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Founding events influence genetic population structure of sockeye salmon (*Oncorhynchus nerka*) in Lake Clark, Alaska

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Abstract

Bottlenecks can have lasting effects on genetic population structure that obscure patterns of contemporary gene flow and drift. Sockeye salmon are vulnerable to bottleneck effects because they are a highly structured species with excellent colonizing abilities and often occupy geologically young habitats. We describe genetic divergence among and genetic variation within spawning populations of sockeye salmon throughout the Lake Clark area of Alaska. Fin tissue was collected from sockeye salmon representing 15 spawning populations of Lake Clark, Six-mile Lake, and Lake Iliamna. Allele frequencies differed significantly at 11 microsatellite loci in 96 of 105 pairwise population comparisons. Pairwise estimates of F_{ST} ranged from zero to 0.089. Six-mile Lake and Lake Clark populations have historically been grouped together for management purposes and are geographically proximate. However, Six-mile Lake populations are genetically similar to Lake Iliamna populations and are divergent from Lake Clark populations. The reduced allelic diversity and strong divergence of Lake Clark populations relative to Six-mile Lake and Lake Iliamna populations suggest a bottleneck associated with the colonization of Lake Clark by sockeye salmon. Geographic distance and spawning habitat differences apparently do not contribute to isolation and divergence among populations. However, temporal isolation based on spawning time and founder effects associated with ongoing glacial retreat and colonization of new spawning habitats contribute to the genetic population structure of Lake Clark sockeye salmon. Nonequilibrium conditions and the strong influence of genetic drift caution against using estimates of divergence to estimate gene flow among populations of Lake Clark sockeye salmon.

Keywords: bottleneck, founder effect, Lake Clark, *Oncorhynchus nerka*, population structure, sockeye salmon

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Introduction

Populations are products of opposing evolutionary forces. Gene flow promotes homogeneity among populations, genetic drift promotes divergence, and selection may act in either direction. The relative effects of these forces shape the genetic population structure (pattern of genetic variation among and within populations) of a species. Understanding genetic population structure is critical for effective management as it provides a basis for defining management units, can identify populations of unusual genetic composition, and may identify populations at risk of extinction as a result of low genetic diversity (Avise 1994). Population structure is positively associated with genetic diversity and resilience to disturbance such that large, highly structured populations have high genetic diversity and probability of persistence (Giesel 1974; Altukhov 1981). In contrast, small, panmictic populations are vulnerable to inbreeding, demographic stochasticity, genetic drift and thus, reduced evolutionary potential, and increased probability of extinction (Luikart *et al*. 1998; Soulé & Mills 1998).

A genetic bottleneck effect occurs when a population experiences a severe reduction in effective population size (Avise 1994). During a bottleneck event, genetic drift (random

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changes in allele frequencies as a result of imperfect sampling of alleles between generations) reduces genetic variation within and increases genetic divergence among populations. Founder effects are bottleneck effects that are associated with the founding of a new population. Thus, genetic drift may affect genetic population structure through founding events. Populations that have been in stable environments and connected by dispersal over long periods of time will reach a genetic equilibrium where the loss of alleles as a result of drift is balanced by the introduction of new alleles through migration (Wright 1951).

Assessing the relative effects of gene flow and genetic drift in shaping contemporary genetic population structure is difficult. If populations are in equilibrium, genetic population structure reflects recent processes and the amount of gene flow among populations can be approximated with the equation: $F_{ST} \sim 1/4Nm + 1$ (Wright 1931; Mills & Allendorf 1996). When populations are not at equilibrium, genetic population structure reflects historical processes and estimates of current gene flow based on F_{ST} are biased and potentially misleading (Hutchison & Templeton 1999). Most natural populations are not in genetic equilibrium (McCauley 1993) because of disrupted dispersal among populations and bottleneck effects (Hutchison & Templeton 1999; Kinnison *et al*. 2002). Populations found at the periphery of a species range (Lesica & Allendorf 1995; Costello *et al*. 2003) and in formerly glaciated regions (Congdon *et al*. 2000; Hewitt 2000; Turgeon & Bernatchez 2001; Castric & Bernatchez 2003) are particularly prone to nonequilibrium conditions because of recent range expansions and founder effects. When the rate of approach to equilibrium is slow compared to disturbance regimes, for example fire (England *et al*. 2002) and tectonic activity (Jollivet *et al*. 1999), populations may never achieve equilibrium.

