al. 1988, Zhivotovsky et al. 1994). Spawning aggregates within each lineage also reveal significant population structure (Gharrett et al. 1988; Shaklee et al. 1991; Varnavskaya and Beacham 1992; Shaklee and Varnavskaya 1994; Hard et al 1996; but see Omelchenko 1994). Recent studies of mitochondrial DNA variation in Russian (Brykov et al. 1996) and Alaskan (Seeb et al. 1996) pink salmon also show evidence of genetic structure with the greatest variation occurring between odd- and even-year populations.

Estimates of genetic distance among large coastal aggregates of odd-year pink salmon (e.g. Russia, Alaska, British Columbia and Washington) generated from allozyme frequency data appear to support a model of genetic isolation by distance (Varnavskaya and Beacham 1992; Shaklee and Varnavskaya 1994). Phenetic analyses suggest North American odd-year populations form two distinct groups; populations from Alaska and Northern British Columbia

and populations from southern British Columbia and Washington State (Varnavskaya and Beacham 1992; Shaklee and Varnavskaya 1994). However, the statistical validity of these putative regional groupings and genetic isolation by distance have not been tested. In addition, no studies have examined the genetic structure of odd-year populations from a broad geographic range (e.g. North America) using a different class of genetic marker. Concordant results from a different genetic marker would strengthen support for inferred genetic relationships based solely on allozymes (Avise 1994).

Microsatellites are a class of nuclear DNA markers that are highly polymorphic and abundant in eukaryotic genomes surveyed to date (Tautz 1989). They consist of 1-5 base pair (bp) repeating sequences that form arrays <300 bp in length and exhibit high levels of co-dominant allelic variation in repeat number (Wright 1992; O'Reilly and Wright 1995). In vitro studies suggests microsatellites mutate via a process of slipped-strand mispairing during DNA replication, resulting in length changes of one or more repeat units (Levinson and Gutman 1987). This process is probably best described by a modification of the single step mutation model called the two phase mutation model (Di Rienzo et al. 1994). The mutation rate is exceptionally high (10^{-3} to 10^{-5} per generation) for many loci (Weber and Wong 1993; Ellegren 1995).

Microsatellites have potential for a variety of genetic studies of salmonids including population genetics and kinship analysis (Bentzen et al. 1994; O'Reilly and Wright 1995). However, it is not clear how to make best use of microsatellite data given the apparent high rate and stepwise mode of mutation (see Jarne and Lagoda 1996). Recent theoretical efforts have focused on developing new statistics to estimate genetic distance and genetic population structure from microsatellite data based on a stepwise mutation model (Slatkin 1995; Goldstein et al. 1995). Slatkin (1995) introduced the parameter R_{ST} as an index of subpopulation structure

analogous to θ defined by Weir and Cockerham (1984). In contrast to θ, R_{ST} accounts for differences in allele size under a stepwise mutation model rather than simple identity or non-identity of allelic states under an infinite allele model (Slatkin 1995). Simulation studies reveal that R_{ST} is a more accurate estimate of genetic structure when migration is small, the difference in average coalescence times within and between populations is large, and the mutation rate is high (e.g. 10^{-3}) (Slatkin 1995). However, R_{ST} and θ approach equality as migration increases, the average coalescence times converge, and the mutation rate decreases (Slatkin 1995). Unfortunately, these simulations do not capture the complexity of interactions between continuous population parameters such as migration rate and average coalescence time, complicating the choice of which statistic to use. Further, these parameters are rarely known with any certainty. Only empirical analysis, comparing both statistics for estimating genetic population structure, will clarify which makes best use of the microsatellite data for a particular taxa (Forbes et al. 1995).

The purpose of this study was to use microsatellite loci to extend our knowledge of genetic variation in North American odd-year pink salmon. We sampled five loci in 13 populations (12 odd- and 1 even-year) from six geographic regions. Our objectives were to test for significant inter-population allelic variation; compare θ and R_{ST} to determine which is the most appropriate measure of population subdivision; test for genetic isolation by distance among odd-year populations; test for significant regional and population level genetic structure. Our results provide one of the first broad-scale estimates of intra-specific microsatellite polymorphism in Pacific salmon.

Methods

Sample collection and preparation

We sampled 12 populations comprising one pair from each of six geographic regions representing the North American range of odd-year pink salmon (Figure 1). In addition, we included one even-year population from Southcentral Alaska. Sample sizes ranged from 20 to 52 (Figure 1). Tissue samples (heart and liver) from adult pink salmon were stored at -80°C prior to DNA extraction. Total genomic DNA was extracted from approximately 50-100 mg of frozen heart or liver tissue using a Gentra Systems™ (Minneapolis MN) Puregene DNA isolation kit. Precipitated DNA was hydrated in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) and heated at 55°C for approximately 12 h. The DNA concentration was measured by spectrophotometry and diluted to 100 ng/μL for use in the polymerase chain reaction (PCR).

Microsatellite selection and multilocus genotyping

Primer pairs for 21 microsatellite loci were chosen among 51 previously screened in pink salmon (see Olsen et al. 1996; Scribner et al. 1996) and tested in at least four individuals to re-evaluate amplification potential. All PCRs were performed with a Perkin Elmer 9600 thermocycler in a 10 μL volume (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.5 units *Taq* polymerase, 0.1-0.5 μM each primer, and 100 ng DNA template). Primers were purchased from Operon Inc. (Alameda, CA). One primer of each pair contained one of three fluorescent labels for fragment detection by the Perkin Elmer-Applied Biosystems Inc. (ABI) 377 Prism™ semi-automated fluorescent detection system.

We were able to amplify 11 loci, five of which were selected for use in this study based on quality of the PCR product and the presence of at least two alleles in at least four fish. Four loci (*One*μ3, Scribner et al. 1996; *Ots*1, D. Hedgecock, U.C.D., personal communication; μ*Sat*60,

Estoup et al. 1993; *Ssa*85, O'Reilly et al. 1996) consisted of arrays comprised of dinucleotide repeats, and one (*Ssa*197, O'Reilly et al. 1996) of primarily tetra-nucleotide repeats. We tested PCR co-amplification (multiplexing) using various primer pair combinations following the methods described in Olsen et al. (1996). The multiplex system consisted of three PCRs (*One*μ3 and *Ots*1, anneal at 52°C; μ*Sat*60 and *Ssa*197, anneal at 58°C; *Ssa*85, anneal at 58°C). The following PCR profile was used: 1 cycle of (94°C (2 min)) + 7 cycles of (94°C (1 min) + X°C (30 sec) + 72°C (15 sec)) + Y cycles of (94°C (30 sec) + X°C (30 sec) + 72°C (15 sec)), where X (annealing temperature) and Y (cycles) varied among microsatellites.

Microsatellites were size fractionated using the ABI 377 Prism™ in GeneScan™ mode (ABI 1996). For each sample approximately 0.5 μL from each of three PCRs was combined with 2.5 μL formamide, 0.50 μL 25 mM EDTA and 0.5 μL (1.0 fmol) Perkin-Elmer GS350 internal size standard in a 0.5 mL microcentifuge tube. The samples were denatured at 95°C for approximately 3 min, chilled on ice, and loaded on a 4.5% denaturing polyacrylamide gel. Approximately 2.5 μL from each sample was loaded per well. Each gel was run for approximately 2 h at 3000 V. Data were analyzed using the internal lane sizing standard and local Southern sizing algorithm in the GeneScan analysis software, ver. 2.1 (ABI 1996). Scoring of alleles for each locus and tabulation of data for importing into statistical software was performed with Genotyper software, ver. 1.1 (ABI 1994).

Statistical analysis

The number of alleles and allelic range were computed for each locus and population. Tests for conformity to Hardy-Weinberg equilibrium (HWE), genotypic linkage disequilibrium, and independence between populations and allele frequency were performed for all populations using the probability test in GENEPOP ver. 3.1 (Raymond and Rousset 1995). A Markov chain

method was used to provide an unbiased estimate of the "exact" *P*-value (Guo and Thompson 1992) except for tests of HWE at loci with fewer than 5 alleles. Statistical significance levels (α) for the probability tests were determined using sequential Bonferroni adjustments for simultaneous tests (Rice 1989).

