

DNA and allozyme markers provide concordant estimates of population differentiation: analyses of U.S. and Canadian populations of Yukon River fall-run chum salmon (*Oncorhynchus keta*)

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Abstract: Although the number of genetic markers available for fisheries research has steadily increased in recent years, there is limited information on their relative utility. In this study, we compared the performance of different “classes” of genetic markers (mitochondrial DNA (mtDNA), nuclear DNA (nDNA), and allozymes) in terms of estimating levels and partitioning of genetic variation and of the relative accuracy and precision in estimating population allocations to mixed-stock fisheries. Individuals from eight populations of fall-run chum salmon (*Oncorhynchus keta*) from the Yukon River in Alaska and Canada were assayed at 25 loci. Significant differences in mitochondrial haplotype and nuclear allele frequencies were observed among five drainages. Populations from the U.S.–Canada border region were not clearly distinguishable based on multilocus allele frequencies. Although estimates of total genetic diversities were higher for the DNA loci ($H_t = 0.592$ and $h = 0.647$ for nDNA and mtDNA, respectively) compared with protein allozymes ($H_t = 0.250$), estimates of the extent of population differentiation were highly concordant across marker classes (mean $\theta = 0.010$, 0.011 , and 0.016 for allozymes, nDNA, and mtDNA, respectively). Simulations of mixed-stock fisheries composed of varying contributions of U.S. and Canadian populations revealed a consistent bias for overallocation of Canadian stocks when expected Canadian contributions varied from 0 to 40%, due primarily to misallocations among genetically similar border populations. No single marker class is superior for differentiating populations of this species at the spatial scale examined.

Résumé : Ces dernières années, de nouveaux marqueurs génétiques sont régulièrement venus enrichir la panoplie d'outils dont dispose la recherche dans le domaine des pêches; toutefois, l'utilité relative de ces instruments reste en grande partie à déterminer. Nous avons comparé la performance de différentes « classes » de marqueurs génétiques (ADN mitochondrial (ADNmt), ADN nucléaire (nADN) et alloenzymes) d'après leurs propriétés de différenciation et le degré de variation génétique qu'ils permettent de mettre en évidence et d'après leur exactitude et leur précision relatives pour l'estimation de la distribution des populations dans les pêche de stocks mixtes. Nous avons étudié 25 locus chez des spécimens prélevés dans huit populations de saumon kéta (*Oncorhynchus keta*) à montaison automnale du fleuve Yukon, en Alaska et au Canada. L'analyse des haplotypes mitochondriaux et des fréquences alléliques nucléaires a permis de mettre en évidence des différences significatives entre cinq bassins hydrographiques. La mesure de la fréquence allélique de locus multiples n'a pas permis de mettre en évidence des différences nettes entre les populations de la région limitrophe canado-américaine. La diversité génétique totale mesurée d'après les locus d'ADN ($H_t = 0,592$ et $h = 0,647$, respectivement, pour l'ADNn et pour l'ADNmt) était plus grande que celle mise en évidence au moyen des alloenzymes ($H_t = 0,250$); par ailleurs, pour ce qui est de la différenciation des populations, tous les marqueurs concordaient fortement (la moyenne du facteur θ étant respectivement de $0,010$, $0,011$ et $0,016$ pour les alloenzymes, l'ADNn et l'ADNmt). Dans des simulations reproduisant la pêche de stocks mixtes diversement composés de populations américaines et canadiennes, nous avons constaté un biais constant dénotant la sur-allocation des stocks canadiens lorsque la part des populations canadiennes prévue était comprise entre 0 et 40%, ce phénomène étant dû avant tout à des erreurs d'allocation concernant des populations limitrophes génétiquement ressemblantes. À l'échelle spatiale considérée, aucune des classes de marqueurs évaluées n'a surpassé les autres pour la différenciation des populations de saumon kéta.

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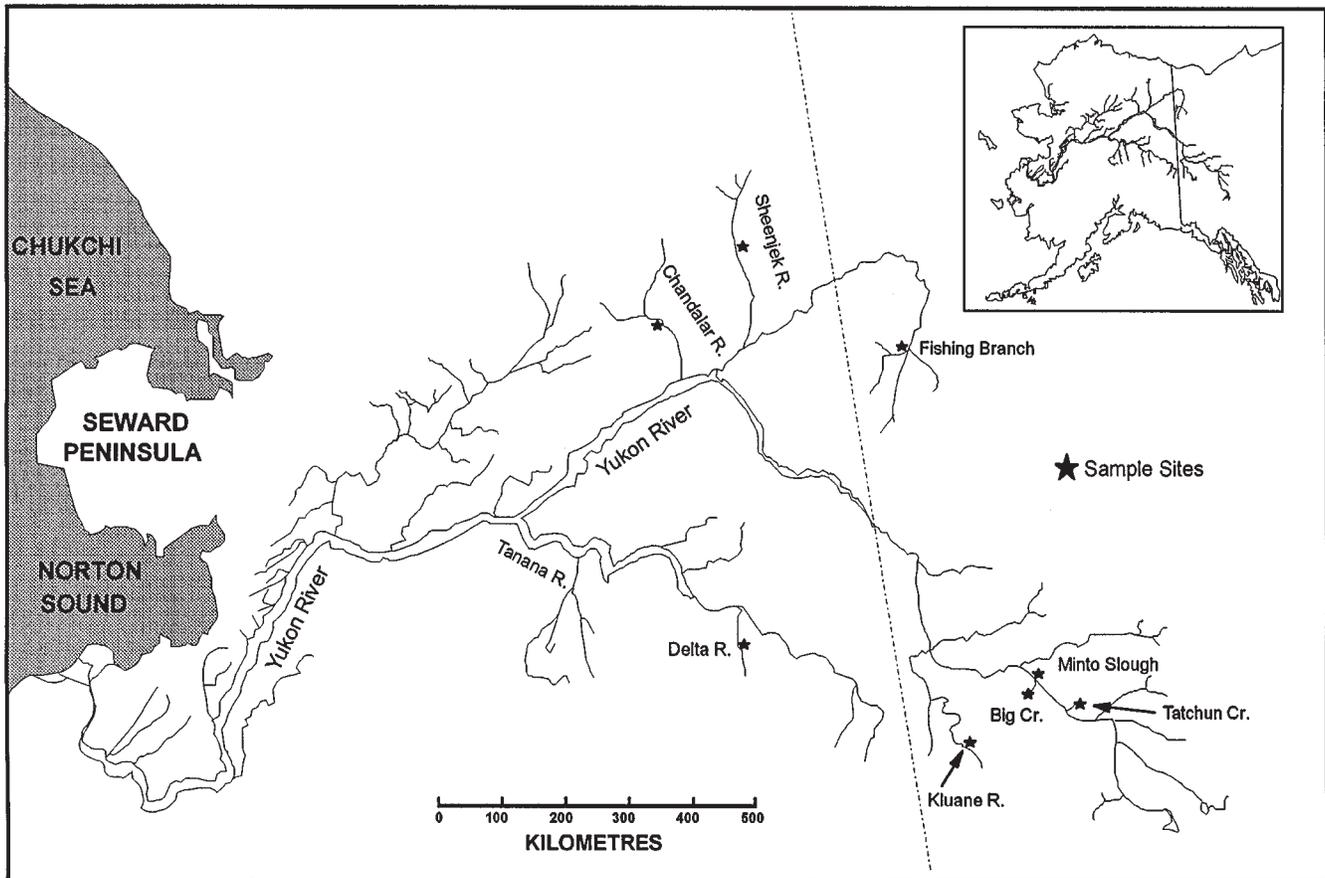
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Fig. 1. Map of the Yukon River drainage showing sampling locations.