Specific natal homing promotes reproductive isolation and genetic structuring between populations of sockeye salmon (*Oncorhynchus nerka*; Ricker 1972; Quinn 1985; Quinn & Dittman 1990). Lakes are focal points of homing and genetic divergence is typically greater among populations spawning in different lakes than among spawning populations within lakes (Wood 1995; Seeb *et al*. 2000; Withler *et al*. 2000). However, there is often significant genetic divergence among spawning populations within lakes as a result of restricted gene flow among fish spawning in beach and tributary habitats (ecological isolation), geographically distant habitats (spatial isolation), or differing in their time of return or spawning (temporal isolation; Ricker 1972; Wilmot & Burger 1985; Varnavskaya *et al*. 1994a, 1994b; Wood 1995; Woody *et al*. 2000; Ramstad *et al*. 2003). Sockeye salmon are excellent colonizers of newly created habitats because their homing fidelity exists in a dynamic balance with a tendency to stray (Quinn 1984, 1985) and they can quickly establish spawning populations with only a few individuals (Milner 1987; Milner & Bailey 1989; Milner *et al*. 2000). Thus, sockeye salmon are vulnerable to founder effects, and genetic drift is expected to influence the genetic population structure of sockeye salmon.

We tested the relative importance of ecological, temporal and spatial isolation (restricted gene flow) and founder effects (genetic drift) on the genetic population structure of sockeye salmon of Lake Clark, Alaska. Populations spawn in a variety of habitat types (beach, inlet tributary, outlet tributary), across a broad range of spawning times (mid-August to early November), and in varying geographical proximities to one another (from 3 km to > 300 km) throughout this system. In addition, there is the potential for founder effects because Lake Clark is geologically young, having been created by glacial retreat approximately 12 000–15 000 years ago (Stilwell & Kaufman 1996). Spawning habitats within Lake Clark vary in time since deglaciation, suggesting that they similarly vary in time since first colonization. For example, sockeye salmon spawn in an area of the Upper Tlikakila River that was deglaciated approximately 100–200 years ago (unpublished data, Dr Patricia Heiser, University of Alaska, Anchorage). Lake Clark sockeye salmon that spawn in younger habitats may have experienced recent founder effects.

This study provides an empirical test of the role of founder effects in shaping the genetic population structure of a colonizing species. We address three primary questions. (i) What is the pattern of genetic divergence among spawning populations of Lake Clark, Six-mile Lake, and Lake Iliamna sockeye salmon? (ii) Is there evidence of genetic bottleneck effects among spawning populations of sockeye salmon within Lake Clark relative to Six-mile Lake and Lake Iliamna? (iii) What physical and biological factors best explain the patterns of genetic divergence among and genetic variation within these spawning populations of sockeye salmon?

Materials and methods

Sample collection

Fin tissue was collected from 11 Lake Clark, two Six-mile Lake and two Lake Iliamna spawning populations (Fig. 1, Table 1). Samples (~100) from three Lake Clark populations (Currant Creek, Priest Rock Creek, Kijik River) and both Lake Iliamna populations (Lower Talarik Creek, Fuel Dump Island) were collected in a single year. Samples (~50) from all other populations were collected in each of two years to test for interannual variation in allele frequencies within populations. Post-spawning and spawning fish were captured on their spawning grounds by seine and tangle net. A fin clip (approximately 5 mm^2) was collected and stored in 100% ethanol from each fish sampled.