We compared the statistics $\hat{\theta}$ and $\hat{\rho}_{ST}$ as measures of subpopulation structure in pink salmon. Rousset (1996) showed that $\hat{\rho}_{ST}$ is analogous to $\hat{R}_{ST}$ but uses the same sample weighting scheme as $\hat{\theta}$. Each statistic was computed for all odd-year population pairs (66 pair) and all odd- x even-year population pairs (12 pair) using GENEPOP. A Wilcoxon signed ranks test (Zar 1984, pg. 153-154) was used to determine if $\hat{\rho}_{ST}$ was significantly greater than $\hat{\theta}$ for the within lineage (odd- x odd-year) and between lineage (even- x odd-year) population pairs. We computed the coefficient of variation of $\hat{\theta}$ and $\hat{\rho}_{ST}$ for all between region population pairs of odd-year pink salmon to estimate the precision of each statistic.

We tested for isolation by distance between odd-year populations from each regional sample location using $\hat{\theta}$ and $\hat{\rho}_{ST}$ as measures of genetic distance. Geographic distance was measured as the shortest straight line coastal route between regions on a map of coastal North America (U.S. Geological Survey 1976). Statistical significance was tested using a Spearman rank correlation coefficient implemented in the "Mantel" test module in GENEPOP, and was based on 15,000 permutations of the data. The probability value of the observed data given the null hypothesis (no correlation between genetic distance and geographic distance) was calculated from the distribution of test statistics following the permutations.

We tested statistical significance of allele frequency clines at two loci (*One*μ3, μ*Sat*60). Both loci exhibited alleles at high frequencies that appeared to be geographically correlated.

Such a pattern could result when migration follows a linear stepping stone model and mutation rate is much smaller than migration rate (Hartl and Clark 1989). We plotted frequency of the common allele versus geographic distance from Northwest Alaska and tested the significance of the slope of a best fit linear model using the statistical software STATISTICA (StatSoft Inc., Tulsa, OK.).

We conducted a hierarchical gene diversity analysis using an analysis of molecular variation (AMOVA) for diploid data (see Michalakis and Excoffier 1996) as implemented in ARLEQUIN ver. 1.0 (Schneider et al. 1996). A distance matrix of the number of different alleles for each pair of haplotypes was used to compute a global estimate of percent genetic variation within and between populations and $\hat{\theta}$ for all twelve odd-year samples. We then tested for regional genetic structure at two levels by grouping odd-year populations and partitioning variation into within region ($\hat{\theta}_{WR}$) and between region ($\hat{\theta}_{BR}$) components. Two aggregation strategies were used for grouping populations based on geographic location. First, we grouped pairs of populations from the six sample locations to form six regional groups. Second, we grouped eight populations from the northern sample locations (Northwest Alaska, Southcentral Alaska, Southeast Alaska, North British Columbia) and four populations from the southern sample locations (South British Columbia, Northwest Washington) to form two regional groups. The second strategy emulated the genetic structure hypothesized in earlier allozyme studies (Varnavskaya and Beacham 1992; Shaklee and Varnavskaya 1994). Significance testing of the different gene diversity components followed the scheme in Excoffier et al. (1992): first, individuals are permuted among populations to obtain a null distribution of $\hat{\theta}$. Second, the regions are assumed real and individuals are permuted within each region without regard to

population to obtain a null distribution of $\hat{\theta}_{WR}$. Third, the populations are assumed real and whole populations are permuted across regions to obtain a null distribution of $\hat{\theta}_{BR}$. Fifteen thousand permutations were run to estimate the probability of having a higher value of $\hat{\theta}$, $\hat{\theta}_{WR}$ and $\hat{\theta}_{BR}$ than those observed by chance alone.

Results

Microsatellite genotyping

Microsatellite loci *Ots*1, μ*Sat*60 and *Ssa*197 were scored automatically using Genotyper. *One*μ3 amplification products exhibited single base "stutter" bands typical of variable adenylation (Magnuson et al. 1996); alleles at this locus were scored manually by selecting the longest fragment (size in nucleotide bases) as the "true" allele. *Ssa*85 amplification products exhibited extensive "stutter" so we developed criteria based on allelic patterns of parents and offspring from three full-sib families to manually score each individual. *Ssa*197 exhibited several unique characteristics so its results are described separately below.

*Genetic variation at microsatellite Ssa*197

Two observations distinguished *Ssa*197 from the other loci. First, we identified one tetra- and 16 tri-allelic individuals in eight populations. Such an outcome is possible under certain scenarios given the tetraploid ancestry of Pacific salmon. However, other hypothesis may also explain these results including a tandem duplication event and non-specific primer annealing. Second, we found an apparent null allele as indicated by statistically significant heterozygote deficiency in eight populations (Table 1) and incomplete transmission of parental alleles in two full-sib families (J. Seeb, unpublished data). Microsatellite loci exhibiting null alleles may be useful genetic markers in some contexts (see for example Paetkau and Strobeck 1995; Brookfield

1996); however, sequencing is necessary to fully verify their presence. Alternatively, primers can be relocated away from the sequence mutation. Both options were beyond the scope of this study. For this reason, and because the explanation for apparent tri- and tetra-allelism in *Ssa*197 was not resolved, we did not include it in further statistical analysis of microsatellite variation.

*Genetic variation at microsatellites One*μ3, μ*Sat*60, *Ots*1 *and Ssa*85

The total number of alleles per locus for all odd-year samples ranged from four (*One*μ3) to 53 (*Ssa*85) (Table 1). Two microsatellites, μ*Sat*60 and *One*μ3, exhibited reciprocal latitudinal trends in frequency for alleles 109 and 162 respectively (Appendix 1). The 109 allele at μ*Sat*60 was most frequent in the Snake River population in Northwest Alaska (0.87), least frequent in the Gray Wolf River population in Northwest Washington (0.44) and averaged 0.66 among all odd-year samples. Conversely, the 162 allele at *One*μ3 was least frequent in the Nome River population in Northwest Alaska (0.53), most frequent in the Gray Wolf River population in Northwest Washington (0.85) and averaged 0.66 among all odd-year populations. The frequency of both alleles in the single even-year sample from Koppen Creek in Southcentral Alaska (μ*Sat*60 = 0.91 and *One*μ3 = 0.49) was most similar to the two Northwest Alaska populations.

The mean expected heterozygosity ($\hat{H}_E$) at each locus for all odd-year pink salmon ranged from 0.44 (*One*μ3) to 0.96 (*Ssa*85) and averaged 0.68. *One*μ3 and μ*Sat*60 exhibited moderate polymorphism ($\hat{H}_E$ = 0.44 and 0.46) while *Ots*1 and *Ssa*85 were highly polymorphic ($\hat{H}_E$ = 0.86 and 0.96). The mean $\hat{H}_E$ among populations was less variable, ranging from 0.64 (Snake River) to 0.71 (Khyex River) (Table 1). $\hat{H}_E$ for the even-year population ranged from 0.16 (μ*Sat*60) to 0.97 (*Ssa*85) and averaged 0.63. Tests for HWE revealed two populations (Babine River and Gray Wolf River) with significant heterozygote deficiencies for *Ssa*85 (Table 1).

Probability tests of non-random associations between genotypes for all pairs of loci for each population resulted in only two probability values less than 0.05. Neither value was significant when the critical value (0.05) was adjusted for 78 simultaneous tests.

We found significant heterogeneity in allele frequencies among the 13 pink salmon samples ($P < 0.001$), among all odd-year samples ($P < 0.001$), and among pooled odd-year samples from each geographic region ($P < 0.001$). Allele frequencies in the pooled odd-year sample varied significantly from the single even-year sample ($P < 0.001$). We found significant allele frequency heterogeneity among the two northwest Washington samples ($P < 0.001$) but not the other intra-regional pairs of odd-year samples.

Comparison of θ *and* ρ_{ST}

Values of $\hat{\rho}_{ST}$ and $\hat{\theta}$ were generally quite low (Table 2). We found no significant difference between $\hat{\rho}_{ST}$ and $\hat{\theta}$ (Wilcoxon signed ranks test, $P > 0.50$) for the 66 odd-year population pairs (open circles, Figure 2). However, $\hat{\rho}_{ST}$ was larger than $\hat{\theta}$ (Wilcoxon signed ranks test, $P < 0.001$) for the cross lineage population pairs (solid squares, Figure 2). Global values for $\hat{\rho}_{ST}$ and $\hat{\theta}$ over all loci were 0.026 and 0.022 for all odd-year samples and 0.169 and 0.032 for the pooled odd- x even-year sample. Values of $\hat{\rho}_{ST}$ and $\hat{\theta}$ for each locus for all odd-year samples were 0.020 and 0.022 (*One*μ3), 0.007 and 0.019 (*Ots*1), 0.071 and 0.058 (μ*Sat*60), 0.028 and 0.007 (*Ssa*85). Estimates of the coefficient of variation (CV) for $\hat{\rho}_{ST}$ were more than twice the estimate for $\hat{\theta}$ for all but the Southcentral Alaska x North British Columbia odd-year population pairs (Table 3).