Introduction

Recent advances in molecular technology have dramatically increased the number of markers capable of resolving variation at the DNA level (see reviews by Park and Moran 1994; Ferguson et al. 1995). Different techniques including mitochondrial DNA (mtDNA) (Meyer 1993), minisatellites (O'Reilly and Wright 1995), and microsatellites (Scribner et al. 1996) have the ability to target DNA sequences that have different patterns of inheritance, different modes and rates of mutation, and, concomitantly, different levels of variation. It is not intuitively clear which genetic markers afford the greatest utility for elucidating population variation. Can DNA markers improve discrimination among salmon populations beyond the obvious advantage of providing more loci? Can the relative utility of different markers vary at different spatial scales of analysis? Unfortunately, little attempt has been made to survey the same set of populations for multiple classes of genetic markers or to assess the accuracy and precision of various genetic markers (or mixtures of different genetic loci analyzed using protein- and DNA-based methodologies) for mixed-stock analysis (MSA).

Chum salmon (*Oncorhynchus keta*) have received considerable attention due to their broad geographic distribution and abundance in U.S.–Canada border drainages. This important international fisheries resource can be used as a model system for comparative genetic studies. Many fisheries for this species are conducted in locations far removed from the breeding

locations from which individuals have originated, and as such are typically composed of a mixture of populations from a variety of geographic locales.

The Yukon River drainage is the largest drainage used by chum salmon in the northern portion of their range and is one of two drainages in western Alaska that support both summer- and fall-run populations. Summer-run chum salmon spawn during July and early August in the lower and middle portions of the river (Buklis 1981), while fall-run fish generally spawn in September and October in the middle and upper portions of the river (Buklis 1981). Fall-run chum salmon are somewhat unique because while most chum salmon do not migrate great distances in freshwater, Yukon River fall-run chum salmon migrate nearly 3000 km to spawning areas in both Alaska and the Yukon Territory of Canada. This extensive geographic distribution within the drainage may promote a high degree of population genetic structuring, which in turn may be used in MSA.

Much of the current information on population genetic characteristics of chum salmon and of stock composition of chum salmon fisheries has come from allozyme loci (Phelps et al. 1994). These studies and references cited therein reveal that chum salmon vary in their genetic characteristics both spatially and temporally (e.g., based on run-timing). Surveys of mtDNA (Park et al. 1993) and minisatellite DNA variation (Beacham 1996) provide additional insights into the extent of population structuring at local and regional geographic scales.

Significant differences in allozyme allele frequencies were

observed between fall- and summer-run populations in the Yukon River (Wilmot et al. 1994). Allozyme data could successfully be used to separate fall- and summer-run chum salmon when they occurred together in mixtures. However, allozyme data were not sufficient to separate fall-run chum salmon of U.S. versus Canadian origin due to genetic similarities among populations spawning in drainages near the U.S.–Canada border (Porcupine River and Yukon River mainstem; Fig. 1). Development and screening of additional genetic markers was deemed appropriate to increase statistical power (Wilmot et al. 1992).

The objectives of this study were to (i) describe the magnitude and patterns of population differences in allele and haplotype frequency among fall-run chum salmon within the Yukon River drainage using allozyme, nuclear DNA (nDNA), and mtDNA markers, (ii) examine the utility of various genetic markers for population separation and MSA and discuss options and criteria for choosing subsets of markers for particular descriptive and analytical purposes, (iii) compare equal and proportional weighting strategies for genetic MSA, and (iv) address issues related to definitions of reporting groups based on political (United States and Canada) and biological (i.e., tributary of origin or geographic proximity) criteria.

Methods and materials

Sampling locations and tissue collections

Samples were obtained from >50 adult fall-run chum salmon from each of eight populations (Fig. 1) within the tributaries or mainstem of the Yukon in the United States and Canada. Specific sampling locales and years of collection include three populations from the United States (Delta River (1990), Chandalar River (1989), and Sheenjek River (1989)) and five populations from Canada (Big Creek (1992), Minto Slough (1989), Tatchun Creek (1992), Fishing Branch Creek (1989), and Kluane River (1992)). Collections were made on the spawning grounds during September–November of each year. Although samples were not all obtained from the same year, previous studies (Wilmot et al. 1992; Kondzela et al. 1994) have shown that for natural populations, allozyme allele frequencies within a sampling locale are temporally stable (in the absence of hatchery influences, e.g., Phelps et al. 1994). We assumed that allele frequencies at the DNA loci surveyed in the present study were also temporally stable.

Four tissues (skeletal muscle, liver, eye, and heart) were taken from each individual and frozen immediately in individually labeled tubes. All samples were kept at -70°C in the laboratory until analyzed. DNA was extracted from muscle or heart for each individual using proteinase-K followed by standard phenol–chloroform extraction and alcohol precipitation (Sambrook et al. 1989). DNA concentrations were determined using fluorimetry, and stocks of 50 ng/ μL were made for all individuals. DNA samples were kept at -20°C .

Allozymes

Protein electrophoresis followed standard techniques as described by Aebersold et al. (1987). Locus nomenclature followed recommendations adopted by the American Fisheries Society (Shaklee et al. 1990). Allelic designations were standardized following previous studies of chum salmon (e.g., Kondzela et al. 1994). Twenty loci (including two isoloci) which have previously been described as polymorphic for Yukon River chum salmon from these locales (Wilmot et al. 1992) were used for analysis. Loci include *sAAT-1,2**, *sAAT-3**, *mAAT-1**, *mAH-3**, *ALAT**, *ESTD**, *G3PDH-2**, *bGLUA**, *mIDHP-1**, *sIDHP-2**, *LDH-A1**, *sMDH-A1**, *sMDH-B1,2**, *mMEP-2**, *MPI**, *PEP-B1**, *PEPLT**, and *PGDH**.