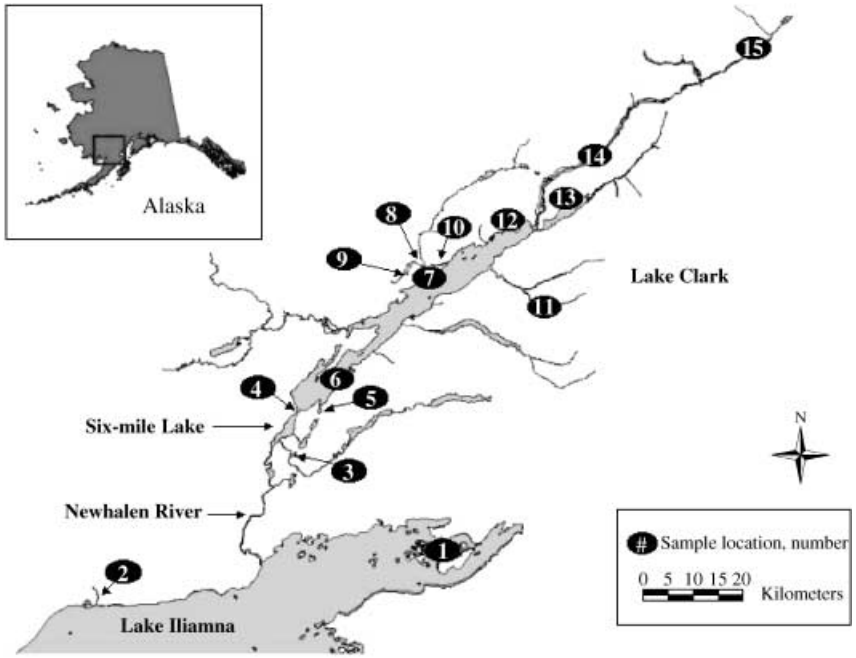

Fig. 1 Map of Lake Clark, Six-mile Lake, and Lake Iliamna with sample sites shown. Refer to Table 1 for population numbers.

Table 1 Site description, mean peak spawning date, sample size, heterozygosity, mean number of alleles and allelic diversity (number of alleles corrected for sample size), and mean *M* of Lake Clark, Six-mile Lake and Lake Iliamna sockeye salmon populations

		Site	Lake	Habitat type	Peak spawning date	*N*	H_E	No. of alleles	Allelic richness	*M*
1	FDI	Fuel Dump Island	Iliamna	B	23-Aug	87	0.483	7.1	6.5	0.780
2	TCI	Talarik Creek	Iliamna	T	28-Aug	97	0.500	6.5	6.0	0.660
3	TAZ	Tazimina River	Six-mile	T	25-Aug	99	0.507	6.5	5.8	0.760
4	OUT	Lake Clark Outlet	Six-mile	T	13-Sep	100	0.518	6.3	5.7	0.766
5	SBL	Sucker Bay Lake	Clark	B	30-Aug	100	0.502	4.3	4.2	0.615
6	CHI	Chi Point	Clark	B	24-Sep	99	0.475	6.0	5.4	0.765
7	KR	Kijik River	Clark	T	20-Sep	99	0.484	5.3	4.9	0.690
8	LKR	Little Kijik River	Clark	T	18-Sep	98	0.457	5.0	4.6	0.721
9	KLSB	Kijik Lake South Beach	Clark	B	25-Sep	100	0.452	5.0	4.6	0.654
10	PRC	Priest Rock Creek	Clark	T	11-Oct	65	0.483	4.9	4.9	0.678
11	CC	Currant Creek	Clark	T	25-Sep	100	0.480	5.5	5.0	0.640
12	HPB	Hatchet Point Beach	Clark	B	12-Oct	99	0.501	5.9	5.3	0.726
13	LLCB	Little Lake Clark Beach	Clark	B	7-Oct	100	0.474	5.6	5.1	0.637
14	LTLK	Lower Tlikakila	Clark	T	7-Oct	100	0.464	5.5	4.9	0.699
15	UTLK	Upper Tlikakila	Clark	T	25-Sep	100	0.480	5.2	4.8	0.595

Spawning habitat type is coded as beach (B) and tributary (T). Allelic richness is standardized to the lowest sample size ($N = 65$).

We attempted to obtain a sample size of 100 for all populations, which provides a 95% probability of detecting an allele at a frequency of 0.015 or greater. We generally met this goal with the exception of Priest Rock Creek ($N = 65$) where a lower sample size reduced the ability to detect rare alleles. However, the number of fish sampled represents a significant fraction of fish present at this site (~150).