Geographic patterns: Isolation by distance and allele frequency clines

Three Mantel tests were performed using $\hat{\theta}$ and $\hat{\rho}_{ST}$ values from all odd-year population pairs. Geographic distances used for all inter-regional pairs of populations are shown in Table 3. The first test indicated a highly significant correlation between $\hat{\theta}$ and geographic distance ($P < 0.0005$) for population pairs from all sample regions (Figure 3). For the second test we removed the Northwest Alaska populations and tested for correlation among the five southern regions. The probability value increased but was still significant ($P < 0.003$). The third test indicated a marginally significant correlation between $\hat{\rho}_{ST}$ and geographic distance ($P = 0.051$) for all population pairs from all regions.

A best fit linear model was used to explain the relationship between allele frequency and geographic location for microsatellites *One*μ3 (allele 162, $R^2 = 0.63$, $P < 0.002$) and μ*Sat*60 (allele 109, $R^2 = 0.74$, $P < 0.001$) (Figure 4). We removed the Northwest Alaska populations to test the geographic basis of allele frequency clines among the five southern regions. The probability values increased but were still significant (allele 162, $P < 0.004$; allele 109, $P = 0.034$).

Genetic population structure

The hierarchical gene diversity analysis indicated significant genetic population structure (Table 4). The estimate of percent genetic variation that occurred among all odd-year populations was 2.25% ($p < 0.001$), with the remainder (97.75%) occurring within populations. Both regional pooling schemes revealed significant between region ($\hat{\theta}_{BR}$) variation (two regions, $P < 0.008$; six regions, $P < 0.001$). The within region component ($\hat{\theta}_{WR}$) was significant for the two region scheme ($P < 0.001$) but not for the six region scheme ($P > 0.24$).

Discussion

Comparison of broad-scale polymorphism among loci

The wide range in polymorphism among the four dinucleotide loci in odd-year pink salmon is consistent with data from other salmonids (O'Reilly and Wright 1995) and is suggestive of widely varying mutational properties among loci. Allele length variants differ by multiples of two bp which is consistent with length change via replication slippage (of one, two or more repeats) and could favor a single or two phase mutation model (Schlotterer and Tautz 1992; DiRienzo et al. 1994). Mutation is generally considered a diversifying force enhancing variation among populations. However, if microsatellite array length is limited, a high mutation rate may act as a homogenizing factor, counteracting the diversifying effects of genetic drift (Garza et al. 1995; Nauta and Weissing 1996). Nauta and Weissing (1996) showed the rate at which allele frequency distributions converge is a function of the mutation rate, population size, time since divergence and maximum number of alleles. Using simulations, Nauta and Weissing (1996) demonstrated that measures of population divergence may be underestimated when the number of allelic states is constrained and mutation rate is high (say 10^{-3} to 10^{-4}). This relationship is likely oversimplified because the mutational properties of microsatellites are poorly understood. However, some empirical evidence may support their theoretical conclusions. Bowcock et al. (1994) showed that microsatellite loci with the highest heterozygosity (presumably due to higher mutation rates) had significantly lower $\hat{F}_{ST}$ values in a broad-scale study of human populations. The present study, while not containing enough loci for statistical analysis, showed a similar trend. The two most polymorphic loci (*Ots*1 and *Ssa*85) exhibited the lowest values of $\hat{\theta}$. These results must be interpreted with caution though because they are based

on a single statistic applied to loci with potentially different mutational properties. Loci with higher mutation rates (more polymorphic) may tend to have lower values of $\hat{\theta}$, which measures variance in allele frequency, irrespective of constraints on allele size. The fact that we saw no trend between locus heterozygosity and $\hat{\rho}_{ST}$, that measures variance in allele size, suggests this is the case in our study. Analysis of additional loci and larger sample sizes are needed to adequately test the theoretical relationship between mutation rate and estimates of population divergence. This is especially true for teleost fishes in which microsatellite loci vary widely in heterozygosity (Brooker et al. 1994; O'Reilly and Wright, 1995) and the number of alleles often exceeds 20, well beyond the limitations imposed by Nauta and Weissing (1996).

Genetic population structure and patterns of gene flow

Our comparison of $\hat{\rho}_{ST}$ and $\hat{\theta}$ suggests the latter is the better index of population structure in odd-year pink salmon, regardless of geographic distance between populations. The values of the two measures were not significantly different among regions, but $\hat{\theta}$ was more precise (lower coefficient of variation) presumably because relatively few new mutations have accrued within populations Slatkin (1995). Slatkin (1995) showed $\hat{\theta}$ approaches $\hat{\rho}_{ST}$ as migration increases, average coalescence times within and between populations converge and mutation rate decreases. We could not estimate all three parameters from the present data. However, it was instructive to consider results of the odd-year pairs in light of results from the odd-year/even-year pairs in which no migrants are exchanged and the time since divergence may be as great as 1 million years (Brykov et al. 1996, but see also Zhivotovsky et al. 1994). The fact that $\hat{\rho}_{ST}$ was significantly greater than $\hat{\theta}$ for the twelve odd-year/even-year pairs suggests the overall mutation rate is high and therefore a high migration rate and/or small difference in average coalescence

times are influencing genetic population structure in odd-year pink salmon. Further analysis should incorporate additional even-year populations and more loci to validate the relationship between $\hat{\rho}_{ST}$ and $\hat{\theta}$ within and between lineages.

We estimated over 97.7% of the total genetic variation was common to all populations sampled. The estimate of subpopulation structure ($\hat{\theta} = 0.023$, $P < 0.0001$) was low given the broad geographic range of our study. However, this values falls within the range reported for odd-year populations in earlier allozyme studies which is among the lowest for Pacific salmon (Beacham et al. 1988; Varnavskaya and Beacham 1992; Hard et al. 1996).

The geographic range to which pink salmon exhibit philopatry is a matter of debate (Varnavskaya and Beacham 1992; Omelchenko 1994; Zhivotovsky et al. 1994;). Some suggest that pink salmon are composed of unstable populations that fluctuate in time by citing the lack of regional heterogeneity in allele frequency at allozyme loci as well as tagging evidence of inter-population migration (see Omelchenko 1994 and references therein). Others support the concept of temporally stable pink salmon populations and show evidence of significant regional heterogeneity in allozyme allele frequency using a different suite of loci (Varnavskaya and Beacham 1992). The latter view is supported by recent studies of mtDNA variation in pink salmon showing little variation among rivers within regions but significant genetic heterogeneity between regions (Brykov et al. 1996; Seeb et al. 1996). We showed statistical support for genetically distinct regional spawning aggregates under two population aggregation schemes for North American odd-year pink salmon. The fact that $\hat{\theta}_{BR}$ was significant for both regional aggregation schemes suggest hierarchical structure may exist beyond the two levels tested here. However, further refinement of the ARLEQUIN program and additional sampling is needed to

test this. The fact that $\hat{\theta}_{BR}$ was significant for the two region aggregation scheme lends some statistical support to the two putative North American population clusters in the dendrogram by Shaklee and Varnavskaya (1994). Our microsatellite data suggests North American populations are temporally stable, at least on a broad scale (~ 500-5000 km) but that regional differentiation is weak presumably due to migration. A better understanding of the dynamics of pink salmon populations will require further genetic analysis as well as additional migration studies.

We did not detect significant intra-regional genetic variation for the six region pooling scheme using AMOVA. The probability test of allele frequency independence among population pairs within regions revealed only one instance of significant heterogeneity (Stillaguamish River and Gray Wolf River in Northwest Washington). Allozyme data show the Stillaguamish River and Gray Wolf River populations are part of two distinct lineages, Puget Sound and Hood Canal/Strait of Juan de Fuca, respectively (Busack and Shaklee 1995). In fact, the Puget Sound populations appear to be more closely related to Fraser River populations (South British Columbia populations in this study) than to Hood Canal/Strait of Juan de Fuca (Shaklee et al. 1991). The lack of differences in allele frequencies in any of the other intra-regional comparisons differs from other allozyme data (Beacham et al 1985; Beacham et al. 1988; Varnavskaya and Beacham 1992). This difference is best explained by the fact that our study included only two populations from each region and employed only four microsatellite loci. A more extensive analysis of microsatellite variation in a narrower geographic range using more populations and more loci may reveal significant intra-regional genetic heterogeneity.