Nuclear DNA loci

Five microsatellite loci were used for analysis (*One μ 1*, *One μ 10*, and *One μ 18*, Scribner et al. 1996; *Ots1*, Banks et al. 1996; *Ssa14*, McConnell et al. 1995). Loci were screened for variation using polymerase chain reaction (PCR) conditions optimized for each locus. All PCR reactions were conducted in 25- μL volumes using about 100 ng of template DNA, 0.25 unit of Taq DNA polymerase (Perkin Elmer), and buffer (10 mM Tris–HCl, pH 8.3, 1.5 mM MgCl_2 , 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 0.01% Triton X-100) for 35 cycles. Primer and dNTP concentrations were 1 pmol of each primer and 25 μM of each dNTP (*One μ 1*, *One μ 10*, and *One μ 18*), 8.3 pmol of each primer and 40 μM of each dNTP (*Ots1*), and 10 pmol of each primer and 200 μM of each dNTP (*Ssa14*). Samples were screened for variation using [γ - ^{32}P]ATP end-labeled primers. One primer from each pair was end-labeled using T4 polynucleotide kinase according to manufacturer's specifications (Pharmacia). PCR conditions were as described above using locus-specific annealing temperatures (*One μ 1*, *One μ 10*, and *One μ 18*: 55°C ; *Ots1*: 58°C ; *Ssa14*: 50°C). Each PCR reaction was mixed with 10 μL of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% xylene cyanol, 0.05% bromophenol blue) and heated for 5 min at 95°C before loading onto a 6% denaturing sequencing gel. An M13 control sequencing reaction (USB) was run adjacent to the samples to provide an unambiguous size marker for the microsatellite alleles. Gels were dried and autoradiographed overnight at -70°C using intensifying screens.

Primers GH2ex4 and GH2ex5a described in Park et al. (1995) were used to amplify intron D of the growth hormone 2 gene (*GH2D**). PCR reactions were conducted in a total volume of 100 μL with a final concentration of 2 mM MgCl_2 , 0.8 mM each dNTP, 0.2 mM of each primer, 2.5 units of AmpliTaq DNA polymerase (Perkin Elmer), and 0.1–0.4 μM genomic DNA template. Cycling conditions included an initial denaturation at 94°C for 1 min followed by 40 cycles at 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min. A final extension was performed at 72°C for 5 min. Polymorphisms were detected by restricting the PCR product with *Hinf* I following manufacturer's recommendations (New England Biolabs). Digested products were run on a 4% Amplisize agarose gel (BioRad) at 50 V for 4.5 h. Resulting banding patterns were visualized using ethidium bromide under ultraviolet light.

mtDNA variation

PCR was used to amplify a portion of the ND5/6 region of the mitochondrial genome (about 2400 base pairs) using primers 5'-AATAGCTCATCCATTGGTCTTAGG-3' and 5'-TAACAACGG-TGGTTTTTCAAGTCA-3' reported in Cronin et al. (1993). PCR reactions were conducted in 50- μL volumes using 100–500 ng of DNA, 10 \times buffer (0.1 M Tris–HCl, pH 8.5, 0.025 M MgCl_2 , 0.5 M KCl, 1 mg bovine serum albumin/mL), 200 mM of each dNTP, 10 pmol of each primer, and 1.25–2.5 units of Taq DNA polymerase (Perkin Elmer). Amplification consisted of one denaturation cycle of 95°C for 4 min followed by 32 cycles of 95°C for 50 s, 50°C for 40 s, and 70°C for 2.5 min.

PCR products were digested with seven restriction enzymes (*Alu* I, *Ase* I, *Bsa* II, *Dpn* II, *Rsa* I, *Sau* 96I, and *Taq* I) under conditions specified by the manufacturer (New England Biolabs). Digested PCR products were run on 2% agarose gels, stained with ethidium bromide, and photographed under ultraviolet light (312 nm). Restriction fragment sizes were estimated by comparison with a 100 base pair ladder (Pharmacia) and a phage lambda standard (Lambda c1857 Sam 7 digested with *Hind* III and PhiX174 digested with *Hae* III; Pharmacia). Composite haplotypes were defined based on presence/absence of restriction sites across all restriction enzymes (Lansman et al. 1981).

Statistical analyses

Allele and haplotype frequencies were calculated for each of the eight populations. Tests for departures from Hardy–Weinberg

equilibrium were conducted for each nuclear locus ($\alpha = 0.05$, adjusted for multiple testing using the Bonferroni criterion, e.g., Manly 1985). Estimates of total gene diversity (Nei 1973) and the number of alleles were estimated for each nuclear locus across the eight populations. For microsatellite loci *Ots1* and *Ssa14*, rare alleles of similar size were binned into composite allelic classes. All statistical analyses were based on composite allele frequencies.

Summarization with *F*-statistics (Cockerham 1969) the partitioning of allelic variance at each of the 24 nuclear loci among individuals within and among populations was conducted using the program FSTAT (Goudet 1995). Tests of significance were conducted using permutations ($N = 1000$) of alleles within samples (f) and of alleles or multilocus genotypes among samples (θ). In a complimentary analysis of spatial heterogeneity in mtDNA haplotype frequencies, the analysis of molecular variance (AMOVA) program (Excoffier et al. 1992) was used to estimate θ . To facilitate comparisons across the three marker classes (allozymes, nDNA, and mtDNA), the same genetic distance metric (Cavalli-Sforza and Edwards chord distances (Cavalli-Sforza and Edwards 1967)) was used in metric multidimensional scaling (MDS) (Krzanowski and Marriott 1994) to describe genetic relationships among populations. MDS analyses were performed using each of the three marker classes separately and on the combined data set. Spatial variation was further summarized for the nuclear loci using a hierarchical likelihood ratio analysis (*G*-statistic, Sokal and Rohlf 1995). In a complimentary analysis for mtDNA, geographic heterogeneity at a series of hierarchical levels was assessed using Monte Carlo simulations (Roff and Bentzen 1989) with 10 000 replicate simulations.

Simulated mixed-stock assessment of U.S.–Canada allocations

The accuracy and precision of population allocations to simulated U.S.–Canada mixed-stock fisheries were evaluated. Simulations were performed using the Statistical Package for Analyzing Mixtures developed by the Alaska Department of Fish and Game using the GIRLS (Masuda et al. 1991) and CONJA-S (Pella et al. 1996) algorithms. Analyses were conducted for each marker class separately and on the combined data set. The program used both the iteratively reweighted least squares (Pella and Milner 1987) and expectation maximization (Pella and Milner 1987) maximum likelihood algorithms in simulations of possible mixture scenarios to evaluate the performance of MSA for the eight populations. The estimation procedure also includes parametric (Monte Carlo) bootstrapping to evaluate bias (accuracy) and variance (precision) due to mixture sampling error and to sampling error in the baseline. Sample sizes of 400 fish were used for each simulation. Estimates of precision were based on 100 bootstrap replicates, where both the mixtures and baselines were resampled each iteration and are presented for each marker class and for the combined analysis by 1 SD about the mean estimate. Simulations were run for each of six mixed-stock data sets, constructed so that the proportion of U.S. and Canadian reporting groups varied incrementally by 20% from 0% U.S. to 100% U.S.