Microsatellite genotyping

Total DNA was extracted using the Puregene® DNA Isolation Tissue Kit (Gentra Systems). Concentration of DNA was measured with a DyNA Quant 200 Fluorometer (Hoefer) after rehydration in Tris-EDTA. Working stocks for each sample were diluted with deionized water to concentrations of 50 ng/μL.

Table 2 Microsatellite loci analysed including multiplex annealing temperatures and allelic variation per locus and population

Locus	Annealing temperature (°C)	Allelic size range	Total no. alleles per locus	Mean no. alleles per population	Reference
Oki1–1	56	106–122	5	3.1	Smith *et al*. (1998)
Oki1–2	56	140–164	7	3.0	Smith *et al*. (1998)
Omy325	56	118–174	21	10.9	O'Connell *et al*. (1997)
μSat60	58	112–136	12	5.6	Estoup *et al*. (1993)
Oneμ21	58	110–152	16	10.1	Scribner *et al*. (1996)
Ots3	48	74–100	12	4.0	Banks *et al*. (1999)
Oneμ18	52	163–189	12	6.1	Scribner *et al*. (1996)
Oneμ13	52	154–174	11	5.9	Scribner *et al*. (1996)
One105	52	124–144	7	4.5	Olsen *et al*. (2000)
Ots107	48	90–130	9	4.1	Nelson & Beacham (1999)
Omy77	48	85–121	14	5.4	Morris *et al*. (1996)

Fish were genotyped at 11 microsatellite loci (Table 2). Primers were directly labelled with infrared fluorophore IRD700 and IRD800 (LI-COR). The DNA was amplified in 10-μL polymerase chain reactions (PCR; 200 μmol each dNTP, 4 pmol each primer, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% each of gelatin, nonidet P-40 and Triton-X 100, and 0.5 units of DNA polymerase (Promega and/or Perkin-Elmer) in a series of five PCRs (Table 2). Profiles for PCR were 94 °C for 2 min followed by 35–40 cycles of 15 s to 1 min at 94 °C, 15 s to 1 min at annealing temperature (Table 2) and 30 s to 1 min at 72 °C. Blank reactions (all constituents present but template DNA) were included in each PCR to detect sample contamination.

DNA was electrophoresed on a 6% denaturing polyacrylamide gel and PCR products were scored relative to a known size standard on a LI-COR DNA Analyser Global Edition IR2 and a LI-COR DNA Sequencer Long Reader 4200 using V4.03 GENE IMAGIR software (Scanalytics, Inc.). An individual fish with known allele sizes was included on every gel and a second gel reader proofed allele sizes to ensure accuracy and consistency of scoring across gels. Individuals representing 10% of genotyped fish were reamplified and scored a second time. Comparison of initial and repeated scores revealed a genotyping error rate of less than 2%.

Statistical analysis — genetic population structure

Departures from Hardy–Weinberg proportions (Guo & Thompson 1992) and heterogeneity of allele frequencies were tested using GENEPOP version 3.2 (Raymond & Rousset 1995). Tests of significance were combined over all loci using Fisher's combined probability test (Sokal & Rohlf 1981). The proportion of genetic variation due to population subdivision was estimated as F_{ST} and computed in FSTAT, version 1.2 (Goudet 1995) according to Weir & Cockerham (1984). Principal component analysis was performed using the covariance matrix of allele frequencies in MINITAB, version 11 (State College, PA) after omitting the largest allele at each locus to allow for the nonindependence of allele frequencies within a locus (Johnson 1998). Sequential Bonferroni adjustments were made for all multiple comparisons (Rice 1989).

Statistical analysis — bottleneck effects

Recently bottlenecked populations may exhibit gametic disequilibrium (nonrandom association of alleles at different loci; Waples 1991, 2002), reduced allelic diversity, loss of rare alleles (Allendorf 1986), a mode shift in allele frequency distributions (Luikart *et al*. 1998), increased heterozygosity relative to that expected at mutation–drift equilibrium (Maruyama & Fuerst 1985; Cornuet & Luikart 1996; Luikart & Cornuet 1998), and a reduced value for the statistic *M* (Garza & Williamson 2001). Therefore we tested for the presence of bottleneck effects with a suite of measures.