Our microsatellite data revealed two significant broad-scale trends that suggest the extent and direction of migration among North American odd-year pink salmon populations is related to

distance and location. Significant allele frequency clines at two loci (Oneμ3, μSat60) suggest migration may operate under a linear stepping stone model where the allele frequency differences at the northern and southern ends of the geographic range are due to historical accident. It is possible that populations from Northwest Alaska and Northwest Washington / South British Columbia are descendent from two distinct lineages that persisted through glaciation in different refugia (Aspinwall 1974). Genetic evidence supporting northern and southern refugial populations have been reported for sockeye (Varnavskaya et al. 1994) and chum salmon (Seeb and Crane in press). Equal support for this hypothesis in odd-year pink salmon will require additional sampling, including populations from Asia, and more loci. The significant relationship between $\hat{\theta}$ and geographic distance for all odd-year population pairs provided, to our knowledge, the first statistical support for genetic isolation by distance (IBD) in pink salmon. Examples of significant correlation between genetic and geographic distance have been shown for chum (Kijima and Fujio 1982) and chinook salmon (Utter et al. 1993). In fact, Kijima and Fujio (1982) used their data to test hypothetical migration routes. Our data suggests no more than the pattern of broad-scale gene flow is structured such that migration among regions increases as geographic distance decreases. In total our examination of microsatellite polymorphism provides additional support to a model of temporally stable population aggregations in odd-year pink salmon.

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Table 1. Expected heterozygosity ($\hat{H}_E$) and number of alleles per locus (A) for each population/locus pair[a].

Region	Population (sample year)		*One*μ3	*Ots*1	μ*Sat*60	*Ssa*85	Avg.	*Ssa*197
Northwest AK.	Nome River (1991)	$\hat{H}_E$	0.51	0.86	0.25	0.97	0.65	0.89*
		A	2	14	3	34	13	15
	Snake River (1991)	$\hat{H}_E$	0.50	0.84	0.24	0.97	0.64	0.92
		A	3	16	4	34	14	13
Southcentral AK.	AFK Hatchery (1995)	$\hat{H}_E$	0.49	0.85	0.41	0.96	0.68	0.93*
		A	3	17	4	30	14	17
	Koppen Creek (1995)	$\hat{H}_E$	0.48	0.87	0.48	0.97	0.70	0.91*
		A	2	17	5	29	13	15
Southeast AK.	LPW Hatchery (1993)	$\hat{H}_E$	0.49	0.84	0.49	0.96	0.70	0.91*
		A	2	10	4	20	9	12
	Gastineau Hatchery (1993)	$\hat{H}_E$	0.45	0.89	0.49	0.96	0.70	0.91*
		A	2	13	5	19	10	13
North B.C.	Babine River (1993)	$\hat{H}_E$	0.47	0.81	0.51	0.95*	0.69	0.90*
		A	2	8	4	21	9	14
	Khyex River (1993)	$\hat{H}_E$	0.47	0.85	0.54	0.96	0.71	0.91*
		A	2	13	4	30	12	15
South B.C.	Fraser River (1995)	$\hat{H}_E$	0.39	0.89	0.55	0.96	0.70	0.94
		A	2	13	4	28	12	17
	Cayoosh Creek (1995)	$\hat{H}_E$	0.44	0.87	0.43	0.94	0.67	0.93
		A	2	15	4	23	11	17
Northwest WA.	Stillaguamish River (1995)	$\hat{H}_E$	0.37	0.87	0.56	0.94	0.69	0.93
		A	2	17	5	24	12	17
	Gray Wolf River (1995)	$\hat{H}_E$	0.26	0.87	0.62	0.94*	0.67	0.93*
		A	2	10	3	18	8	15
	Odd-year average[b]	$\hat{H}_E$	0.44	0.86	0.46	0.96	0.68	0.92
		A	2 (4)	14 (25)	4 (9)	26 (53)	11	15 (21)
Even-year	Koppen Creek (1994)	$\hat{H}_E$	0.51	0.86	0.16	0.97	0.63	0.94
		A	2	13	2	35	13	17

[a] Population/locus pairs with significant heterozygote deficiency based on HWE following sequential Bonferroni adjustment (initial $\alpha = 0.004$) are marked with an (*).

[b] Total number of alleles for all odd-year samples are shown in parentheses.

Table 2. Pairwise $\hat{\varrho}_{ST}$ (upper diagonal) and $\hat{\theta}$ (lower diagonal) at four microsatellite loci in twelve odd- and one even-year pink salmon populations from six regions in North America.

Region	Population (sample year)	1	2	3	4	5	6	7
Northwest AK.	1. Nome River (1991)	----	0.001	0.113	0.097	0.067	0.006	0.109
	2. Snake River (1991)	-0.002	----	0.040	0.026	0.005	-0.023	0.035
Southcentral AK.	3. AFK Hatchery (1995)	0.017	0.009	----	-0.008	-0.010	0.034	-0.006
	4. Koppen Creek (1995)	0.018	0.013	-0.004	----	-0.017	0.021	-0.009
Southeast AK.	5. LPW Hatchery (1993)	0.010	0.006	-0.005	-0.007	----	-0.002	-0.019
	6. Gastineau Hatchery (1993)	0.015	0.006	-0.004	-0.005	-0.014	----	0.030
North B.C.	7. Babine River (1993)	0.047	0.038	0.009	0.002	-0.001	0.004	----
	8. Khyex River (1993)	0.037	0.029	0.005	-0.004	-0.003	-0.005	-0.005
South B.C.	9. Fraser River (1995)	0.042	0.028	0.014	0.007	0.010	0.000	0.018
	10. Cayoosh Creek (1995)	0.036	0.028	0.017	0.008	0.008	0.004	0.022
Northwest WA.	11. Stillaguamish River (1995)	0.088	0.075	0.036	0.023	0.030	0.026	0.016
	12. Gray Wolf River (1995)	0.105	0.092	0.051	0.041	0.046	0.035	0.029
Even-year	13. Koppen Creek (1994)	0.005	0.006	0.014	0.023	0.017	0.023	0.049

Table 2. Extended

Region	Population (sample year)	8	9	10	11	12	13
Northwest AK.	1. Nome River (1991)	0.050	0.009	0.028	0.056	0.005	0.236
	2. Snake River (1991)	0.000	-0.014	-0.010	0.001	-0.004	0.151
Southcentral AK.	3. AFK Hatchery (1995)	0.010	0.060	0.029	0.020	0.106	0.057
	4. Koppen Creek (1995)	-0.002	0.044	0.017	0.006	0.093	0.077
Southeast AK.	5. LPW Hatchery (1993)	-0.016	0.025	0.004	-0.009	0.075	0.068
	6. Gastineau Hatchery (1993)	-0.009	-0.021	-0.020	-0.009	-0.007	0.154
North B.C.	7. Babine River (1993)	0.002	0.057	0.032	0.011	0.107	0.056
	8. Khyex River (1993)	----	0.008	-0.002	-0.010	0.036	0.109
South B.C.	9. Fraser River (1995)	0.008	----	-0.005	0.009	-0.002	0.196
	10. Cayoosh Creek (1995)	0.013	0.004	----	-0.001	0.016	0.162
Northwest WA.	11. Stillaguamish River (1995)	0.012	0.010	0.024	----	0.040	0.137
	12. Gray Wolf River (1995)	0.030	0.029	0.045	0.019	----	0.255
Even-year	13. Koppen Creek (1994)	0.044	0.053	0.046	0.097	0.119	----

Table 3. Geographic distance and coefficient of variation (CV) of $\hat{\theta}$ and $\hat{\varrho}_{ST}$ for all between region population pairs of odd-year pink salmon.

