

Concordance of Nuclear and Mitochondrial DNA Markers in Detecting a Founder Event in Lake Clark Sockeye Salmon

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ABSTRACT Genetic bottleneck effects can reduce genetic variation, persistence probability, and evolutionary potential of populations. Previous microsatellite analysis suggested a bottleneck associated with a common founding of sockeye salmon *Oncorhynchus nerka* populations of Lake Clark, Alaska, about 100 to 400 generations ago. The common founding event occurred after the last glacial recession and resulted in reduced allelic diversity and strong divergence of Lake Clark sockeye salmon relative to neighboring Six Mile Lake and Lake Iliamna populations. Here we used two additional genetic marker types (allozymes and mtDNA) to examine these patterns further. Allozyme and mtDNA results were congruent with the microsatellite data in suggesting a common founder event in Lake Clark sockeye salmon and confirmed the divergence of Lake Clark populations from neighboring Six Mile Lake and Lake Iliamna populations. The use of multiple marker types provided better understanding of the bottleneck in Lake Clark. For example, the Sucker Bay Lake population had an exceptionally severe reduction in allelic diversity at microsatellite loci, but not at mtDNA. This suggests that the reduced microsatellite variation in Sucker Bay Lake fish is due to consistently smaller effective population size than other Lake Clark populations, rather than a more acute or additional bottleneck since founding. Caution is urged in using reduced heterozygosity as a measure of genetic bottleneck effects because stochastic variance among loci resulted in an overall increase in allozyme heterozygosity within bottlenecked Lake Clark populations. However, heterozygosity excess, which assesses heterozygosity relative to allelic variation, detected genetic bottleneck effects in both allozyme and microsatellite loci.

A genetic bottleneck occurs when a population experiences a temporary severe reduction in effective population size (N_e ; Avise 1994). Reduced N_e promotes loss of genetic variation due to genetic drift (random changes in allele frequencies as a result of imperfect sampling of alleles between generations) and an increase in genetic divergence between bottlenecked populations and those at mutation-drift equilibrium (nonbottlenecked). Founder effects are genetic bottleneck effects that are associated with the founding of a new population.

Populations that have experienced acute bottleneck effects (e.g., $\leq 20 N_e$) are important to identify because their low genetic vari-

ation may result in reduced evolutionary potential and probability of persistence (Luikart et al. 1998; Soulé and Mills 1998;

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Briskie and Mackintosh 2004). Differentiating between populations that experienced ancient bottlenecks and those that had more recent bottlenecks (e.g., within the past 100 generations) is also important because the latter may be experiencing problems due to small population size (demographic stochasticity, inbreeding, fixation of deleterious alleles) and may respond to mitigative management (Cornuet and Luikart 1996; Luikart et al. 1998). The two scenarios are not mutually exclusive. For example, populations that experienced bottleneck events in the distant past may continue to lose genetic variation over time due to low N_e and lack of genetically diverse immigrants (Crow and Aoki 1984; Slatkin 1994).

A suite of statistical tests is available to identify genetic signals of bottlenecked populations. These include tests for reduced allelic diversity in general, loss of rare alleles in particular (Allendorf 1986; Luikart et al. 1998), and heterozygosity excess (Maruyama and Fuerst 1985; Cornuet and Luikart 1996; Luikart and Cornuet 1998). Multiple genetic markers, including microsatellites, allozymes, and mitochondrial DNA (mtDNA), may be similarly tested to effectively detect genetic bottleneck effects.

Microsatellites are sensitive to recent bottleneck effects because they have large numbers of alleles as a result of high mutation rates (Weber and Wong 1993; Ellegren 2000). Rare alleles (frequency < 0.1) tend to be abundant at microsatellite loci and a reduction in their proportion relative to higher frequency alleles is a sensitive indicator of genetic bottleneck effects.

Allozymes generally have fewer alleles than microsatellites because of their lower mutation rates (Nei 1987). This lack of allelic diversity suggests allozyme loci will be less sensitive to bottleneck effects. However, rare alleles are expected to be more abundant than alleles of intermediate frequency at neutral loci regardless of the mutation rate or model

(Nei et al. 1976), and natural populations typically exhibit an abundance of rare allozyme alleles (Chakraborty et al. 1980; Luikart et al. 1998). In a survey of natural populations, allozyme loci successfully identified bottlenecked populations but with a lower success rate than microsatellites (Luikart et al. 1998).

Mitochondrial DNA generally has one-quarter the effective population size of nuclear DNA (nDNA; e.g., microsatellite and allozyme loci) because it is haploid and maternally inherited (Nei and Tajima 1981; Birky et al. 1983). Thus, genetic drift is amplified at mtDNA relative to nDNA, resulting in greater loss of mtDNA variation during a bottleneck, greater divergence between bottlenecked and nonbottlenecked populations, and slower recovery of genetic variation following a bottleneck than nDNA. Patterns of mtDNA variation also contain information about sex-biased dispersal and immigration because mtDNA is maternally inherited. For example, many studies have interpreted strong mtDNA divergence and little or no nDNA divergence among populations as evidence of male mediated gene flow and female philopatry (Palumbi and Baker 1994; Pope et al. 2000; Waits et al. 2000; Tiedemann et al. 2004).

Microsatellites, allozymes, and mtDNA, therefore, all contain information about the bottleneck history and genetic structure of populations. However, the nature of this information differs among the three marker types and comparing bottleneck signals in mtDNA and nDNA can provide information on the duration and timing of bottleneck events (Wilson et al. 1985; Birky 1991). For example, a population that experienced an acute, brief bottleneck in the distant past may currently exhibit little or no reduction in nDNA variation but significantly reduced mtDNA variation. Similarly, a population that experienced a prolonged bottleneck effect will exhibit a significant reduction in both nDNA and mtDNA variation because of the contin-

ued loss of nDNA variation at the rate of $\frac{1}{2N_e}$ per generation.

In this study, we use all three marker types to provide a comprehensive picture of genetic bottleneck effects and population structure of sockeye salmon *Oncorhynchus nerka* populations of Lake Clark, Alaska (Figure 1). Lake Clark sockeye salmon comprise 1% to 75% of the annual return to the Kvichak River watershed, the largest sockeye salmon spawning and rearing system in the world (Rogers et al. 1999; Woody 2004). The Kvichak system was historically the largest contributor of sockeye salmon to the Bristol Bay fishery, valued at up to US\$350 million (Fair 2003; Link et al. 2003). However, Kvichak River sockeye salmon are now listed as a “stock of management concern” by the Alaska Department of Fish and Game due to a chronic inability to meet minimum escapement goals over the last 10 years (Westing et al. 2005). Lake Clark

sockeye salmon also support subsistence and recreational fisheries and provide critical marine derived nutrients to the Lake Clark ecosystem, the centerpiece of Lake Clark National Park and Preserve (Kline et al. 1993; Willson and Halupka 1995).