The effects of differences in the relative abundance of stocks contributing to a mixture were evaluated using two population weighting strategies (equal and proportional weighting). Since researchers often lack information on stock size, we initially assumed that each population contributed equally to the simulated mixture. Simulations were also conducted weighting the population contributions to the mixture by estimates of escapement. Estimates of escapement for each sampling area were 100 000 or 0.288 for the upper Tanana River (Delta), 58 600 or 0.169 for the Chandalar River, 86 400 or 0.249 for the Sheenjek River, 34 900 or 0.100 for Fishing Branch Creek, 58 600 or 0.169 for the Canadian mainstem (Tachun Creek, Big Creek, and Minto Slough), and 8700 or 0.025 for the Kluane River (L. Barton, Alaska Department of Fish and Game, 1300 College Road, Fairbanks, AK 99701, U.S.A., personal communication).

Previous data (Wilmot et al. 1992) suggested that populations of fall-run chum salmon could be grouped based on biological criteria

(e.g., distance between populations, glacial history of spawning areas, bank orientation during migration) and that these groupings may confer greater accuracy and precision in MSA than have previously been realized using political boundaries (United States and Canada) as the sole criterion for establishment of reporting groups. To test alternative criteria, we grouped the eight populations into three reporting groups (Delta River, Kluane River, and the U.S.–Canada border and Canadian mainstem).

Finally, to assess alternative marker selection strategies, MSA simulations were conducted using the United States and Canada as reporting groups and using 10 loci that were selected using different criteria. Selection criteria included 10 loci with (i) the largest estimates of total gene diversity (H_t), (ii) the largest estimates of F_{st} , (iii) the largest among-population *G*-statistic, (iv) and five sets of 10 loci selected randomly without replacement. For all simulations, mean mixture estimates were derived from 100 simulations using a mixture composition of 100% Canadian populations, with each population contributing equally to the mixture.

Results

Levels of genetic diversity

Twenty four nuclear loci (18 allozyme loci, one gene intron, and five microsatellite loci) were polymorphic across the eight chum salmon populations (Table 1). Variation was also found within the ND5/6 coding region of mtDNA (Table 1). Levels of genetic variability in terms of allelic diversity and H_t varied among marker classes and among loci within each class (Table 1). Sixteen of 18 allozyme loci were characterized by two alleles, and for nine loci, the frequency of the common allele was >0.85 across all populations. In contrast, the nDNA loci were generally characterized by higher numbers of alleles (range 2–20) and allele frequencies that were not dominated by a single allele (Table 1). The ND5/6 region of mtDNA was also highly polymorphic with seven haplotypes resolved. Estimates of H_t (Table 1) were generally higher for nDNA loci (mean 0.592) than for allozyme loci (mean 0.250), although considerable heterogeneity was observed among loci within each marker class (Fig. 2). Levels of mtDNA haplotype diversity (0.647) were generally comparable with H_t values for the nDNA loci.

Population genotypic frequencies did not deviate from Hardy–Weinberg expectations for most loci. Six percent (12 of 192 tests) were significant at $\alpha = 0.05$, and no test was significant after nominal α levels were corrected for multiple comparisons (24 tests for each population), suggesting that variation at each nuclear locus was consistent with disomic inheritance at single loci. No indication of null alleles was observed at the microsatellite loci.

Spatial variation

Significant differences in allele frequency among the eight populations were observed for eight of 18 allozyme loci (mean $\theta = 0.010$, $P < 0.01$), four of six nDNA loci (mean $\theta = 0.011$, $P < 0.01$), and for mtDNA ($\theta = 0.016$, $P < 0.05$). No one marker class consistently exhibited higher levels of θ for all loci (Fig. 2). Although a larger proportion of the total genetic variation at the nDNA loci appears to be segregating within populations as compared with the allozyme loci, estimates of mean f , F , and θ were highly concordant. Estimates of θ were also similar to results from mtDNA. These results suggest that all three marker classes appear to capture the same signature of

Table 1. Analysis of genetic diversity within and among eight populations of fall-run chum salmon from the Yukon River drainage.

Marker class	Locus	No. of alleles	Range of common allele frequencies ^a	<i>F</i> -statistics ^a			
				<i>H</i> _t ^b	<i>F</i>	<i>f</i>	θ
Allozymes	<i>sAAT-1,2</i> *	2	0.935–0.971	0.079	0.018	0.019	0.000
	<i>sAAT-3</i> *	2	0.543–0.678	0.472	0.164	0.164**	0.000
	<i>mAAT-1</i> *	2	0.878–0.970	0.151	0.026	0.023	0.003
	<i>mAH-3</i> *	2	0.667–0.924	0.362	–0.044	–0.075	0.028**
	<i>ALAT</i> *	2	0.925–0.992	0.010	0.034	0.034	0.000
	<i>EST-D</i> *	2	0.575–0.820	0.441	0.046	0.032	0.017*
	<i>G3PDH-2</i> *	2	0.840–0.941	0.205	–0.018	–0.022	0.003
	<i>bGLU-A</i> *	2	0.910–0.958	0.118	0.011	0.014	0.000
	<i>mIDHP-1</i> *	2	0.980–1.000	0.011	–0.004	–0.008	0.004
	<i>sIDHP-2</i> *	3	0.400–0.602	0.531	0.046	0.036	0.010*
	<i>LDHA-1</i> *	2	0.432–0.726	0.457	–0.020	–0.036	0.015*
	<i>sMDHA-1</i> *	2	0.899–0.958	0.131	0.025	0.020	0.004
	<i>sMDHB-1,2</i> *	2	0.996–1.000	0.001	0.000	0.000	0.000
	<i>mMEP-2</i> *	2	0.840–0.992	0.129	0.070	0.044	0.027**
	<i>MPI</i> *	2	0.875–0.950	0.143	–0.054	–0.064	0.009*
	<i>PEPB-1</i> *	3	0.742–0.892	0.266	0.069	0.061	0.008*
	<i>PEPLT</i> *	2	0.942–1.000	0.046	–0.021	–0.031	0.010*
<i>PGDH</i> *	2	0.960–0.992	0.045	0.075	0.079	0.000	
Mean ± SE	All loci			0.250	0.034	0.024±0.003*	0.010±0.003**
nDNA	GTHD*	3	0.463–0.663	0.540	–0.028	–0.030	0.002
	<i>Onew1</i>	2	0.630–0.930	0.370	0.005	–0.032	0.036**
	<i>Onew10</i>	6	0.367–0.493	0.668	0.003	0.004	0.000
	<i>Onew18</i>	3	0.570–0.735	0.495	0.027	0.019	0.009*
	<i>Ots1</i>	20	0.076–0.305	0.853	0.126	0.114**	0.013**
	<i>Ssa14</i>	12	0.440–0.730	0.622	0.026	0.010	0.016**
Mean ± SE	All loci			0.592	0.030	0.025±0.004	0.011±0.004**
mtDNA	ND5/6	14	0.063–0.556	0.647 ^c		0.016 ^d	

Note: **P* < 0.05; ***P* < 0.01.