Regional pair	No. pop. pairs	Dist. (km)	CV $\hat{\theta}$	CV $\hat{\varrho}_{ST}$	CV $\hat{\varrho}_{ST}$ / CV $\hat{\theta}$
South B.C. x Northwest WA.	4	150	0.530	1.495	2.8
Southeast AK. x North B.C.	4	500	3.349	6.715	2.0
Southcentral AK. x Southeast AK.	4	700	0.271	3.501	12.9
North B.C. x South B.C.	4	800	0.386	1.098	2.8
North B.C. x Northwest WA.	4	950	0.412	1.415	3.4
Southcentral AK. x North B.C.	4	1200	1.773	4.385	2.5
Southeast AK. x South B.C.	4	1300	0.806	7.949	9.9
Southeast AK. x Northwest WA.	4	1450	0.262	3.294	12.6
Southcentral AK. x North B.C.	4	2000	0.428	0.492	1.1
Southcentral AK. x Northwest WA.	4	2150	0.308	0.899	2.9
Northwest AK. x Southcentral AK.	4	2500	0.297	0.617	2.1
Northwest AK. x Southeast AK.	4	3200	0.447	2.795	6.3
Northwest AK. x North B.C.	4	3700	0.190	0.934	4.9
Northwest AK. x South B.C.	4	4500	0.197	5.996	30.5
Northwest AK. x Northwest WA.	4	4650	0.137	1.919	14.0

Table 4. Hierarchical gene diversity analysis over all loci for three population grouping strategies[a]: None = one group consisting of all populations; Six regions = six groups consisting of population pairs from each region; Two regions = two groups consisting of the eight northern populations and four southern populations.

Grouping strategy	Source of variation	σ^2	% of total	$\hat{\theta}$	$\hat{\theta}_{BR}$	$\hat{\theta}_{WR}$
None	Total	1.37299	100.00			
	Within populations	1.34207	97.75			
	Between populations	0.03092	2.25	0.0225*		
Six regions	Total	1.37609	100.00			
	Within populations	1.34207	97.53			
	Between populations	0.03402	2.47	0.0247*		
	Between regions	0.03239	2.35		0.0235*	
	Between pop. within regions	0.00163	0.12			0.0012
Two regions	Total	1.38512	100.00			
	Within populations	1.34207	96.89			
	Between populations	0.04305	3.11	0.0311*		
	Between regions	0.02447	1.77		0.0177*	
	Between pop. within regions	0.01858	1.34			0.0137*

[a] (*) denotes $P < 0.01$ of not greater than zero.

Appendix 1. Allele frequencies for each population[a,b]

Locus-Allele (bp)	*Northwest Alaska* nom91	sna91	*Southcentral Alaska.* afk95	kop95	*Southeast Alaska* lpw93	gas93	*North British Columbia* bab93	khy93	*South British Columbia* fra95	cay95	*Northwest Washington* sti95	gra95	*Even-year* kop94
*One*μ3 n =	39	31	52	44	20	20	40	40	40	40	40	40	40
154	0	0.016	0	0	0	0	0	0	0	0	0	0	0
156	0	0	0.010	0	0	0	0	0	0	0	0	0	0
162	0.526	0.597	0.605	0.614	0.600	0.675	0.638	0.638	0.738	0.688	0.762	0.850	0.488
168	0.474	0.387	0.385	0.386	0.400	0.325	0.362	0.362	0.262	0.312	0.238	0.150	0.512
*Ots*1 n =	39	31	52	44	19	20	40	40	40	40	40	40	40
214	0	0	0	0	0	0	0	0.013	0	0	0	0	0
216	0	0.016	0.010	0	0	0.025	0	0.013	0.025	0	0.013	0.075	0
218	0	0.032	0	0	0	0	0.038	0.037	0	0	0.025	0	0
220	0.090	0.016	0.038	0.045	0.026	0.050	0	0.025	0	0.013	0.013	0.013	0.050
222	0	0.016	0	0.011	0.026	0.025	0	0	0.150	0.087	0.188	0.024	0.025
224	0.205	0.194	0.144	0.193	0.263	0.225	0.225	0.225	0.125	0.224	0.150	0.212	0.124
226	0.064	0.016	0.010	0.011	0	0	0	0	0	0.012	0	0	0.050
228	0.244	0.275	0.183	0.171	0.185	0.125	0.113	0.087	0.150	0.062	0.037	0.088	0.237
230	0.076	0.032	0	0.023	0	0.025	0.024	0.038	0.100	0.037	0.024	0.050	0.012
232	0.154	0.226	0.279	0.239	0.238	0.200	0.287	0.263	0.200	0.162	0.250	0.112	0.225
234	0.037	0.016	0.076	0.046	0.079	0.100	0.087	0.050	0.025	0.038	0.050	0.175	0.050
236	0.026	0.016	0.114	0.091	0.105	0.100	0.213	0.138	0.025	0.025	0.112	0.188	0.138
238	0.013	0.016	0.019	0.057	0	0.025	0.013	0.025	0.100	0.213	0.037	0.063	0.025
240	0.026	0.032	0.029	0.023	0.026	0.050	0	0.062	0.063	0.050	0.037	0	0
242	0	0	0	0.011	0	0	0	0	0.013	0.013	0.012	0	0.038
244	0.013	0	0.010	0.023	0	0.025	0	0.024	0.012	0	0.013	0	0.013
246	0.026	0.065	0.029	0.023	0	0.025	0	0	0.012	0.013	0.013	0	0
248	0	0	0.010	0.011	0.026	0	0	0	0	0.038	0.013	0	0.013
250	0	0	0.010		0.026	0	0	0	0	0.013	0.013	0	0
252	0.013	0.016	0	0	0	0	0	0	0	0	0	0	0
254	0	0.016	0	0	0	0	0	0	0	0	0	0	0
256	0	0	0	0.011	0	0	0	0	0	0	0	0	0
260	0.013	0	0.010	0.011	0	0	0	0	0	0	0	0	0
264	0	0	0.010	0	0	0	0	0	0	0	0	0	0
268	0	0	0.019	0	0	0	0	0	0	0	0	0	0
μ*Sat*60 n =	40	31	52	44	19	20	40	40	40	40	40	40	40
103	0.063	0.016	0	0	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0.088	0.012	0.012	0	0
107	0	0	0	0.011	0	0	0	0	0.024	0	0.012	0	0
109	0.863	0.871	0.731	0.670	0.684	0.700	0.600	0.588	0.613	0.713	0.488	0.438	0.913
111	0	0	0	0.023		0.025	0.012	0.025	0	0	0.025	0.137	0
113	0.074	0.097	0.240	0.284	0.211	0.175	0.375	0.337	0.275	0.263	0.463	0.425	0.087
115	0	0.016	0	0	0.053	0.025	0	0	0	0	0	0	0
117	0	0	0.019	0.012	0.052	0.075	0.013	0.050		0.012	0	0	0
121	0	0	0.010	0	0	0	0	0	0	0	0	0	0