A previous study (Ramstad et al. 2004) based on microsatellite analysis reported a founder event in Lake Clark sockeye salmon. Spawning populations of Lake Clark sockeye salmon exhibited reduced genetic variation and strong genetic divergence from populations of neighboring Six Mile Lake and Lake Iliamna (Figure 1). The Sucker Bay Lake population of Lake Clark exhibited the most extreme bottleneck signal with lower allelic richness ($A: 4.2$) and proportion of rare alleles (0.45) than all other populations surveyed ($A = 4.6-6.5$; proportion of rare alleles = 0.53-0.70), and a significant excess of heterozygosity relative to that expected at mutation-

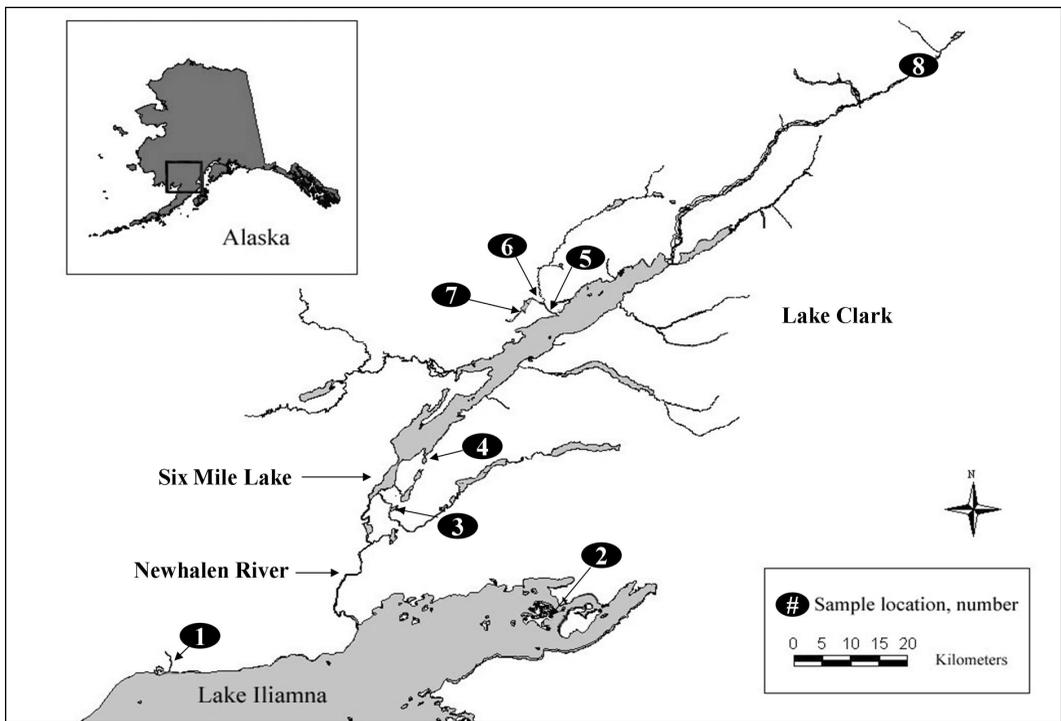


Figure 1. Map of Lake Clark, Six Mile Lake, and Lake Iliamna with sample sites shown. Sites are numbered from downstream to upstream, refer to Table 1 for population names.

drift equilibrium. In addition, Six Mile Lake populations were more similar in diversity and frequency of microsatellite alleles to Lake Iliamna populations than they were to Lake Clark populations. This result is surprising because Six Mile Lake is geographically proximate to Lake Clark and separated from Lake Iliamna by the Newhalen River (~39 km in length) which is a barrier to salmon migration at high water velocities (Poe and Mathisen 1982).

Given the economic, ecological, and cultural importance of Lake Clark sockeye salmon, it is important to verify the reduced genetic variation detected by microsatellite analysis. Testing this pattern with additional markers may also provide insight into the nature and timing of bottleneck events in Lake Clark sockeye salmon and determine if mitigation is warranted by managers to conserve recently bottlenecked populations.

In this study, we used two additional genetic markers (allozymes and mtDNA) to further examine patterns of genetic bottleneck effects and population structure in Lake Clark sockeye salmon. We addressed three specific questions:

1. Do allozyme and mtDNA data sug-

gest the same pattern of founder effects as microsatellites in Lake Clark sockeye salmon?

2. Is the reduced genetic variation of Sucker Bay Lake sockeye salmon due to a recent, acute bottleneck effect or consistently low effective population size?

3. Does the Six Mile Lake population share similar allozyme allele and mtDNA haplotype frequencies with Lake Iliamna or Lake Clark populations?

Methods

Microsatellites

The microsatellite data used here are a subset of those reported in Ramstad et al. (2004). We drew on these data to provide direct comparisons between marker types in samples of the same populations and, where possible, individual fish. Samples from the eight populations included in this study (Figure 1; Table 1) are identical to those in Ramstad et al. (2004). Data from all three marker types (microsatellites, allozymes, mtDNA) were collected from the same individuals for the Lower Talarik Creek (TCI), Fuel Dump Island (FDI—also

Table 1.

Sample description and size (*N*), mean heterozygosity (H_E), and mean allelic/haplotypic diversity (*A*) for microsatellite loci, allozyme loci, and mtDNA of Lake Clark, Six Mile Lake, and Lake Iliamna sockeye salmon populations. Allelic/haplotypic diversity is the number of alleles or haplotypes per population, standardized to the lowest sample size per locus and population. Sampling dates available upon request from corresponding author.

	Sample description		Microsatellites ^a			Allozymes			Mitochondrial DNA			
	Site	Lake	<i>N</i>	H_E	<i>A</i>	<i>N</i>	H_E	<i>A</i>	<i>N</i>	H_E	<i>A</i>	
1	TCI	Lower Talarik Creek	Iliamna	97	0.500	6.4	100	0.097	1.7	24	0.746	4.0
2	FDI	Fuel Dump Island	Iliamna	87	0.483	7.1	99	0.101	1.7	30	0.662	3.9
3	TAZ	Tazimina River	Six Mile	99	0.507	6.3	100	0.098	1.7	23	0.708	4.0
Six Mile Lake and Lake Iliamna populations pooled				283	0.507	9.3	299	0.101	2.1	77	0.708	5.0
4	SBL	Sucker Bay Lake	Clark	100	0.502	4.2	---	---	---	23	0.609	3.0
5	KR	Kijik River	Clark	99	0.484	5.1	100	0.115	1.5	23	0.237	2.0
6	LKR	Little Kijik River	Clark	98	0.457	4.9	100	0.110	1.5	26	0.542	3.0
7	KLSB	Kijik Lake South Beach	Clark	100	0.452	4.9	100	0.107	1.6	26	0.517	2.0
8	UTLK	Upper Tlikakila River	Clark	100	0.480	5.0	100	0.120	1.5	28	0.362	3.0
Lake Clark populations pooled				497	0.488	6.7	400	0.114	1.7	126	0.473	3.0

^a From Ramstad et al. 2004.

known as Flat Island), Kijik River (KR), and Upper Tlikakila River (UTLK) populations. Both microsatellite and mtDNA data were collected from the same individuals in the Kijik Lake South Beach (KLSB) and Sucker Bay Lake (SBL) populations while mtDNA and allozyme data were collected from the same fish in the Tazimina River (TAZ) population.

Allozymes

Heart, muscle, liver, and eye tissues were collected from approximately 100 adult sockeye salmon captured by seine and tangle net from seven Lake Clark, Six Mile Lake, and Lake Iliamna spawning sites in 2000 and 2001 (Figure 1; Table 1). Sucker Bay Lake fish were not sacrificed for tissue samples out of concern for the extremely small size of this population (< 500 fish observed per sampling year) and an inability to ensure sampling of only postspawning fish. Samples were immediately placed on dry ice and kept frozen (-80°C) until used in allozyme analysis. The tissue and gel protocols were those of Seeb et al. (2000); allele and locus nomenclature followed the American Fisheries Society standard (Shaklee et al. 1990).