Gametic disequilibrium was assessed for all pairwise locus comparisons within each population using a Fisher exact test in GENEPOP version 3.2 (Raymond & Rousset 1995). The number of alleles observed in a population is highly dependent on sample size. Therefore, allelic diversity was assessed as allelic richness, which is a measure of the number of alleles per population corrected for sample size (El Mousadik & Petit 1996). Allelic richness was calculated and compared among major population groups in FSTAT version 1.2 (Goudet 1995). Mode shifts in allele frequencies within populations were assessed graphically (Luikart *et al*. 1998). Heterozygosity excess relative to a nonbottlenecked population in mutation–drift equilibrium having the same number of alleles was tested in BOTTLENECK, version 1.2.02 (Cornuet & Luikart 1996). We assume an infinite alleles model of mutation (IAM) for this analysis although microsatellites are expected to conform more closely to a stepwise mutation model (Shriver *et al*. 1993).

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

The statistic M was calculated according to Garza & Williamson (2001). Three of the 11 loci surveyed were not included in this analysis (*Oki-1*, *One105*, *Ots107*) because their allelic distributions did not meet the test requirement of having rare alleles or empty allelic states within the range of common alleles (> 0.1 frequency). Significance was assessed by comparison of mean M across loci, for each population with both a critical value (M_C) and the more conservative rule that $M < 0.68$ suggests a bottleneck effect (Garza & Williamson 2001). Because M is simply the ratio of the number (k) to the size range (r) of microsatellite alleles, its value does not rely on any mutation model or population size assumptions. However, the critical value M_C was calculated with the assumption of large prebottleneck population size ($N_e = 5000$), a constant microsatellite mutation rate ($\mu = 5 \times 10^{-4}$/locus/generation), and a two-phase mutation model where 90% of mutations are single-step mutations and the average size of all other mutations is 3.5 repeats. Assuming an N_e to N ratio of 0.2 (Allendorf *et al*. 1997; Reiman & Allendorf 2001) and a generation interval of 4 years, the prebottleneck effective population size of 5000 translates to 6250 spawning fish observed per year. While this value seems high, it results in a conservative test because the value of M_C is negatively related to the prebottleneck N_e and plateaus above an N_e of 5000. Thus, a large prebottleneck N_e of 5000 suggests fewer bottleneck effects than a lower N_e and larger values of N_e would not change the qualitative results of this analysis. Both the mean M and the mean proportion of rare alleles (frequency < 0.1) were compared among major population groups with a one-tailed, nonparametric Mann–Whitney test (Zar 1984). Comparisons of allelic richness between Sucker Bay Lake (treating each of two sampling years as a population) and major population groups were made in FSTAT version 1.2 (Goudet 1995).

Statistical analysis — factors shaping genetic population structure

Correlations between pairwise genetic population divergence (F_{ST}) and spawning habitat type, geographical distance, difference in spawning time, and difference in allelic richness among populations were assessed by simple and partial Mantel tests in FSTAT version 1.2 (Goudet 1995) according to Manly (1991). Four predictor variable matrices were defined (i) spawning habitat type (HAB), (ii) geographical distance (GEODIST), (iii) spawning time (SPTIME), and (iv) allelic richness (ALLRICH). The spawning habitat matrix coded for populations spawning in similar (0) and different (1) habitat types (beach or tributary). Geographic distance is the minimum distance (in km) through water between all sampling sites and was assessed in ARCINFO. Spawning time was estimated as the mean date of peak spawning across survey years and was based on the presence of gravid females during sampling. Difference in allelic richness between populations serves as a measure of genetic bottleneck effects. All variables but habitat type were log transformed after values of zero were reassigned the smallest pairwise value detected in all population comparisons (spawning time = 1 day, $N = 4$ comparisons; $F_{ST} = 0.0001$, $N = 8$ comparisons). Tests were conducted among all surveyed populations (Lake Clark, Six-mile Lake and Lake Iliamna) and populations within Lake Clark.