Appendix 1. Extended

Locus-Allele (bp)	*Northwest Alaska*		*Southcentral Alaska.*		*Southeast Alaska*		*North British Columbia*		*South British Columbia*		*Northwest Washington*		*Even-year*
	nom91	sna91	afk95	kop95	lpw93	gas93	bab93	khy93	fra95	cay95	sti95	gra95	kop94
*Ssa*85 n =	38	31	52	44	18	19	40	40	40	40	40	40	40
137	0	0	0	0	0	0	0	0	0	0	0	0	0.013
141	0	0	0	0	0	0	0	0	0	0	0	0	0.013
147	0	0	0	0	0	0	0	0	0	0.013	0	0	0.013
151	0	0	0	0	0	0	0	0	0	0	0	0	0.025
153	0	0.016	0	0.011	0	0	0	0	0	0	0	0	0.013
155	0.013	0	0	0	0	0	0	0	0	0	0	0	0
157	0	0	0.058	0	0	0	0.013	0.025	0	0	0.013	0	0.025
159	0	0	0	0	0	0	0.075	0.013	0.013	0	0	0	0
161	0	0.016	0	0	0	0	0.013	0.025	0	0	0	0	0.050
163	0	0.016	0.010	0	0	0	0	0	0	0	0	0	0.025
165	0	0	0.010	0	0.028	0	0.050	0	0	0	0	0	0
167	0	0	0.010	0	0.056	0.053	0	0.013	0	0	0	0	0.038
169	0	0	0	0	0	0	0	0	0	0	0	0	0.038
171	0	0	0	0.057	0.028	0	0	0	0	0	0	0	0
173	0.013	0.016	0	0.011	0	0	0	0.013	0	0	0	0	0.013
175	0.013	0.016	0	0	0	0	0	0	0	0	0.025	0	0
177	0.013	0.016	0.010	0	0	0	0	0	0	0	0.013	0	0
179	0	0	0.010	0.034	0.056	0	0	0	0	0	0	0	0.013
181	0.013	0.016	0.029	0	0	0.026	0	0.025	0	0	0	0	0.013
183	0.039	0	0	0	0	0	0	0	0	0.013	0	0	0.038
185	0.013	0.016	0.029	0.034	0	0	0	0.013	0.013	0	0	0	0
187	0.026	0.048	0.010	0.011	0	0	0	0	0.013	0	0.013	0	0.025
189	0.013	0.032	0.048	0.034	0.028	0.026	0.013	0.038	0.025	0	0.013	0.013	0.038
191	0.013	0.032	0.019	0.034	0	0	0.013	0.038	0.038	0.013	0.050	0	0.088
193	0.039	0.032	0.058	0.034	0.028	0	0	0	0.050	0.038	0	0.100	0.013
195	0	0.032	0.029	0.023	0	0	0	0	0.013	0.113	0	0.025	0.050
197	0.066	0	0.029	0.091	0.028	0.026	0.075	0.063	0.013	0.063	0.113	0.013	0.013
199	0.039	0.016	0.038	0.034	0.056	0.079	0.025	0.050	0.088	0.075	0.050	0	0.038
201	0.013	0	0.106	0.034	0.083	0.079	0.025	0	0.063	0.063	0.088	0.075	0.063
203	0.013	0.032	0.048	0.057	0.056	0.079	0.075	0.075	0.100	0.125	0.063	0.075	0
205	0.013	0.048	0.048	0.057	0.056	0.105	0.050	0.113	0.075	0.013	0.088	0.075	0
207	0.026	0.016	0.019	0.045	0.139	0.026	0.113	0.050	0.025	0.075	0.088	0.050	0.025
209	0.026	0.016	0.038	0.068	0.028	0.053	0.075	0.075	0.075	0.113	0.150	0.063	0
211	0	0.032	0.058	0.068	0	0.053	0.088	0.038	0.050	0.013	0.025	0.100	0.088
213	0.053	0.113	0	0.023	0.083	0.079	0.063	0.038	0.038	0.063	0.050	0.013	0.025
215	0.053	0.032	0.038	0.034	0.056	0.053	0.075	0.075	0.050	0.025	0.013	0.063	0
217	0.079	0.048	0.019	0.045	0.028	0.026	0.038	0.025	0.038	0	0.025	0.075	0.013
219	0.053	0.032	0.067	0.034	0.056	0.026	0.063	0.025	0.038	0.025	0.013	0.138	0.025
221	0.013	0.065	0.048	0.023	0	0	0	0.038	0.013	0.038	0.025	0.038	0.038
223	0.026	0.032	0.029	0.011	0	0.105	0.038	0.013	0.025	0	0.013	0.013	0.025
225	0.066	0.048	0.010	0	0	0	0.013	0	0.013	0	0	0.025	0.013
227	0	0.016	0.029	0.023	0	0	0	0.025	0	0	0	0	0.013
229	0.039	0.016	0	0.011	0.028	0.026	0	0.013	0.038	0.038	0.013	0	0.050
231	0.013	0.032	0	0	0	0.053	0.013	0.013	0	0	0	0	0.013

Appendix 1. Extended

Locus-Allele (bp)	*Northwest Alaska* nom91	sna91	*Southcentral Alaska.* afk95	kop95	*Southeast Alaska* lpw93	gas93	*North British Columbia* bab93	khy93	*South British Columbia* fra95	cay95	*Northwest Washington* sti95	gra95	*Even-year* kop94
*Ssa*85 n =	38	31	52	44	18	19	40	40	40	40	40	40	40
233	0.013	0	0.038	0.034	0	0	0	0.013	0.013	0.025	0	0	0
235	0.053	0.016	0	0	0.056	0	0	0.013	0	0	0.013	0	0
237	0.039	0.016	0	0.011	0	0	0	0.025	0.038	0	0	0	0.013
239	0.026	0	0	0	0.028	0	0	0	0	0	0	0	0
241	0	0	0	0	0	0	0	0	0.025	0.013	0	0	0
243	0	0	0	0	0	0	0	0	0.013	0	0.025	0	0
245	0	0	0	0	0	0	0	0	0	0	0.025	0	0.013
247	0	0	0.010	0	0	0	0	0	0	0	0	0.050	0
249	0.013	0	0	0	0	0.026	0	0.013	0.013	0.013	0	0	0
251	0.026	0.032	0	0	0	0	0	0	0	0.025	0	0	0
253	0	0.016	0	0.011	0	0	0	0.013	0	0	0	0	0
255	0.026	0	0	0	0	0	0	0	0	0.013	0	0	0
263	0	0.016	0	0	0	0	0	0	0	0	0	0	0
*Ssa*197 n =	39^{1}	29^{1}	49^{2}	40^{0}	19^{0}	18^{1}	37^{1}	37^{0}	40^{0}	35^{5}	37^{3}	37^{3}	40^{0}
124	0	0.017	0	0	0	0	0	0.027	0	0	0	0	0.013
128	0.026	0	0.041	0	0.026	0	0	0	0	0.014	0	0	0
132	0.064	0.069	0.061	0.050	0	0.028	0.014	0.054	0.063	0.071	0.054	0	0.063
136	0.013	0.052	0.020	0.038	0.053	0.028	0.095	0.041	0.025	0.114	0.027	0.054	0.038
140	0.167	0.103	0.082	0.113	0.079	0.028	0.041	0.041	0.063	0.029	0.014	0.095	0.088
144	0.192	0.155	0.092	0.175	0.079	0.194	0.203	0.149	0.113	0.014	0.122	0.041	0.100
148	0.192	0.121	0.102	0.125	0.132	0.083	0.041	0.041	0.075	0	0.027	0.054	0.100
152	0.077	0.121	0.092	0.088	0.105	0.083	0.041	0.068	0.063	0.029	0.095	0.041	0.075
156	0.038	0.121	0.071	0.075	0.211	0.111	0.149	0.068	0.075	0.129	0.122	0	0.100
160	0.051	0.052	0.112	0.100	0.132	0	0.068	0	0.025	0.071	0.068	0	0.088
164	0.051	0.069	0.092	0.100	0.026	0.028	0.014	0.176	0.038	0.057	0.122	0.081	0.088
168	0.026	0.017	0.051	0.025	0	0.028	0.122	0.054	0.050	0.143	0.081	0.081	0.050
172	0.013	0.052	0.061	0.013	0.105	0.167	0.095	0.135	0.075	0.057	0.041	0.108	0.075
176	0.038	0.052	0.031	0	0.026	0.083	0.068	0.014	0.063	0.043	0.041	0.108	0.038
180	0.026	0	0.051	0.013	0.026	0.056	0.041	0.068	0.100	0.014	0.054	0.108	0.013
184	0.026	0	0.010	0.038	0	0.083	0	0.041	0.088	0.086	0.095	0.014	0
188	0	0	0.010	0.025	0	0	0.014	0	0.038	0.043	0.014	0.041	0.025
192	0	0		0.025	0	0	0	0.027	0.038	0.071	0.014	0.122	0.038
196	0	0	0.020	0	0	0	0	0	0.013	0.014	0.014	0	0
200	0	0	0	0	0	0	0	0	0	0	0	0.041	0
204	0	0	0	0	0	0	0	0	0	0	0	0.014	0.013

[a] Population codes were derived using the name (first three letters) and sample date (last two numbers) from figure 1.