Six of the 41 allozyme loci surveyed are isoloci (*mAH-1,2*; *sMDH-A1,2*; *sMDH-B1,2*; *TPI-1,2*; *G3PDH-1,2*; *GPI-B1,2*) which are duplicate loci that share alleles with identical electrophoretic mobility (Allendorf and Thorgaard 1984). Individuals possess four alleles at each isolocus and their genotypes cannot be accurately resolved because it is impossible to assign observed variation to a particular locus of the pair without extensive experimental matings (Waples 1988). In this study, every individual had at least two copies of the most frequent allele at each isolocus. Therefore, we treated isoloci as single, disomic loci that contained all of the variation observed within each individual, at each isolocus.

Twenty-two monomorphic allozyme loci were excluded from further analysis (*sAAT-*

1,2; *ADA-1*; *mAH-4*; *CK-A1*; *CK-A2*; *FDH*; *FH*; *PEPA*; *G3PDH-1,2*; *G3PDH-3*; *G3PDH-4*; *mIDHP-1*; *mIDHP-2*; *sIDHP-1*; *LDH-A1*; *LDH-A2*; *LDH-B1*; *LDH-C*; *PEPB-1*; *PEPLT*; *PGDH*; *sSOD-1*). Of the 19 loci included in subsequent analyses, 10 had a common allele frequency of at least 0.99 in all populations surveyed (*sAAT-3*; *sAH*; *GPI-B1,2*; *GPI-A*; *sIDHP-2*; *sMDH-A1,2*; *sMDH-B1,2*; *PEPD-1*; *TPI-3*; *TPI-4*; Table 2). The common allele at each locus was *100 except for *mAAT-1* and *TPI-1,2* where the common allele was *-100 and *PGM-1* where a null allele was most common. Frequencies of the null allele at *PGM-1* were estimated by treating the absence of product as homozygous for the null allele and assuming Hardy-Weinberg proportions (Allendorf and Seeb 2000).

Mitochondrial DNA

Dorsal fin tissue clips (approximately 5 mm²) were collected from fish from eight spawning populations (Figure 1; Table 1) and immediately stored in 100% ethanol. Total genomic DNA was extracted from fin clips according to Spruell et al. (1999).

Restriction fragment length polymorphism (RFLP) analysis was performed on 23–30 fish per population using the ND1/ND2 region of mtDNA. This region was selected because it is variable and representative of genetic variation patterns found in other regions of sockeye salmon mtDNA (Churikov et al. 2001; Doiron et al. 2002). The ND1/ND2 region (~2,600bp) was amplified in 35 µL polymerase chain reactions (PCR) using primers given by Carney et al. (1997) and Gharrett et al. (2001). The reaction mixture contained 10 mM tris-HCl (pH 9.0), 50 mM KCl, 0.2 mM each dNTP, 2.0 mM MgCl₂, 0.2 µM each primer, and 1.75 units of Taq DNA polymerase (Fisher Scientific, Fair Lawn, NJ). Amplification was carried out in an MJ Research PTC-200 Peltier thermal cycler with the following PCR profile:

Table 2.

Allozyme allele frequencies in sockeye salmon of Lake Clark, Six Mile Lake, and Lake Iliamna. Frequencies are given for the most common allele for diallelic loci. Loci for which the frequency of the common allele is at least 0.99 in all populations are not listed. Refer to Table 1 for population names.

Sample	mAH-1,2			mAH-3	LDH-B2			mAA-1	TPI-1,2		
	*100	*75	*133		*100	*110	*85		*-100	*54	*-173
1 TCI	0.850	0.125	0.025	1.000	0.930	0.070	—	0.923	0.995	—	0.005
2 FDI	0.783	0.187	0.030	1.000	0.894	0.106	—	0.948	0.990	0.005	0.005
3 TAZ	0.845	0.140	0.015	1.000	0.855	0.145	—	0.969	0.985	0.015	—
5 KR	0.795	0.170	0.035	0.980	0.820	0.180	—	0.765	1.000	—	—
6 LKR	0.855	0.110	0.035	0.979	0.855	0.145	—	0.833	1.000	—	—
7 KLSB	0.825	0.170	0.005	0.990	0.910	0.080	0.010	0.800	1.000	—	—
8 UTLK	0.779	0.189	0.032	1.000	0.765	0.230	0.005	0.670	1.000	—	—

Sample	ALAT				PGM-1	PGM-2	MPI			
	*100	*91	*108	*95			*100	*105	*91	*96
1 TCI	0.372	0.541	0.015	0.071	0.805	0.758	0.980	0.015	—	0.005
2 FDI	0.414	0.525	—	0.061	0.832	0.778	0.995	—	0.005	—
3 TAZ	0.725	0.181	—	0.093	0.697	0.775	1	—	—	—
5 KR	0.781	0.073	—	0.146	0.640	0.815	1	—	—	—
6 LKR	0.707	0.131	—	0.162	0.716	0.730	1	—	—	—
7 KLSB	0.735	0.150	—	0.115	0.720	0.755	1	—	—	—
8 UTLK	0.708	0.078	—	0.214	0.773	0.815	1	—	—	—

95°C for 2 min followed by 30 cycles of denaturing at 95°C for 1 min, annealing at 50°C for 1 min, and elongation at 72°C for 3 min; amplification was completed with a final extension step of 72°C for 3 min.

Amplified product was digested with each of four restriction endonucleases (*Bst*N I, *Bst*U I, *Ban* II, *Sau*96 I; New England BioLabs, Beverly, MA) in separate, 20 µL reactions. Reactions consisted of a 5 µL sub sample of the PCR product, 2 µL of 10x restriction buffer, and 3 units of restriction enzyme. Digests with *Bst*N I also included 0.2 µL of 100X bovine serum albumin (BSA). Digest reactions were incubated at either 60°C (*Bst*N I, *Bst*U I) or 37°C (*Ban* II, *Sau*96 I) for 3 h, the latter was inactivated by exposure to 80°C for 20 min. Digest products were electrophoresed on 1.5% agarose gels prepared with SeaKem LE agarose and ethidium bromide in 0.5× TBE buffer (pH~8.0; 0.045M tris, 0.045M Boric acid, and 0.001M EDTA). Gels were scanned on Hitachi FMBIO 100 and Hitachi FMBIO II

and band sizes scored relative to a 100bp DNA ladder size standard (Invitrogen Life Technologies, Carlsbad, CA) using Hitachi FMBIO Analysis software version 8.0. Blank reactions (all constituents present but template DNA) and uncut PCR product were included in each gel to detect sample contamination.

A letter code was assigned to each distinct banding pattern for each enzyme reaction and these were used in combination (order: *Bst*N I, *Bst*U I, *Ban* II, *Sau*96 I) to describe composite haplotypes as in Churikov et al. (2001; Table 3). Fragments less than 140bp in length could not be resolved with the method described above, but were diagnostic of only a single banding pattern (C) of a single enzyme (*Sau*96 I) previously described by Churikov et al. (2001). This pattern was identified by the presence of a larger, easily viewed fragment. Thus, we expect that only novel haplotypes distinguished by bands less than 140bp would go undetected with this method.

Table 3.

Sample size (*N*) and composite mtDNA haplotype frequencies of Lake Clark, Six Mile Lake, and Lake Iliamna sockeye salmon populations. Refer to Table 1 for population names.