^aAllele frequency data for individual populations are available from the senior author upon request.

^bTotal gene diversity (Nei 1973) across the eight stocks.

^cHaplotypic diversity (Nei and Tajima 1981).

^dEstimates derived as described by Excoffier et al. (1992).

microevolutionary events that have given rise to the present spatial allelic diversity.

The eight populations could be combined into five groups (Delta River representing the Tanana drainage, Chandalar River, Sheenejk River, and Fishing Branch Creek on the Porcupine River, Klwane River, and the three populations from the Yukon River mainstem). Twelve of the 24 nuclear loci (cumulative $G = 517.55$, $P < 0.01$; Table 2) and mtDNA ($\chi^2 = 54.46$, $P < 0.05$) differed significantly among these five groups. Hierarchical analyses of heterogeneity in allele frequency revealed little evidence of significant spatial differentiation among populations of the same drainage (Table 2). Two of the 24 nuclear loci (*mAH-3** and *GH2D**) showed significant heterogeneity between the two populations from the Porcupine River (Sheenejk River and Fishing Branch Creek) at $\alpha < 0.05$, although none were significant when levels were adjusted for multiple testing. No evidence for spatial differentiation was noted among the three Canadian Yukon River mainstem populations (Table 2). Results of analyses of population variation in mtDNA haplotype frequency were concordant with those for both classes of nuclear loci. Significant differences were noted among groups ($\chi^2 = 46.92$, $P < 0.005$), although no

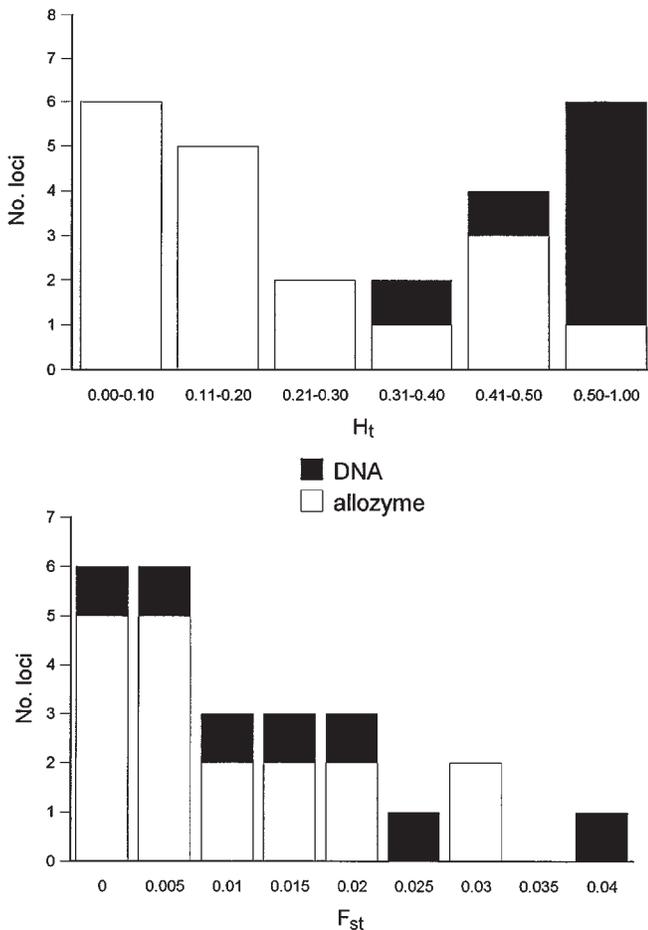
significant differences were observed among populations within a drainage.

Interpopulation relationships based on genetic distance, when summarized in the form of MDS plots, revealed that interpopulation similarity is generally a function of geographic proximity (Fig. 3). This trend was consistent across the three marker classes. Populations from the Canadian mainstem cluster, as do the Sheenejk River and Fishing Branch Creek populations from the Porcupine River. Delta River and Klwane River were genetically distinct. The U.S.–Canada border population from the Chandalar River was more clearly distinguishable from the other border populations based on mtDNA.

Simulation analyses

Accuracy graphs were constructed depicting the accuracy and precision of simulation results of the relative abilities of each marker class to allocate simulated mixtures to each of two reporting groups (United States and Canada). Results of simulations conducted under assumptions of equal stock contribution (Fig. 4) and when stock contributions were weighted proportionally by estimates of escapement (data not shown) were very similar in terms of accuracy and precision. Analyses

Fig. 2. Frequency histograms describing locus distributions of H_t and F_{st} values for allozyme, nDNA, and mtDNA markers from analyses of eight populations of fall-run chum salmon from the Yukon River drainage.



for each marker class revealed a consistent bias toward over-allocation of Canadian stocks in simulated contributions up to about 50%. Greater precision was attained when data for all loci were included in the analysis (full data set, SD range 5–6%) relative to analyses conducted using each marker class separately (allozymes: SD 6.5–10%; nDNA loci: SD 6.5–9%; mtDNA: SD 17.5–22.5%). Bias for simulations conducted at 0, 20, and 40% expected Canadian contributions was due to underallocation of U.S. stocks (particularly Delta River) and overallocation of genetically similar Canadian border and mainstem stocks. Simulations conducted at 60% Canadian contribution were highly accurate. Estimates from simulations conducted at 80 and 100% Canadian contribution consistently underallocated fish to the Canadian border population from Fishing Branch Creek and overallocated fish to U.S. border populations from the Chandalar River and Sheenjek River populations.

Simulation analyses were conducted to examine accuracy and precision of reporting regions defined based on biological criteria. Analyses conducted with and without weighting revealed that individuals from the border and mainstem populations were allocated with high accuracy and precision to the border reporting group (Table 3). However, a high proportion

of Delta River and Kluane River reporting groups were consistently misallocated to the border reporting group.

No clear locus selection criterion was resolved based on comparisons of mixed-stock simulations using a mixture composed of 100% of Canadian populations, with each population contributing equally to the mixture. Subsets of 10 loci that exhibited the highest levels of H_t (mean estimate ± 1 SD = 0.893 ± 0.052), highest estimates of F_{st} (0.893 ± 0.059), or highest G -statistics (0.882 ± 0.069) were no more accurate or precise in allocating stocks based on U.S. and Canadian reporting groups than were random draws of a comparable number of loci (ranges of mean estimates (0.895–0.900) and SDs (0.062–0.070)) or relative to simulation results when all 25 loci were used together (0.907 ± 0.041).