[b] Number of individuals with more than two alleles at Ssa197 are denoted by superscript above the sample size

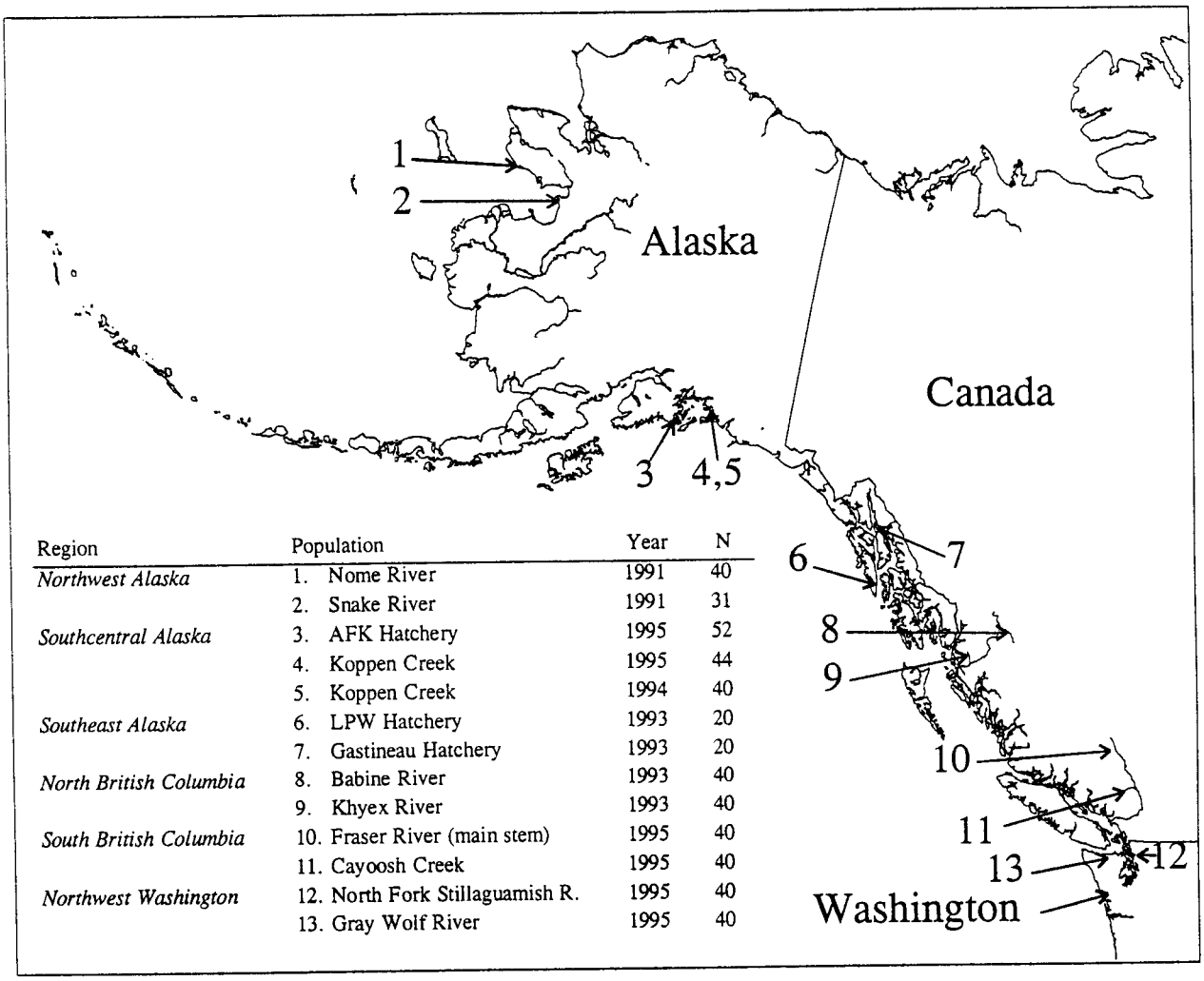
Figure legends

Figure 1. Sample location, year and sample size (N) for pink salmon populations used in this study.

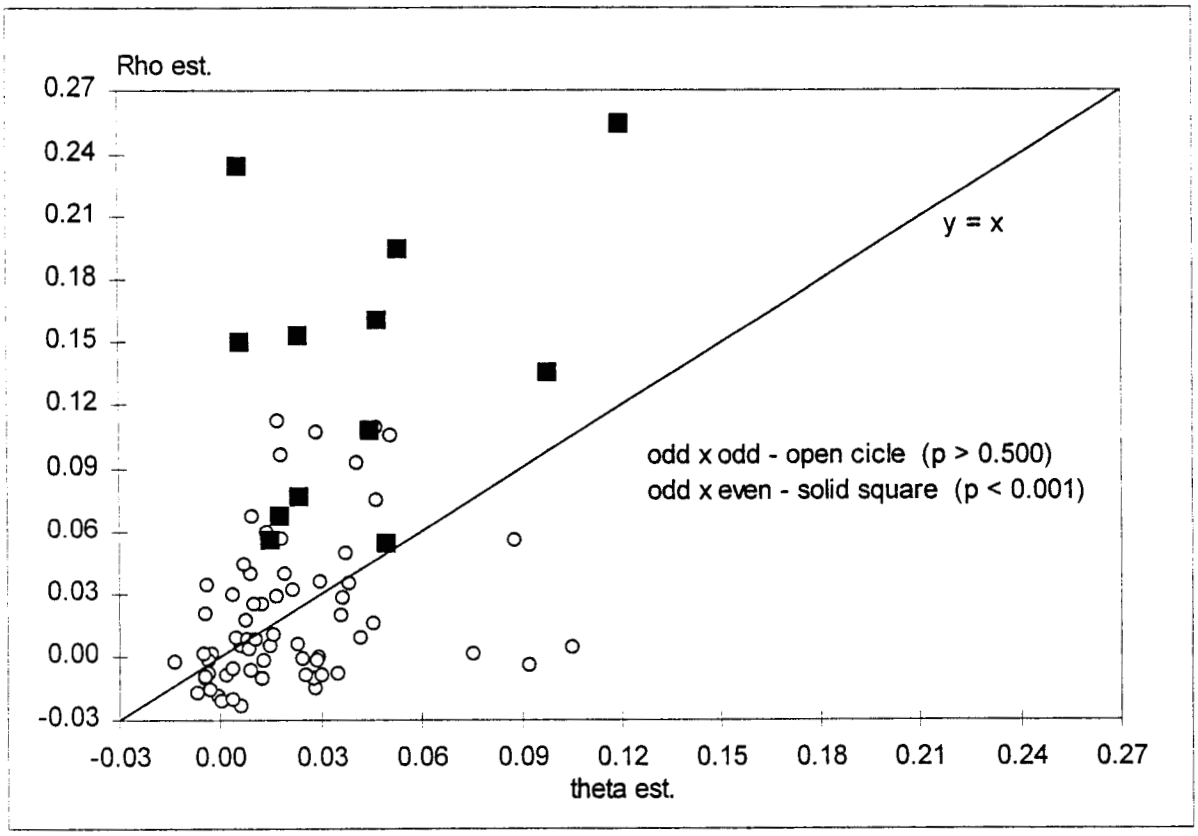
Figure 2. Scatter plot of $\hat{\rho}_{ST}$ versus $\hat{\theta}$ for all pink salmon population pairs. Statistical significance was tested using a Wilcoxon signed ranks test.