	<i>Sample</i>	<i>N</i>	<i>ABDC</i>	<i>AACA</i>	<i>ABBA</i>	<i>ABBC</i>	<i>AAFA</i>
1	TCI	24	0.208	0.292	0.375	0.125	—
2	FDI	30	0.233	0.500	0.233	0.033	—
3	TAZ	23	0.130	0.478	0.217	—	0.174
4	SBL	23	0.565	0.261	0.174	—	—
5	KR	23	0.870	0.130	—	—	—
6	LKR	26	0.615	0.308	0.077	—	—
7	KLSB	26	0.538	0.462	—	—	—
8	UTLK	28	0.786	0.179	0.036	—	—

Statistical Analysis

Gametic disequilibrium was assessed for all pair-wise locus comparisons within each population using a Fisher exact test in GENETPOP version 3.3 (Raymond and Rousset 1995a). Gametic disequilibrium between loci of different marker types was assessed for only a subset of the populations sampled because data for each marker type was not always collected on the same fish from each population. Therefore gametic disequilibrium was assessed between microsatellite and allozyme loci in four populations (all but TAZ, SBL, Little Kijik River[LKR], and KLSB), between microsatellite loci and mtDNA in six populations (all but TAZ and LKR) and between allozyme loci and mtDNA in five populations (all but SBL, LKR and KLSB).

Departures from Hardy-Weinberg proportions (Guo and Thompson 1992) and heterogeneity in population allele frequencies (Raymond and Rousset 1995b) were tested and pair-wise F_{ST} (Wright 1951; Weir and Cockerham 1984) and R_{ST} (Slatkin 1995; Rousset 1996) calculated in GENETPOP version 3.3 (Raymond and Rousset 1995a). Tests of significance were combined over all loci using Fisher's combined probability test (Sokal and Rohlf 1981). Heterogeneity in haplotype frequencies was tested and pair-wise mtDNA F_{ST} was calculated in ARLEQUIN version

2.000 (Schneider et al. 2000). Principal components analysis (PCA) for allozyme and microsatellite allele and mtDNA haplotype frequencies was performed using the covariance matrix in MINITAB, version 13 (State College, PA). One allele at each allozyme and microsatellite locus and the rarest mtDNA haplotype was omitted from these analyses to allow for the nonindependence of allele/haplotype frequencies within loci (Johnson 1998). Sequential Bonferroni adjustments were made for all multiple comparisons (Rice 1989).

A suite of tests was used to detect genetic bottleneck effects. Mean heterozygosity (H_E : heterozygosity expected under Hardy-Weinberg proportions given observed allele frequencies) for all marker types was calculated as Nei's gene diversity (Nei 1987) in FSTAT version 1.2 (Goudet 1995). Because the number of alleles detected in a population is highly dependent on sample size, allelic richness (A : mean number of alleles or haplotypes per population and locus corrected for sample size) was computed and compared among population groups according to El Mousadik and Petit (1996) in FSTAT. Microsatellite, allozyme, and mtDNA allelic richness was standardized to sample sizes of 86, 83, and 23 fish for individual populations and to sample sizes of 233, 283, and 77 fish when populations were pooled (all Six Mile Lake and Iliamna Lake populations, all Lake Clark populations). Mode shift in allele frequency distribution within populations (lower proportion of rare alleles relative to those with a frequency of 0.2–0.3) was assessed graphically for nuclear markers (Luikart et al. 1998). Difference in proportion of polymorphic allozyme loci, mean proportions of rare alleles, and H_E within marker types and between lakes was tested with a one tailed Mann-Whitney U -test

(H_a : lower values among Lake Clark than Lake Iliamna and Six Mile Lake populations) in MINITAB, version 13 (State College, PA).

Bottlenecked populations exhibit greater H_E than expected if the population was at mutation-drift equilibrium (H_{Eq} : heterozygosity expected given Hardy-Weinberg proportions, the observed number of alleles, and a constant population size). This effect can be due to either a greater reduction in allelic diversity than heterozygosity (allelic deficit) or a random increase in H_E due to drift (Luikart et al. 1998; Tarr et al. 1998). Heterozygosity excess relative to a nonbottlenecked population at mutation-drift equilibrium ($H_E > H_{Eq}$) was assessed in BOTTLENECK, version 1.2.02 (Cornuet and Luikart 1996) for nuclear markers. This test is performed with the assumption of the infinite alleles model of mutation (IAM) though microsatellites are expected to generally conform more closely to a step-wise mutation model (SMM; Shriver et al. 1993) or a two phase model of mutation (TPM) incorporating aspects of both the IAM and the SMM (Luikart et al. 1998). In addition to varying among marker types, the appropriate mutation model can vary among loci of the same marker type (Slatkin 2002). In the present context, the use of the IAM is equal to the assumption that the sampled Iliamna populations are not bottlenecked because their observed heterozygosity closely matches that expected at mutation-drift equi-

librium under the IAM (Cornuet and Luikart 1996). Significance of heterozygosity excess over all loci was assessed with a Wilcoxon sign-rank test (Cornuet and Luikart 1996; Luikart and Cornuet 1998).

Results

Genetic Variation within Populations

Nineteen of 41 allozyme loci surveyed were polymorphic (2–4 alleles each; Table 2) and all populations were in Hardy-Weinberg proportions at all polymorphic allozyme and microsatellite loci combined within marker type. Only one locus (*PGM2*) in a single population (KLSB) deviated significantly from Hardy-Weinberg proportions ($P < 0.05$ after sequential Bonferroni correction).

Five haplotypes were observed in the ND1/ND2 region of mtDNA (Figure 2; Table 3). Three of these haplotypes were previously described among sockeye salmon by Churikov et al. (2001; our haplotype AACA =

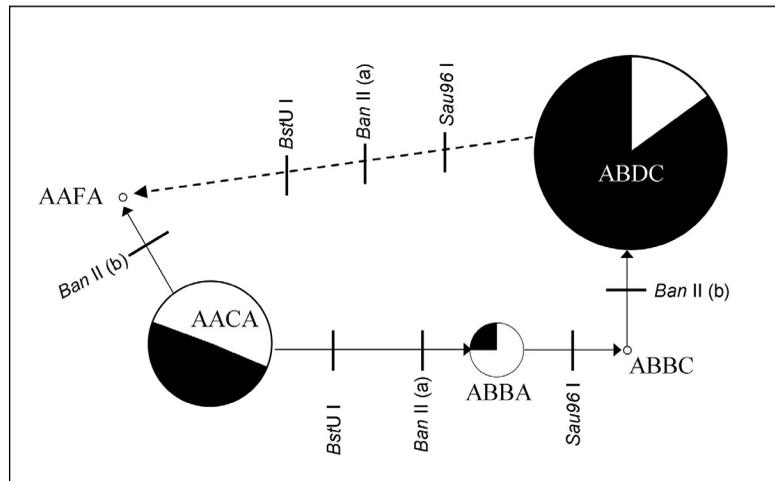


Figure 2. Network of haplotypes detected in this study. Haplotypes represent one *BstU* I site, two *Ban* II sites, and one *Sau96* I site and are coded as ABDC = 1111, AACA = 0000, ABBA = 1100, ABBC = 1101, and AAFA = 0010. Arrows indicate direction of site gain, circle size indicates haplotype frequency over all fish sampled, and shading within circles indicates proportion of fish from Six Mile and Iliamna Lakes (open) and Lake Clark (shaded) with that haplotype. The dashed arrow indicates an alternative, less parsimonious derivation of the AAFA haplotype from the ABDC haplotype.

their haplotypes L and Q , $ABBA = H$, $ABBC = G$), while two others ($ABDC$, $AAFA$) are new. The $ABDC$ and $AAFA$ haplotypes differ from the $ABBC$ and $AACA$ haplotypes by the presence of an additional *Ban* II cut site that results in the absence of a 340bp band and the presence of 190bp and 150bp bands (Figure 2). The $AAFA$ haplotype could also have been derived from the $ABDC$ haplotype with the gain of three cut sites found among other haplotypes (Figure 2).