Discussion

Over the past decade, increasing attention has been focused on examination of genetic polymorphisms at the DNA level. Interest in this area has been motivated in part by the refinement of molecular techniques capable of resolving microevolutionary variation in genetic markers with contrasting modes of inheritance and rates of mutation and by the increased accessibility of technologies to fisheries biologists. Technological developments have given rise to a diverse array of molecular genetic markers (see review by Park and Moran 1994). However, while various molecular genetic markers have been used for chum salmon in several locations in the United States and Canada (e.g., mtDNA, Park et al. 1993; minisatellites, Beacham 1996) and for various other salmonid species (e.g., *Salmo* sp., Ferguson et al. 1995; Atlantic salmon (*Salmo salar*), Galvin et al. 1995; Tessier et al. 1995), few data exist that have simultaneously examined multiple molecular and allozyme loci to address questions of marker suitability for questions of population differentiation and fisheries assessment. As a result, fisheries researchers ask what criteria should be used to determine “the right marker for the right job”? Data drawn from different processes may have different sampling errors. If there is heterogeneity among data sets with respect to some property that affects estimation of population relationships, combining the data can lead to misleading results.

Comparisons among marker classes

Estimates of the extent of population differentiation (θ) were highly concordant between marker classes. Further, accuracy and precision from mixed-stock simulations were similar for allozymes and nDNA. We observed an increase in precision in simulated mixed-stock assessments when all markers were combined (Fig. 4), suggesting that all genetic markers appear to similarly track microevolutionary processes that have given rise to the observed patterns of genetic diversity. Loci with different inherent properties and rates of evolution can be used simultaneously in a GSI context. Secondly, results of simulations were entirely consistent regardless of the assumptions used to establish specific stock contributions to U.S. and Canadian reporting groups in the simulated mixture analysis (i.e., when stocks were assumed to contribute equally or when stocks were weighted in proportion to escapement estimates). Finally, political boundaries appear to poorly differentiate reporting groups when used in the allocation of mixed-stock fisheries. Although the degree of bias was not

Table 2. Hierarchical heterogeneity analysis (modified from Smouse and Ward 1978) using allozyme and nDNA data collected for fall-run chum salmon in the Yukon River drainage.

Hierarchical level	df	df	df	df	df	df				
		<i>sAAT-1,2*</i>	<i>sAAT-3*</i>	<i>mAAT-1*</i>	<i>mAH-3*</i>	<i>ALAT*</i>				
Total	7	5.89	7	8.26	7	10.49	7	33.20***	7	9.31
Among	4	4.69	4	4.39	4	9.61*	4	27.78***	4	8.35
Within	3	1.19	3	3.86	3	0.87	3	5.41	3	0.94
Porcupine	1	0.00	1	3.36	1	0.10	1	3.99*	1	0.52
Canadian mainstem	2	1.19	2	0.50	2	0.77	2	1.42	2	0.42
		<i>ESTD*</i>	<i>G3PDH-2*</i>	<i>bGLUA*</i>	<i>mIDHP-1*</i>	<i>sIDHP-2*</i>				
Total	7	21.34 **	7	9.86	7	5.13	7	10.30	14	27.77*
Among	4	18.96 ***	4	6.71	4	5.04	4	7.89	8	22.21**
Within	3	2.37	3	3.13	3	0.08	3	2.40	6	5.54
Porcupine	1	0.31	1	0.05	1	0.05	1	2.40	2	1.18
Canadian mainstem	2	2.06	2	3.08	2	0.03	2	0.00	4	4.36
		<i>LDH-A1*</i>	<i>sMDH-A1*</i>	<i>sMDH-B1,2*</i>	<i>mMEP-2*</i>	<i>MPI*</i>				
Total	7	18.10 *	7	11.69	7	4.07	7	30.23***	7	13.98
Among	4	14.89**	4	4.44	4	2.87	4	24.25***	4	11.64*
Within	3	3.20	3	7.23	3	1.19	3	5.97	3	2.33
Porcupine	1	0.02	1	0.10	1	1.19	1	0.00	1	0.28
Canadian mainstem	2	3.18	2	7.13	2	0.00	2	5.97	2	2.05
		<i>PEP-B1*</i>	<i>PEPLT*</i>	<i>PGDH*</i>	<i>GH2D*</i>	<i>Oneu1</i>				
Total	14	20.79	7	15.91*	7	3.84	14	27.04*	7	36.46***
Among	8	15.15	4	14.19**	4	3.45	8	14.47	4	32.23***
Within	6	5.63	3	1.71	3	0.37	6	12.56	3	4.22
Porcupine	2	3.18	1	0.58	1	0.34	2	8.17*	1	2.85
Canadian mainstem	4	2.45	2	1.13	2	0.03	4	4.39	2	1.37
		<i>Oneu10</i>	<i>Oneu18</i>	<i>Ots1</i>	<i>Ssa14</i>	<i>Overall</i>				
Total	35	42.76	14	33.25**	56	130.84***	49	119.46***	31	649.97***
Among	20	31.10	8	29.78***	32	95.96***	28	107.40***	18	517.55***
Within	15	11.64	6	3.46	24	34.87	21	12.05	13	132.42
Porcupine	5	2.15	2	2.03	8	9.33	7	5.09	45	47.37
Canadian mainstem	10	9.49	4	1.43	16	25.54	14	6.96	90	85.05

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

great at U.S.–Canadian frequencies in the midrange (i.e., 60%), which mirror actual values in the fisheries (about 60% U.S. composition over an entire season, Wilmot et al. 1992), greater accuracy and precision may be attained when reporting groups are based on biological (i.e., geographic proximity) rather than political criteria.