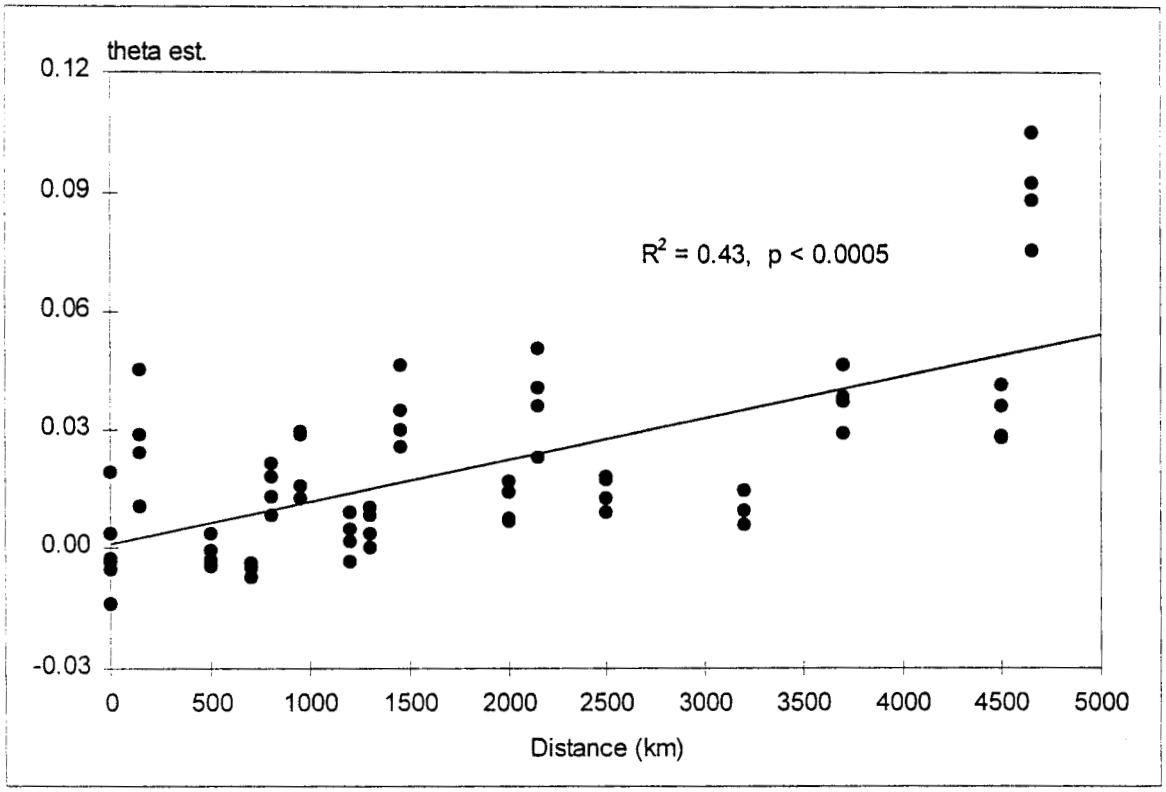
Figure 3. Scatter plot of $\hat{\theta}$ versus geographic distance for all odd-year population pairs. Statistical significance was tested using a Spearman rank correlation coefficient implemented in the "Mantel" test module in GENEPOP, and was based on 15,000 permutations of the data.

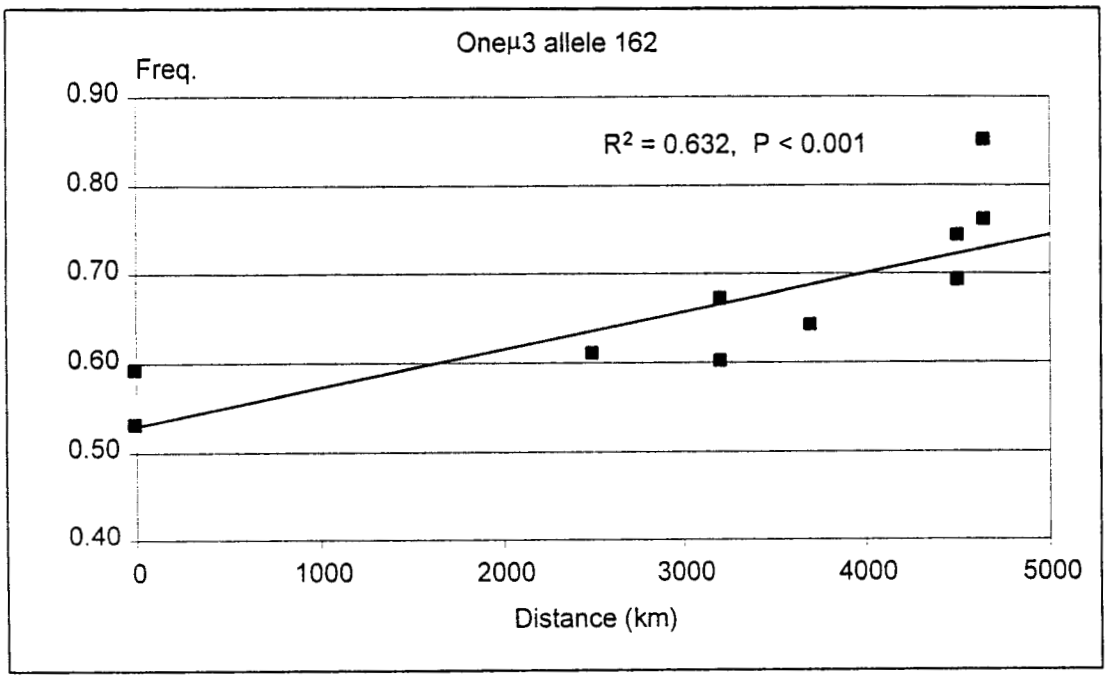
Figure 4. Allele frequency of *One*μ3 allele 162 and μ*Sat*60 allele 109 plotted against geographic distance from Northwest Alaska. The *P*-values were derived by testing the significance of slope of each linear regression.

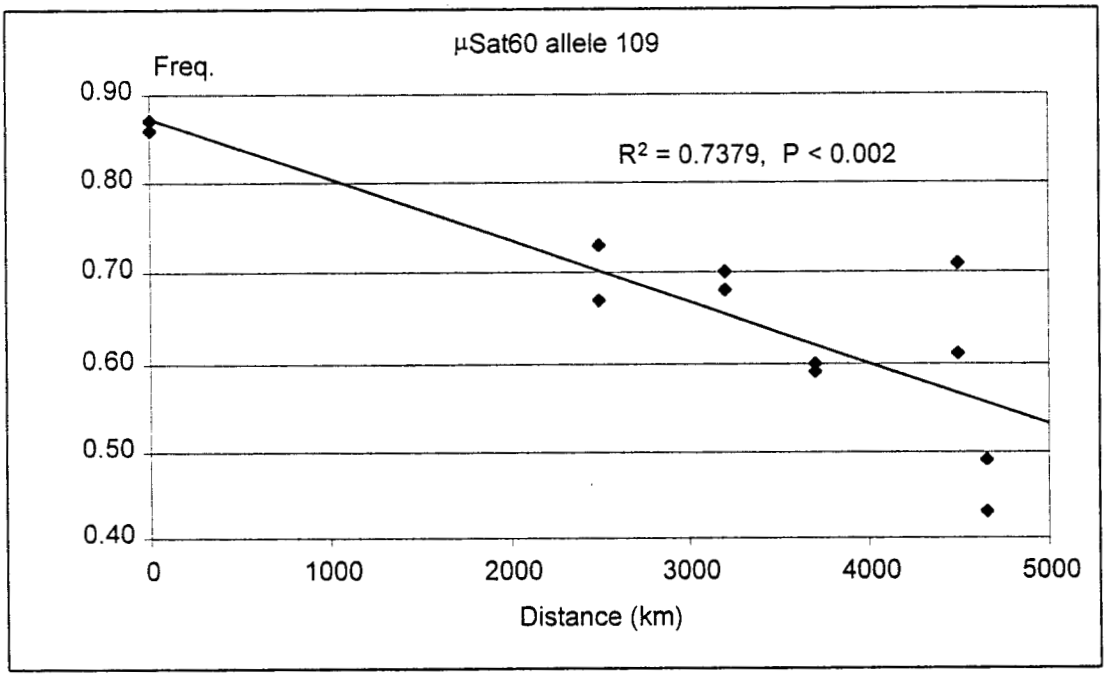


Region	Population	Year	N
Northwest Alaska	1. Nome River	1991	40
	2. Snake River	1991	31
Southcentral Alaska	3. AFK Hatchery	1995	52
	4. Koppen Creek	1995	44
	5. Koppen Creek	1994	40
Southeast Alaska	6. LPW Hatchery	1993	20
	7. Gastineau Hatchery	1993	20
North British Columbia	8. Babine River	1993	40
	9. Khyex River	1993	40
South British Columbia	10. Fraser River (main stem)	1995	40
	11. Cayoosh Creek	1995	40
Northwest Washington	12. North Fork Stillaguamish R.	1995	40
	13. Gray Wolf River	1995	40


Rho est.
0.27
0.24
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
-0.03
y = x
odd x odd - open cicle (p > 0.500)
odd x even - solid square (p < 0.001)
-0.03
0.00
0.03
0.06
0.09
0.12
0.15
0.18
0.21
0.24
0.27
theta est.


theta est.
0.12
0.09
0.06
0.03
0.00
-0.03
R^2 = 0.43, p < 0.0005
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
Distance (km)


Oneμ3 allele 162
Freq.
0.90
0.80
0.70
0.60
0.50
0.40
R² = 0.632, P < 0.001
0
1000
2000
3000
4000
5000
Distance (km)


μSat60 allele 109
Freq.
0.90
0.80
0.70
0.60
0.50
0.40
R² = 0.7379, P < 0.002
0
1000
2000
3000
4000
5000
Distance (km)