There was no tendency toward gametic disequilibrium between any pair of loci or within any population. Significant gametic disequilibrium ($P < 0.05$ after sequential Bonferroni correction across populations) was observed in only 7 of 440 pair-wise microsatellite locus comparisons and 2 of 252 possible pair-wise allozyme locus comparisons. Significant gametic disequilibrium was present in 2 of 39 comparisons between mtDNA and allozyme loci and was not observed between mtDNA and microsatellite loci. Gametic disequilibrium was significant in 7 of 385 possible pair-wise comparisons between microsatellite and allozyme loci. Overall, there was no evidence for biologically meaningful gametic disequilibrium (i.e., significant gametic disequilibrium in multiple loci within the same population or vice versa).

Genetic Bottleneck Effects

A lower proportion of polymorphic allozyme loci was observed in Lake Clark populations (0.41) than Six Mile Lake and Lake Iliamna populations (0.56; $P < 0.05$). Mean allelic richness (A) was significantly reduced in Lake Clark populations relative to Six Mile Lake and Lake Iliamna populations for all three marker types ($P = 0.02$; Table 1). Microsatellite H_E was not lower among Lake Clark populations than Six Mile Lake and Lake Iliamna populations ($P = 0.12$). However, mtDNA H_E was lower among Lake Clark than Six Mile Lake and Lake Iliamna populations ($P =$

0.02). Allozyme H_E was greater in all Lake Clark populations than all Six Mile Lake and Lake Iliamna populations (Table 1) and thus negated the need for a formal one-tailed test of the opposite expectation. These differences persisted when populations were pooled (Table 1).

In addition, fewer alleles and haplotypes were observed in Lake Clark fish than in Six Mile Lake and Lake Iliamna fish despite a greater sample size of Lake Clark fish. Only 82 microsatellite alleles were observed in 497 Lake Clark fish, while 102 were observed in 283 Six Mile Lake and Lake Iliamna fish. Thirty-three allozyme alleles were observed in the 400 Lake Clark fish sampled while 40 were present in 299 fish sampled from Six Mile Lake and Lake Iliamna. Finally, 3 mtDNA haplotypes were present in 126 Lake Clark fish sampled, while 5 haplotypes were observed in 77 Six Mile Lake and Lake Iliamna fish sampled. In total, 118 alleles and haplotypes were detected among 1,023 Lake Clark fish sampled, while 147 alleles and haplotypes were detected in 659 fish sampled from Six Mile Lake and Lake Iliamna (alleles and fish sample sizes summed over loci). Alleles and haplotypes present in Lake Clark populations were generally a subset of those found commonly in Six Mile Lake and Lake Iliamna populations (Figure 2; Table 2, Table 3). Exceptions were a single allozyme allele at each of the *LDH-B2* and *mAH-3* loci (Table 2) and 16 of 120 alleles found in 9 of 11 microsatellite loci surveyed (data not shown). These alleles were all rare (frequency less than 0.1).

There were no mode-shifts in microsatellite allele frequency distribution in any population. Lake Clark populations had a lower mean proportion of rare microsatellite alleles (0.54) than Six Mile Lake and Lake Iliamna populations (0.65; $P = 0.02$). The Sucker Bay Lake population had a lower proportion of rare microsatellite alleles (0.45) than any

other population surveyed (range = 0.53–0.69). All Lake Clark populations had a microsatellite heterozygosity excess relative to Six Mile Lake and Lake Iliamna populations under the IAM (Figure 3). Significant heterozygosity excess was observed in Sucker Bay Lake ($H_E - H_{Eq} = 0.118$; $P < 0.01$), but not in any other Lake Clark population ($H_E - H_{Eq} = 0.024$ – 0.045 ; $P = 0.12$ – 0.26). There was no evidence of microsatellite heterozygosity excess in any population under the SMM or TPM.

The Little Kijik River population of Lake Clark displayed a shift in allozyme allele frequency mode (proportion of rare alleles = 0.125, proportion of alleles with frequency between 0.2 and 0.3 = 0.313) and Lake Clark populations had a significantly reduced mean proportion of rare allozyme alleles (0.22) relative to populations of Six Mile Lake and Lake Iliamna (0.37; $P = 0.03$). All the Lake Clark populations displayed an excess of allozyme heterozygosity relative to Six Mile Lake and Lake Iliamna under the IAM (Figure 3). This effect was statistically significant in two populations (LKR, $H_E - H_{Eq} = 0.087$; UTLK, $H_E - H_{Eq} = 0.098$; $P < 0.05$).

Genetic Divergence among Populations

Twenty-seven of 28 pair-wise population comparisons showed significant differences in microsatellite allele frequencies over all loci ($P < 0.01$; Ramstad et al. 2004). The only populations with statistically equal microsatellite allele frequencies were LKR and KLSB, proximate populations that are part of the same

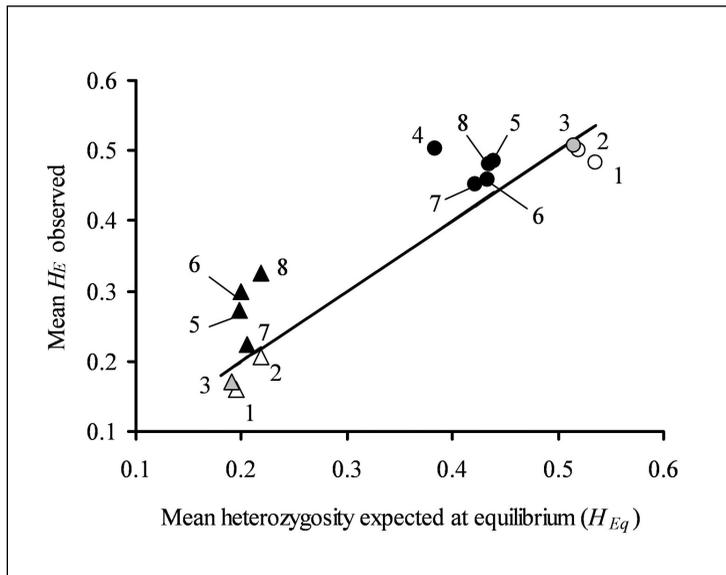
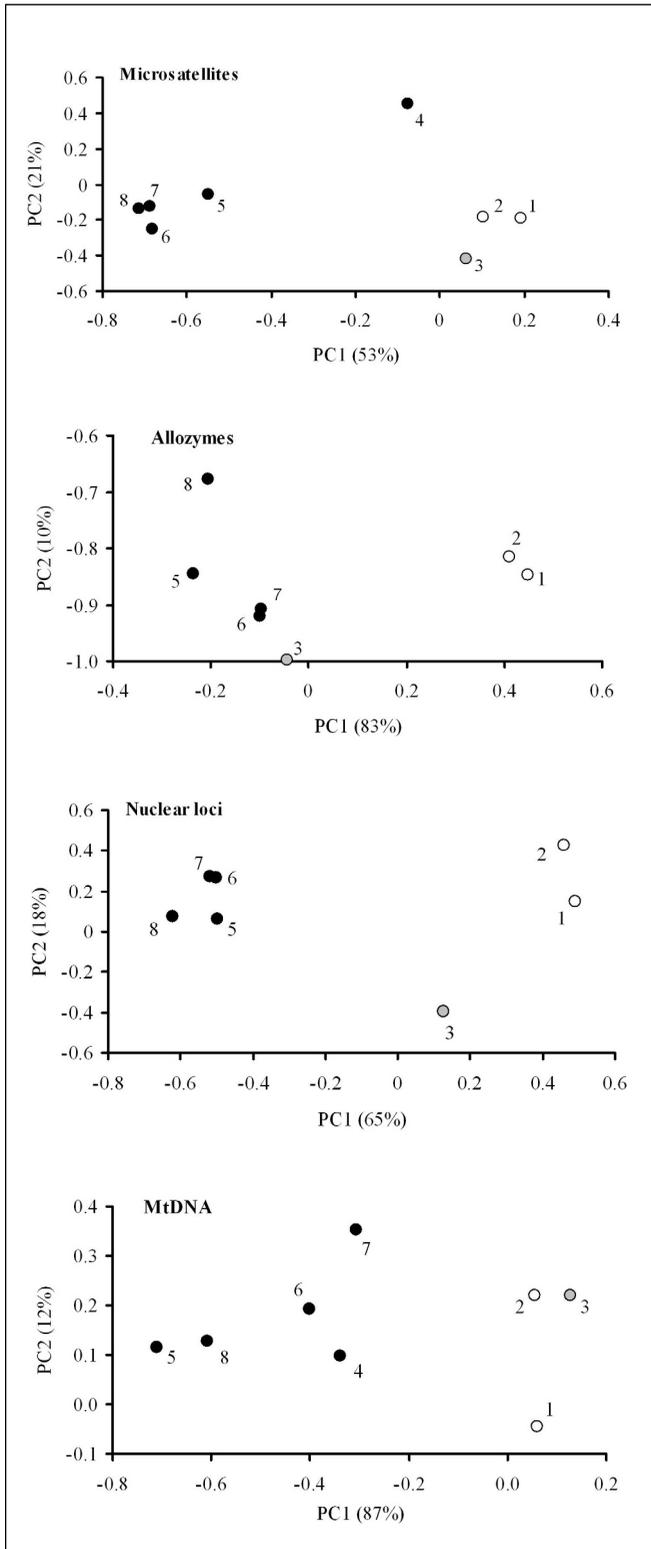