A pattern of isolation by distance (a positive correlation between genetic divergence and geographical distance) is expected among populations in migration–drift equilibrium but not among nonequilibrium populations (Slatkin 1993). In the absence of isolation by distance, a consistently low F_{ST} among populations suggests that historic gene flow has been the primary factor in shaping contemporary genetic population structure, while highly variable F_{ST} values suggests that drift historically overwhelmed gene flow (Hutchison & Templeton 1999). Thus, the relationship between pairwise population F_{ST} and geographical distance (variables untransformed) was evaluated for isolation by distance and the relative historical effects of gene flow and genetic drift.

Differences in the number of alleles and heterozygosity can be a source of bias in estimating $F2_{ST}$ (McDonald 1994; Hedrick 1999). Therefore, we used $F2_{ST}$ to test for a correlation between genetic divergence and genetic diversity. $F2_{ST}$ treats all loci as diallelic by using the frequency of the overall most common allele and pooling all others (McDonald 1994; Allendorf & Seeb 2000).

Results

All loci were polymorphic in all samples. The total number of alleles per locus ranged from five to 21, and the mean number of alleles per population and locus ranged from 3.0 to 10.9 (Table 2). Mean expected heterozygosity ranged from 0.452 to 0.518, and mean number of alleles ranged from 4.3 to 7.1 (Table 1) per population over all loci. There was no evidence of deviation from Hardy–Weinberg proportions at any locus, in any sample. No significant interannual difference in allele frequencies ($P > 0.05$) existed so samples collected from the same population in different years were pooled for further analysis. Significant gametic disequilibrium ($P < 0.05$) was detected in only four of 825 pairwise comparisons among 11 loci after

Table 3 Pairwise F_{ST} (below diagonal) and number of loci with significant allele frequency differences after sequential Bonferroni correction (above diagonal) between sockeye salmon of Lake Clark, Six-mile Lake and Lake Iliamna

	1 FDI	2 TCI	3 TAZ	4 OUT	5 SBL	6 CHI	7 KR	8 LKR	9 KLSB	10 PRC	11 CC	12 HPB	13 LLCB	14 LTLK	15 UTLK
FDI	—	4	7	7	11	8	7	7	8	6	10	6	7	10	8
TCl	0.016	—	8	7	11	8	11	8	7	7	9	9	9	10	10
TAZ	0.043	0.023	—	NS	9	7	9	10	10	8	8	9	10	10	9
OUT	0.026	0.018	0.003	—	9	7	10	8	10	8	7	9	10	10	9
SBL	0.054	0.045	0.065	0.061	—	8	8	9	9	7	8	7	9	8	7
CHI	0.060	0.062	0.052	0.051	0.063	—	NS	2	2	1	0	NS	0	0	NS
KR	0.052	0.055	0.052	0.049	0.044	0	—	4	3	1	1	0	9	10	0
LKR	0.065	0.071	0.069	0.066	0.077	0.014	0.017	—	NS	2	2	3	4	4	4
KLSB	0.067	0.071	0.071	0.069	0.064	0.012	0.011	0.001	—	3	1	2	4	1	2
PRC	0.062	0.057	0.058	0.058	0.050	0.004	0.005	0.012	0.012	—	2	2	2	2	2
CC	0.067	0.065	0.056	0.057	0.057	0	0	0.011	0.008	0.002	—	1	2	NS	1
HPB	0.055	0.060	0.053	0.048	0.060	0.001	0.004	0.013	0.010	0.009	0.004	—	NS	0	0
LLCB	0.070	0.069	0.059	0.061	0.060	0.003	0.005	0.024	0.017	0.013	0.005	0.002	—	0	NS
LTLK	0.089	0.085	0.069	0.074	0.069	0.003	0.006	0.018	0.013	0.007	0	0.006	0.004	—	NS
UTLK	0.076	0.077	0.065	0.066	0.067	0	0.003	0.018	0.013	0.007	0	0.001	0	0	—

NS = comparisons without significant allele frequencies differences over all loci ($P > 0.05$ Fisher's combined probability test). Refer to Table 1 for population numbers.