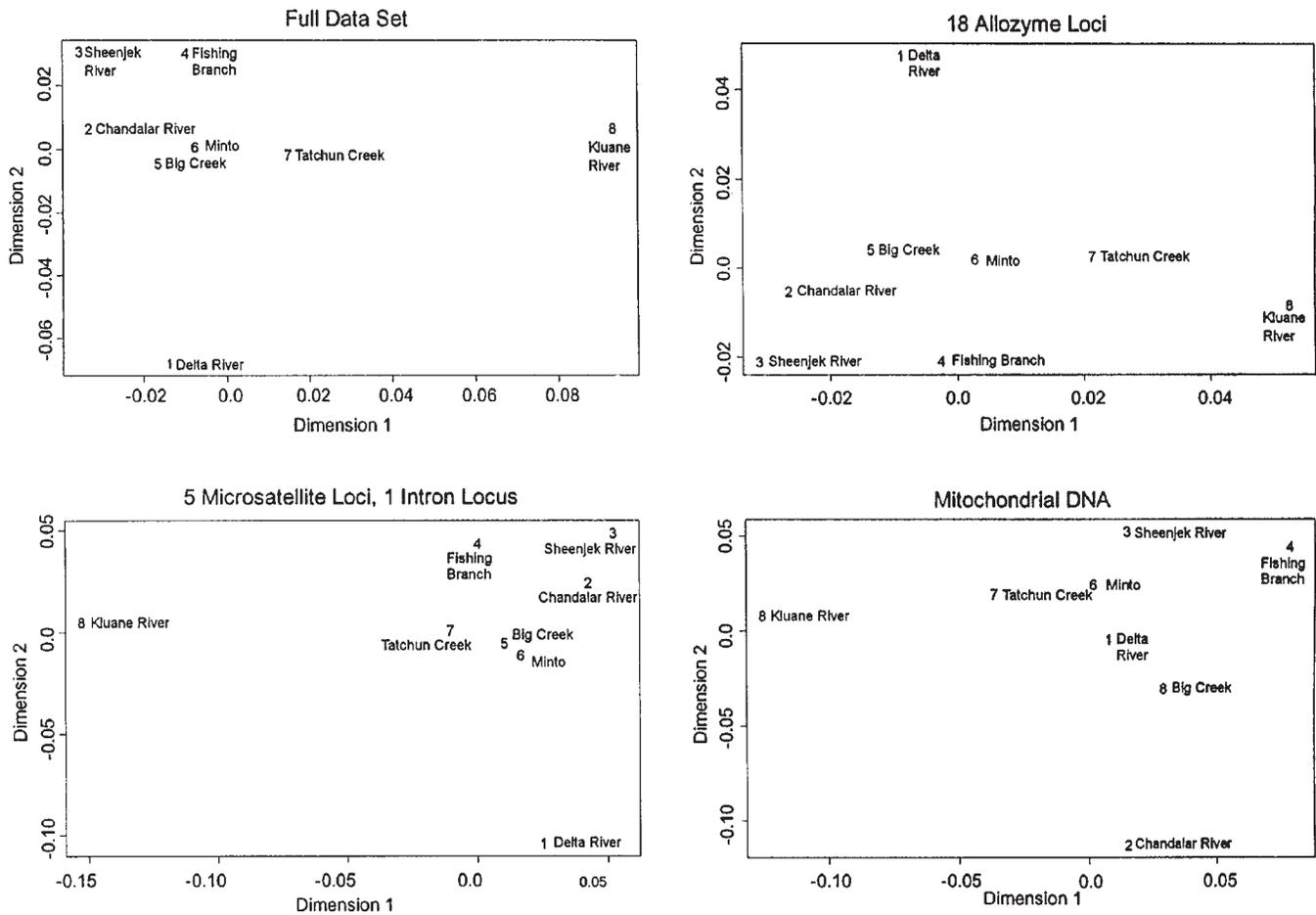
Degree of population differentiation: chum salmon as a model system

This study focuses on only a fraction of the geographic range covered by recent allozyme surveys for chum salmon (e.g., Kondzela et al. 1994; Phelps et al. 1994; Wilmot et al. 1994). These studies have documented significant spatial heterogeneity in allele frequencies over regional and local scales and have emphasized the importance of geographic proximity and run-timing as important variables underlying observed spatial heterogeneity in allele frequency. Results of this study support earlier findings of genetic diversity in the Yukon River based solely on allozymes. Assays of nDNA and mtDNA variation reveal that populations of fall-run chum salmon in the Yukon River drainage have high levels of genetic variability (Table 1). Populations from five population groupings differed

significantly in allele frequency (Table 2) and could be grossly separated into three general groups (Delta River, border and Canadian mainstem populations, and Kluane River; Fig. 3), as had been described earlier based on a more comprehensive population survey that only used allozyme data (Wilmot et al. 1992).

Several biological factors may provide the basis for the three population groupings. The Delta River is in a nonglaciated area. Bank orientation of this and other Tanana River spawning populations is on the south side of the Yukon River near the confluence of the Tanana River during upstream migration. The Delta River population is the latest spawning aggregation in the Yukon River (late October – late November). The border and Canadian mainstem of the Yukon River were also not glaciated. Populations that spawn in this region migrate along the northern bank of the Yukon River during upstream migration and spawn during late September – mid-October. The Kluane River population also migrate upstream along the north bank of the river and spawn during late October. Divergence of fish from the Kluane River could be a function of postglacial founder events and genetic drift. Alternatively, the Kluane River could have been of southeastern

Fig. 3. Multidimensional scaling analysis comparing eight populations of fall-run chum salmon using the Cavalli-Sforza and Edwards (1967) chord distance.



Alaska origin owing to past stream capture events (Wilmot et al. 1994).

DNA analyses of chum salmon have not been conducted for summer-run stocks or for many of the fall-run chum populations. For eight populations of fall-run chum that have been surveyed intensively with several different genetic markers, results suggest that no single marker class is superior in discriminating among populations (Tables 1 and 2) or more accurate and precise in allocating stocks in a mixed-stock fishery (Table 3; Fig. 4). Allozymes and nDNA microsatellite and intron loci were similarly precise, and estimates of precision were highly concordant (Fig. 4). mtDNA alone was both imprecise and inaccurate (Fig. 4), although perhaps comparable results are likely any time a single locus is used. Greater precision was realized when all loci were combined in the simulated MSA.

Why intuitively might one marker class be better than another?

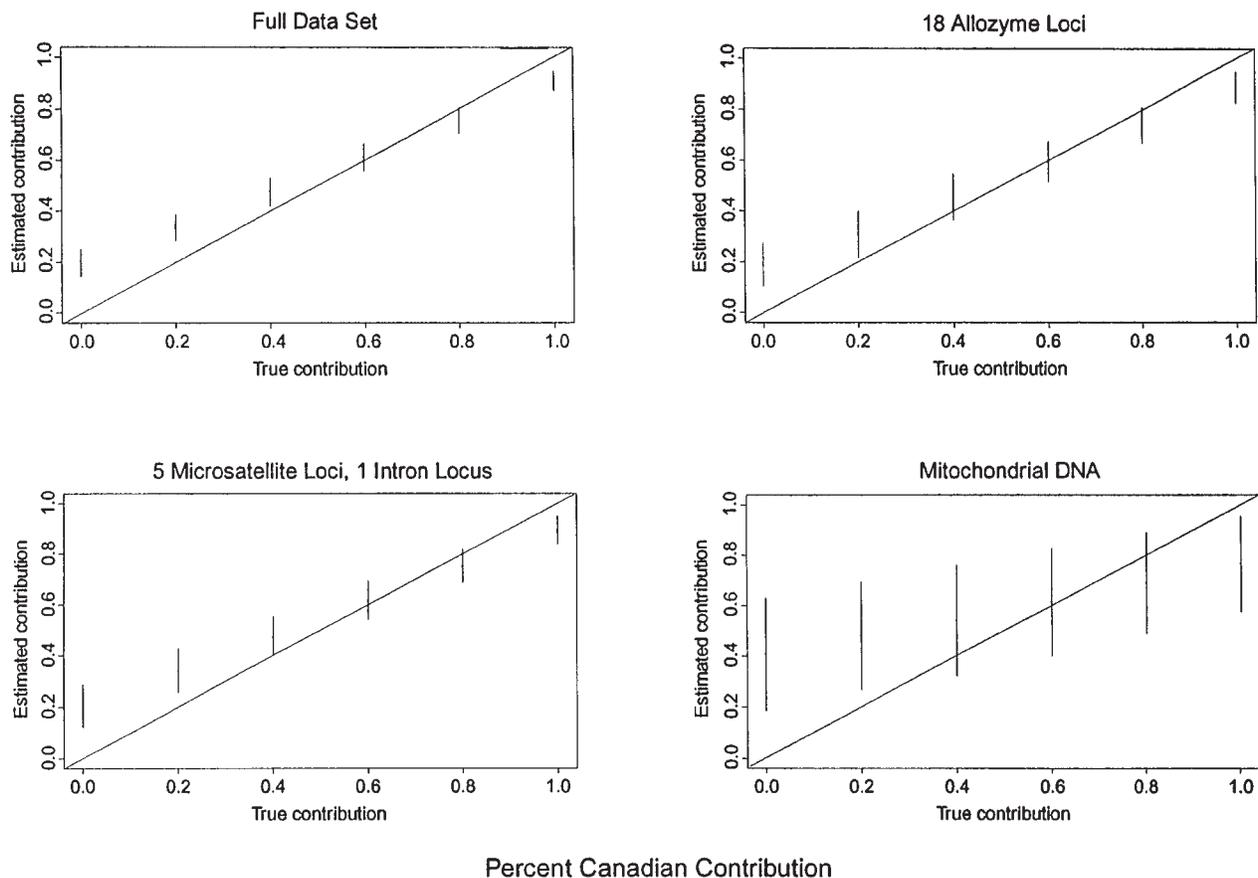
For rapidly evolving loci such as minisatellites and microsatellites, high rates of mutation may be an important factor contributing to patterns and levels of gene diversity within populations as well as variation among them. At equilibrium, the fixation index $F_{st} = 1/(4Nm + 1)$ where N is the effective population size and m is the rate of migration. Wright (1969)