Figure 3. Relationship between mean expected heterozygosity observed (H_E) and expected at mutation-drift equilibrium (H_{Eq}) for 11 microsatellite loci (circles) and 13 allozyme loci (triangles) in Lake Clark (black), Six Mile Lake (gray), and Lake Iliamna (open) sockeye salmon populations. Lake Clark populations have greater H_E than H_{Eq} for both markers due to genetic drift associated with a common founding event. Refer to Table 1 for population names.

small tributary lake system to Lake Clark (Figure 1). F_{ST} ranged from 0.001 (LKR and KLSB) to 0.077 (TCI and UTLK; SBL and LKR; Ramstad et al. 2004). R_{ST} values (range = 0.002–0.110; results not shown) were generally similar to F_{ST} values, and overall F_{ST} and R_{ST} values (all populations and loci) were 0.048 and 0.042, respectively. High F_{ST} and R_{ST} values suggested strong divergence between Lake Clark populations and those in Six Mile Lake and Lake Iliamna and between the Sucker Bay Lake population and all other populations. Principal components analysis of microsatellite data supported this pattern (Figure 4). The first principal component grouped Six Mile Lake and Lake Iliamna populations and differentiated them from Lake Clark populations. The second principal component differentiated the Sucker Bay Lake population from all other populations surveyed.

Significant differences in allozyme allele fre-



frequencies were observed in 18 of 21 pair-wise population comparisons ($P < 0.001-0.03$; Table 4). Populations with equal allozyme allele frequencies were the two Iliamna populations (FDI and TCI) and Kijik Lake populations (LKR and KLSB; KR and LKR). Pair-wise F_{ST} between populations ranged from zero (FDI and TCI; LKR and KLSB) to 0.112 (TCI and KR; TCI and UTLK; Table 4). The pattern of genetic divergence at allozyme loci was similar to that of microsatellite loci, with the greatest F_{ST} values between fish spawning in Lake Clark and Lake Iliamna.

Principal components analysis of allozyme data suggested the Tazimina River population of Six Mile Lake was more similar genetically to Lake Clark than Lake Iliamna populations (Figure 4). However, this finding was largely due to a single allozyme locus (*ALAT*) with a strong influence in the principal components analysis (PC1 loading for *ALAT* allele *100 = -0.576, *91 = 0.717; next highest PC1 loading is for *mAAT-1* allele *-100 = 0.273). With removal of this locus, PCA of the remaining 18 allozyme loci suggested the TAZ population is more similar genetically to Iliamna populations (distance on

Figure 4. Principal components analysis for Lake Clark (black), Six Mile Lake (gray), and Lake Iliamna (open) sockeye salmon populations. Analyses are of 11 microsatellite loci, 13 allozyme loci, nuclear markers combined, and mtDNA. Percentages in parentheses indicate amount of variation explained by each principal component. Refer to Table 1 for population names.

Table 4.

Allozyme pair-wise F_{ST} (below diagonal) and number of loci with significant allele frequency differences after sequential Bonferroni correction (above diagonal) between Lake Clark, Six Mile Lake, and Lake Iliamna sockeye salmon populations. Comparisons without significant allele frequencies differences over all loci ($P > 0.05$ Fisher's combined probability test) are noted as NS. No allozyme data is available for Sucker Bay Lake (population 4, SBL), refer to Table 1 for population names.

	1 TCI	2 FDI	3 TAZ	5 KR	6 LKR	7 KLSB	8 UTLK
TCI	—	NS	1	4	1	2	3
FDI	0	—	2	3	2	2	3
TAZ	0.067	0.060	—	2	1	1	2
KR	0.112	0.106	0.024	—	NS	1	1
LKR	0.074	0.072	0.008	0.008	—	NS	1
KLSB	0.075	0.072	0.013	0.007	0	—	3
UTLK	0.112	0.104	0.050	0.011	0.019	0.020	—

Table 5.

Pair-wise F_{ST} of mtDNA (below diagonal) and significance of haplotype frequency differences (above diagonal) between Lake Clark, Six Mile Lake, and Lake Iliamna sockeye salmon populations. NS = not significant at $P > 0.05$; *significant at $P < 0.05$; **significant at $P < 0.01$; ***significant at $P < 0.001$. Refer to Table 1 for population names.

	1 TCI	2 FDI	3 TAZ	4 SBL	5 KR	6 LKR	7 KLSB	8 UTLK
TCI	—	NS	NS	*	***	**	***	***
FDI	0.014	—	NS	NS	***	*	**	***
TAZ	0.033	0	—	**	***	***	**	***
SBL	0.085	0.088	0.138	—	*	NS	*	NS
KR	0.367	0.369	0.427	0.109	—	NS	*	NS
LKR	0.146	0.120	0.175	0	0.080	—	NS	NS
KLSB	0.163	0.083	0.138	0.022	0.196	0	—	*
UTLK	0.288	0.285	0.344	0.038	0	0.015	0.112	—

the PC1 axis from TAZ to nearest Iliamna population, FDI = 0.025) than to Lake Clark populations (distance on the PC1 axis from TAZ to nearest Lake Clark neighbor, KLSB = 0.084). The *ALAT* locus also displayed disproportionately large F_{ST} values in comparisons between populations of different lakes (mean *ALAT* F_{ST} = 0.164 ± 0.059 , 95% CI; mean F_{ST} all other loci = 0.077 ± 0.022 , 95% CI) but not when comparisons between populations of the same lake are also considered (mean *ALAT* F_{ST} = 0.111 ± 0.052 , 95% CI; mean F_{ST} all other loci = 0.054 ± 0.021 , 95% CI).