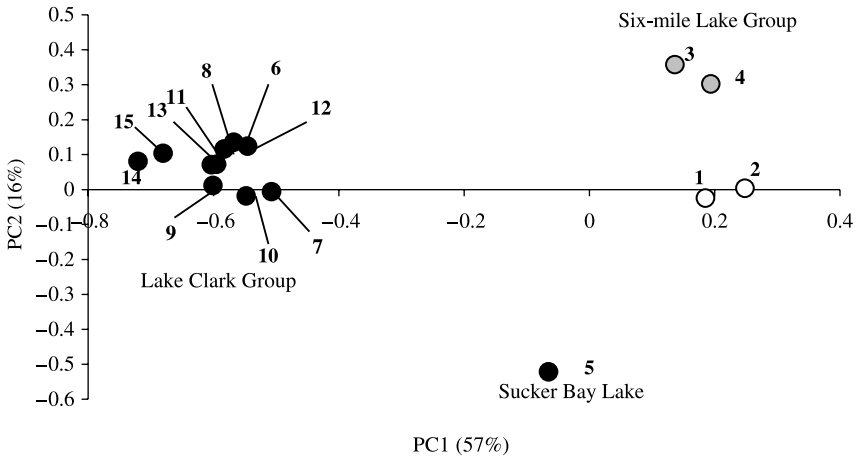

Fig. 2 Principal component analysis of allele frequencies at 11 microsatellite loci. Points represent populations from Lake Clark (black), Six-mile Lake (grey), or Lake Iliamna (white). Percentages in parentheses indicate amount of variation explained by each principal component. Refer to Table 1 for population numbers.

sequential Bonferroni correction within populations. There was no consistent tendency toward gametic disequilibrium between any loci or within any population.

Genetic divergence among all populations

There were significant differences in allele frequencies in 96 of 105 pairwise population comparisons (Table 3). Estimates of F_{ST} ranged from 0 to 0.089 and were greatest between the Lake Clark and Lake Iliamna populations. The Six-mile Lake and Lake Iliamna populations were genetically similar although it is the Six-mile Lake and Lake Clark populations that have historically been grouped for management purposes and that are geographically proximate. The Sucker Bay Lake population was highly divergent from all other populations surveyed. Hereafter we refer to two major population groups: the Six-mile Lake group (populations of Six-mile Lake and Lake Iliamna) and the Lake Clark group (all populations within Lake Clark but Sucker Bay Lake).

The principal component analysis supports this pattern (Fig. 2). The first principal component explained 57% of the total genetic variation and differentiated between the Lake Clark group, Sucker Bay Lake and the Six-mile Lake group. Loadings suggested that this component was primarily influenced by allele frequency differences at four loci (*Omy325*, alleles 152 and 156; *Oneμ18*, alleles 181 and 171; *Oneμ21*, allele 140; *Omy77*, alleles 105 and 109). The second principal component explained 16% of the total genetic variation, further differentiated the Sucker Bay Lake

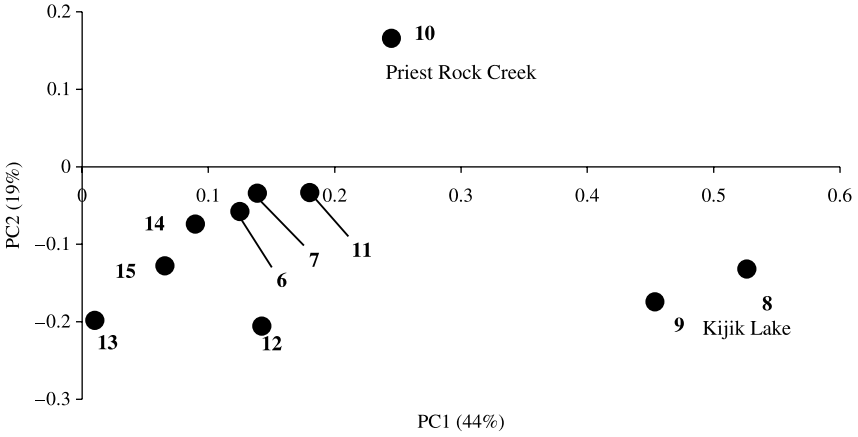

Fig. 3 Principal component analysis of Lake Clark sockeye salmon (excluding Sucker Bay Lake) allele frequencies at 11 microsatellite loci. Highly divergent populations of Kijik Lake and Priest Rock Creek are identified. Percentages in parentheses indicate amount of variation explained by each principal component. Refer to Table 1 for population numbers.