has shown that migration may be supplemented by reversible mutation (u) by substituting m with $(m + u)$. If mutation rates are high relative to the rate of gene flow among populations, then for neutral and highly mutable loci, novel mutations may contribute significantly to increased interpopulation variation. Conversely, if mutation rates are much lower than the rate of migration, F_{st} for neutral alleles has been shown to be nearly independent of mutation rate (Crow and Aoki 1984).

Given that markers may evolve at different rates, one data set may have an inordinately great influence on an analysis simply by virtue of having a larger number of character states. If different markers are evolving at different rates, and under the influence of different evolutionary forces, then estimates of population relationships (and statistical tests of significance) should consider a model of evolution that may vary among marker classes.

Various evolutionary processes could act upon each marker class differently. We assumed that the loci used for this study are selectively neutral. While few empirical or theoretical data have been presented for gene introns, recent literature on microsatellites has discussed that role of mutation in the estimation of population relationships based on genetic distance (Goldstein et al. 1995) and spatial variance in allele frequency (Slatkin 1995; see Scribner et al. 1996 for discussion of techniques and assumptions). Accumulating evidence (Scribner et

Fig. 4. Estimated (based on equal contributions of all populations) versus expected proportion of fall-run chum salmon in simulated mixed-stock fisheries. Estimates are the means of 100 bootstrap iterations and the error bars are 1 SD about the mean.



al. 1996; this study) suggests that for intraspecific comparisons among populations that over recent historical time have or continue to experience some level of gene flow, statistical analyses need not account for mutation rate heterogeneity. The number of generations separating populations is not likely to be sufficient to justify the incorporation of weighting strategies for microsatellites. Further, if microsatellite loci differ markedly in rates of mutation, this heterogeneity should be reflected in greater variances in F_{st} . Comparisons of the SEs about mean F_{st} estimates for microsatellites and allozymes (means \pm SE were 0.011 ± 0.004 and 0.010 ± 0.003 , respectively) do not show this trend.

In instances where few polymorphic allozyme loci are available for population-level analyses (e.g., *Salmo salar*, Galvin et al. 1995; Tessier et al. 1995), loci such as single-locus minisatellites and microsatellites, which are characterized by higher allelic diversities, would be expected to be comparatively more informative than allozymes. However, in situations where comparable numbers of polymorphic allozyme, minisatellite, or microsatellite loci are available, minisatellites and microsatellites would not be expected to exhibit greater levels of interpopulation variance in allele frequency. High levels of variation at these loci are a function of a high rate of mutation. As mutation rates affect only the probability of fixation and not the rate of fixation of neutral alleles, there is no greater likelihood for finding higher frequency stock-specific diagnostic markers at the hypervariable microsatellite

Table 3. Results from a simulation study evaluating biologically defined reporting regions.

Regional allocation	Mixture		
	Delta	Border	Kluane
Population contribution to border mixture weighted equally			
Delta	0.7972 (0.0707)	0.0213 (0.0183)	0.0033 (0.0071)
Border	0.1992 (0.0695)	0.9599 (0.0231)	0.1149 (0.0617)
Kluane	0.0010 (0.0026)	0.0127 (0.0108)	0.8642 (0.0666)
Population contribution to border mixture weighted by escapement			
Delta	0.7972 (0.0707)	0.0194 (0.0189)	0.0033 (0.0071)
Border	0.1992 (0.0695)	0.9646 (0.0236)	0.1149 (0.0617)
Kluane	0.0010 (0.0026)	0.0074 (0.0087)	0.8642 (0.0666)

Note: Mean mixture estimates (SD in parentheses) were derived from 100 simulations where each mixture ($N = 400$) is sampled from a single reporting region. All loci were used.

or minisatellite loci than at polymorphic protein coding loci (Ferguson 1995). Our data support this concept. Estimates of interpopulation variance in allele (or haplotype) frequency were highly concordant across marker classes (Table 1). Further, marker selection criteria based on preferential use of loci with high allelic diversity did not improve simulation estimates of population mixtures.

If no single class of genetic markers proves superior for

stock discrimination and assessments of stock allocation, other factors must be considered in order to forward recommendations to managers. Factors include cost and ease of collection and analysis, versatility, and time and cost necessary to complete baselines necessary for the analyses of fisheries samples of relevance to managers. Further, if data are needed on an in-season basis, are all markers assayable in a time frame necessary to provide data needed for decisions on fisheries openings and closures? Sampling for genetic markers that are assayed using PCR-based technology can be accomplished using extremely small samples (e.g., scales or 10^{-3} g of tissue) acquired through nonlethal sampling. Samples can also be highly degraded, which allows analyses of old samples (e.g., museum specimens, scales saved for many years, spawned-out carcasses). Perhaps the biggest concern involves the time and expense required to accumulate existing baseline information to the point where the necessary sampling of background data (i.e., putative spawning aggregations) is complete and statistical methodologies have been rigorously tested. For chum salmon at the spatial scale examined, each marker class appears to provide the accuracy and precision necessary for MSA and for descriptive and hypothesis-oriented research. Given that comparable levels of polymorphism are expressed by each candidate marker class, the relative merits of any marker class for a particular analysis may ultimately be based on the availability of baseline data. Molecular genetic markers can be added to existing protein allozyme data baselines and used collectively in MSA.

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