Significant differences in mtDNA haplotype frequencies were present in 18 of 28 pair-

wise population comparisons ($P = 0 - 0.05$; Table 5). F_{ST} values ranged from zero (four comparisons) to 0.427 (TAZ and KR) and suggested strong divergence between fish spawning in Lake Clark and those spawning in Six Mile Lake and Lake Iliamna (Table 5). Principal components analysis of the mtDNA data closely approximated that of the microsatellite data in that the first principal component differentiated between fish spawning in Lake Clark and those spawning in Lake Iliamna and Six Mile Lake (Figure 4). Unlike the microsatellite data, however, mtDNA suggested similarity between the Sucker Bay Lake populations and all other Lake Clark populations.

Discussion

We found that 1) patterns of genetic variation at allozyme and mtDNA markers supported the presence of a founder effect associated with the colonization of Lake Clark by sockeye salmon, 2) further reduced microsatellite variation in Sucker Bay Lake sockeye salmon is likely due to persistent low N_e and isolation since the initial founder effect, and 3) variation at allozyme loci and mtDNA supported the conclusion that Six Mile Lake sockeye salmon are more similar genetically to Lake Iliamna than Lake Clark sockeye salmon.

Lake Clark Founder Effect

Lake Clark sockeye salmon populations have reduced genetic diversity at microsatellite loci, allozyme loci, and mtDNA relative to Six Mile

Lake and Lake Iliamna populations. This is evidenced by lower allelic richness, fewer total numbers of alleles, a lower mean proportion of rare microsatellite and allozyme alleles, and a reduced proportion of polymorphic allozyme loci in Lake Clark than Six Mile Lake and Lake Iliamna sockeye salmon populations.

Heterozygosity of Lake Clark populations (H_E) exceeds that expected at mutation-drift equilibrium (H_{Eq}) relative to populations in Six Mile Lake and Lake Iliamna. With microsatellite data, this effect is evident in all Lake Clark populations (but only statistically significant in Sucker Bay Lake) and reflects the deficit of microsatellite alleles in Lake Clark sockeye salmon. Allozyme heterozygosity excess is evident in all Lake Clark populations as well, and is statistically significant in the Little Kijik River and Upper Tlikakila River populations. This trend toward allozyme heterozygosity excess is still evident when the outlier locus *ALAT* is removed and reflects not only reduced allelic diversity but also greater allozyme heterozygosity in Lake Clark than Six Mile Lake and Lake Iliamna populations. Achieving statistical power greater than 0.80 to detect a bottleneck with this test requires 10 polymorphic loci per population (Luikart and Cornuet 1998). In this study, numbers of polymorphic allozyme loci per population range from 7 to 12. However, our findings of bottleneck effects based on allozyme heterozygosity are conservative because at low statistical power it is more likely that a bottlenecked population will be identified as nonbottlenecked than vice versa.

Taken together, these results suggest that a common founding reduced the genetic variation of all Lake Clark populations relative to Six Mile Lake and Lake Iliamna populations of sockeye salmon. The observation that alleles and haplotypes present in Lake Clark populations were generally a subset of those found commonly in Six Mile Lake and Lake Iliamna populations further supports this hypothesis, as

does the difference in A and H_E between pooled Lake Clark and pooled Six Mile Lake and Lake Iliamna populations. Had Lake Clark populations experienced bottlenecks independently, allele frequencies would have drifted independently among populations and pooling them would have produced A and H_E values similar to those observed in the pooled Six Mile Lake and Lake Iliamna populations. The fact that genetic bottleneck signals persist when Lake Clark populations are pooled (reduced A , increased allozyme H_E) suggests these populations experienced a common genetic bottleneck effect rather than multiple independent bottlenecks. A similar pattern was observed among introduced New Zealand Chinook salmon populations *O. tshawytscha* which have reduced allozyme and mtDNA variation relative to putative source populations (Quinn et al. 1996).

Reduced microsatellite variation and lack of mutation-drift equilibrium suggest that Lake Clark was colonized by sockeye salmon within the last 100–400 hundred sockeye salmon generations (~400–1400 years; Ramstad et al. 2004). This timeframe is not surprising given that Lake Clark was created by glacial retreat approximately 12–15,000 years ago and is geologically young (Stilwell and Kaufman 1996). Once a population has lost genetic variation due to drift, it can only be restored by mutation or immigration. Recovery of genetic variation by mutation can take thousands of generations even with the high mutation rate of microsatellites (Crow and Aoki 1984; Birky et al. 1989; Waples 1998; Kinnison et al. 2002). The similar F_{ST} and R_{ST} values between Lake Clark populations suggest a minor role for mutation in promoting genetic variation since a recent, common founding of Lake Clark sockeye salmon (Wenburg et al. 1998).

Sucker Bay Lake

The Sucker Bay Lake population exhibits a strong genetic bottleneck signal (reduced variation, strong divergence) at microsatellite loci

relative to all other populations surveyed (Ramstad et al. 2004). However, this bottleneck signal could not be assessed at allozyme loci (no data are available), and Sucker Bay Lake fish have similar mtDNA variation to all other Lake Clark populations. In addition, the Sucker Bay Lake population exhibits similar haplotype frequencies and divergence from Six Mile Lake and Lake Iliamna fish as other Lake Clark populations. Caution is required when interpreting these results as mtDNA is a single locus while the microsatellite data are from eleven independent loci. However, the contrasting results between the mtDNA and the microsatellite data may lend some insight into the nature of the bottleneck signal in Sucker Bay Lake sockeye salmon.

Mitochondrial DNA is expected to show greater divergence between populations and lower variation than nDNA because of its reduced effective population size (Birky et al. 1983, 1989). An acute, brief bottleneck could greatly reduce mtDNA haplotype diversity while having little effect on nDNA allelic diversity (Wilson et al. 1985; Birky 1991; Hurtado et al. 2004). This is opposite of the pattern observed in Sucker Bay Lake. The discordance between nDNA and mtDNA bottleneck signals in Sucker Bay Lake is likely due to persistent low N_e and isolation since sharing in a common founding with other Lake Clark populations. A similar pattern has been observed in post-fur trade populations of sea otters *Enhydra lutris* where further reduction of nDNA variation relative to mtDNA variation could also be explained by the differential effects of drift on nuclear and mitochondrial DNA (Larson et al. 2002).

Aerial estimates of numbers of spawning fish suggest that Sucker Bay Lake census population size is typically low relative to other Lake Clark populations and fluctuates tremendously from year to year (< 100 to > 7,000 spawners annually between 1955 and 1998; Regnart 1998). In addition, recent evi-

dence from tagging studies suggests isolation of the Sucker Bay Lake population (low straying, different spawning times) from other Lake Clark spawning populations (Young 2004). If these observations reflect historical patterns, they also support the hypothesis that persistent low N_e and isolation have promoted greater loss of nuclear variation in Sucker Bay Lake than other Lake Clark populations.

Greater divergence between populations in nDNA than mtDNA could also be due to greater female immigration or skewed sex ratio in favor of females. However, the bias would have to be extreme (between 7 and 15 females per male; Birky et al. 1989) or female gene flow would homogenize populations in both nuclear and mitochondrial DNA (e.g., Kittles et al. 1999). There is no evidence for female-biased dispersal between spawning populations of sockeye salmon. Nuclear and mitochondrial divergence between Cook Inlet populations is equivalent after correction for the lower N_e of mtDNA (Allendorf and Seeb 2000) and Taylor et al. (1997) suggested restricted female gene flow in nonanadromous *O. nerka*. There is similarly no evidence for a highly skewed sex ratio among Sucker Bay Lake sockeye salmon (personal observation, K. Ramstad). Thus, it is unlikely that the discordance between nuclear and mitochondrial DNA divergence between Sucker Bay Lake and other Lake Clark populations is due to different reproductive contributions of males and females.

Six Mile Lake

All three marker types surveyed show divergence between sockeye salmon spawning in Lake Clark and those spawning in Lake Iliamna. Significant differences in allele and haplotype frequencies, high F_{ST} values between Lake Clark and Lake Iliamna populations, and principal components analysis of variation at 11 microsatellite loci, 19 allozyme loci and mtDNA support this pattern.

All three marker types surveyed also support the genetic similarity between the Tazimina River population of Six Mile Lake and Lake Iliamna populations. A single, highly influential allozyme locus (*ALAT*) suggests the Tazimina River population is more similar genetically to Lake Clark than to Lake Iliamna populations. With the removal of this outlier locus, the remaining 18 allozyme loci support the similarity between Six Mile Lake (Tazimina River) and Lake Iliamna sockeye salmon.

It has been suggested that variation at allozyme loci is more likely to be influenced by natural selection than variation at microsatellite loci (e.g., Karl and Avise 1992; Pogson et al. 1995). Therefore, the exceptionally high F_{ST} values and PCA loadings at the *ALAT* locus may be due to selection at this locus or another to which it is tightly linked. Slatkin and Muirhead (1999) have demonstrated theoretically that slight deviations from neutrality can strongly affect both the allele frequencies and the probability of allelic loss at a given locus. However, the great divergence at *ALAT* may also be caused by genetic drift as fluctuations in effective population size over time are expected to increase the variance of F_{ST} among loci (Beaumont and Nichols 1996). Drift is suggested when outlier behavior of a locus is coupled with evidence of a genetic bottleneck effect while selection is supported if the same loci exhibit similar outlier patterns in multiple and distant sampling locations (Luikart et al. 2003). For example, Allendorf and Seeb (2000) observed an outlier allozyme locus (*sAH*) in their study of sockeye salmon populations of Cook Inlet, Alaska. Had the same locus been an outlier in this study, selection would be supported as the cause of the outlier behavior. However, different loci behaved as outliers in these two studies and in both cases were associated with populations that appeared to have gone through recent genetic bottlenecks. In contrast, the *LDH-B2* locus is likely under selec-

tion as it has exhibited outlier behavior in multiple, distant sampling locations with allele frequencies correlated with differences in spawning habitat (Varnavskaya et al. 1994a). Large variation in allele frequencies at the *ALAT* locus have been observed among sockeye salmon populations in both this and other studies (Varnavskaya et al. 1994a, 1994b; Wood et al. 1994). In other studies, however, variation at the *ALAT* locus did not provided results that were discordant with other loci or were correlated with genetic bottleneck signals. Thus, without additional information, it is impossible to distinguish between the alternative explanations of selection or drift in promoting the outlier behavior of the *ALAT* locus in this study.

The data presented above, and the observation that a population at the confluence of Lake Clark and Six Mile Lake is genetically similar to Lake Iliamna populations (Ramstad et al. 2004), suggest that sockeye salmon of Six Mile Lake (Tazimina River) are genetically similar to Lake Iliamna sockeye salmon. In addition, the Tazimina River population does not display the reduced genetic variation common to Lake Clark populations at the varied markers surveyed here, or in the broader analyses of Ramstad et al. (2004) or Habicht et al. (2004). We conclude that sockeye salmon spawning in Six Mile Lake have a stronger genetic affinity with Lake Iliamna than with Lake Clark sockeye salmon.

Implications

This study demonstrates the importance of using a variety of genetic marker types to test for bottleneck effects and determine genetic population structure. The combination of microsatellite and mtDNA data suggests that isolation and low N_e have promoted further loss of genetic variation in the Sucker Bay Lake population since sharing in a common founder event with other Lake Clark populations. Thus, this combination of data types

provides greater understanding of the nature of the bottleneck effect in the Sucker Bay Lake population than either marker type could have provided alone. In addition, similar results at two additional marker types strongly support the pattern of genetic population structure previously determined with microsatellite data.

Investigators should be cautious when using heterozygosity as an indicator of genetic bottleneck effects. This is because heterozygosity is not as sensitive to drift as allelic diversity (Allendorf 1986), and there is large stochastic variance in heterozygosity among loci, particularly after a reduction in population size (Leberg 1992; Tarr et al. 1998). When a population experiences a bottleneck, a reduction in heterozygosity is expected *on average* across loci. However, for loci with two alleles, there is a nearly equal probability that heterozygosity will increase or decrease at each locus due to random genetic drift of allele frequencies. This is because heterozygosity is maximized when the alleles are equally frequent. For example, Tarr et al. (1998) assayed variation in a recently founded Laysan finch *Telespyza cantans* population and its founding population using nine microsatellite loci with 2–5 alleles per locus. They observed that four of the nine loci exhibited greater H_E in the bottlenecked population than in the founding population though mean H_E overall was reduced.

In this study, 8 of 19 allozyme loci exhibited a greater mean H_E in Lake Clark populations than in Six Mile Lake and Lake Iliamna populations resulting in increased mean H_E over all loci and in every Lake Clark population surveyed. This increase was unexpected, given the observed reduction in H_E at microsatellite loci and mtDNA, and could have been interpreted as evidence that Lake Clark sockeye salmon did not experience a genetic bottleneck effect. However, increased mean allozyme H_E is common among all sur-

veyed Lake Clark populations and coupled with a reduction in allelic diversity. This provides evidence of heterozygosity excess and further suggests a common founding event among Lake Clark sockeye salmon. The effects of genetic drift on heterozygosity can be complex, and it is necessary to assess variation at many loci, particularly with higher numbers of alleles, to detect and quantify these effects (Tarr et al. 1998). Similarly, it is important to assess changes in heterozygosity relative to changes in allelic diversity when assessing evidence of genetic bottleneck effects.

Detection of outlier loci is critical to the accurate assessment of both genetic bottleneck effects and genetic population structure. It is not uncommon to find an outlier locus that profoundly affects results in studies including 20 or fewer loci (Allendorf and Seeb 2000; Landry et al. 2002; Luikart et al. 2003). In this study, the Tazimina River population of Six Mile Lake grouped with the bottlenecked Lake Clark populations when the *ALAT* locus was included but grouped with the nonbottlenecked populations in Lake Iliamna when *ALAT* was not included. Repeating analyses with and without the *ALAT* locus provided evidence of the Lake Clark founder event and the genetic similarity between Six Mile Lake and Lake Iliamna populations. Other studies provide similar examples where outlier loci profoundly affect interpretation of genetic variation relative to geographic distribution, phenotypic variation, and phylogeny (Wilding et al. 2001; Landry et al. 2002).

Lake Clark sockeye salmon are unlikely experiencing deleterious bottleneck effects because their founding event was associated with postglacial colonization and is not recent (< 100 generations; Luikart et al. 1998). In addition, the Lake Clark sockeye salmon founding event has resulted in only a modest reduction in heterozygosity (< 5% at microsatellite loci) and there is no evidence in the

literature of deleterious effects associated with such a small reduction in heterozygosity (Keller and Waller 2002). There are currently at least 32 spawning populations of sockeye salmon in Lake Clark without known barriers to dispersal among them (Young 2004). Given time, genetic variation of these populations will likely increase and mutation-drift equilibrium will be reached.

Isolation and low N_e have apparently promoted further loss of genetic variation in the Sucker Bay Lake population. It is unclear if an increase or further erosion of genetic variation among Sucker Bay Lake sockeye salmon is more likely given time. Conservative management may consider maintaining or increasing effective population size, minimizing disturbance of the Sucker Bay Lake population, and maintaining connectivity between all sockeye salmon spawning populations of Lake Clark.

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