population, and differentiated between the populations of Six-mile Lake and Lake Iliamna. The second principal component was influenced primarily by allele frequency differences at four loci (*μSat60*, alleles 118 and 130; *Oneμ21*, alleles 130 and 140; *Oneμ13*, allele 168; *Omy77*, allele 105). Lake Clark group populations had a pairwise F_{ST} of 0.060 [95% confidence interval (CI) 0.021–0.111] with Sucker Bay Lake and 0.054 (95% CI 0.023–0.086) with the Six-mile Lake group. Between Sucker Bay Lake and the populations of the Six-mile Lake group, F_{ST} was 0.049 (95% CI 0.018–0.082).

Genetic divergence within the Lake Clark group

There was significant genetic structuring within the Lake Clark group although many populations were genetically very similar (Table 3, Fig. 3). There was no difference in allele frequencies between the two Kijik Lake populations sampled (Little Kijik River, Kijik Lake South Beach) and many of the greatest pairwise F_{ST} values within the Lake Clark group were between Kijik Lake and other populations (range from 0.008 to 0.024, Table 3). Priest Rock Creek differed in allele frequencies from all other populations sampled (Table 3). This pattern of divergence within Lake Clark was supported by principal component analysis (Fig. 3). The first principal component explained 44% of the genetic variation within Lake Clark and separated the Kijik Lake populations from all others. Component loadings suggested differences in allele frequencies at seven loci (*Oki1–1*, alleles 110 and 114; *Oki1–2*, alleles 148 and 156; *Omy325*, allele 152; *μSat60*, alleles 118 and 130; *Oneμ21*, allele 140; *Oneμ18*, allele 185; *Oneμ13*, allele 168). The second principal component explained 19% of the genetic variation and differentiated the Priest Rock Creek population. Allele frequency differences at seven loci were also indicated by the second component loadings (*Oki1–2*, alleles 148 and 156; *Omy325*, allele 152; *Oneμ21*, alleles 130 and 140; *Oneμ18*, allele 181; *One*μ13, allele 160; *One105*, alleles 132 and 136; *Omy77*, alleles 105 and 109).

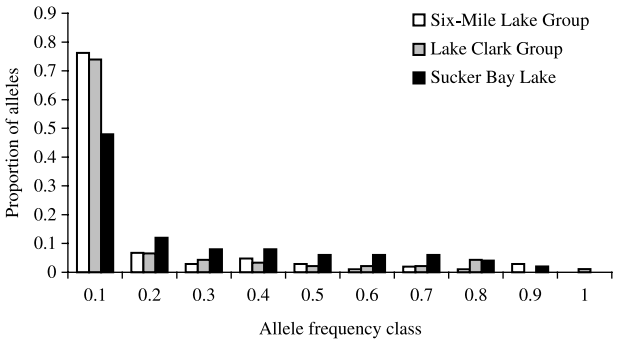

Fig. 4 Allele frequency distributions of Six-mile Lake, Lake Clark, and Sucker Bay Lake sockeye salmon. The far left bar of each plot indicates the proportion of rare alleles (frequency less than 0.1).

Genetic diversity and bottleneck effects

The data suggested a bottleneck among Lake Clark fish relative to fish of the Six-mile Lake group. A total of 92 alleles was found in the Lake Clark group fish (959 sampled) and 105 alleles were found in fish of the Six-mile Lake group (383 sampled), despite our sample sizes greatly favouring finding more alleles in the Lake Clark populations. Mean allelic richness (number of alleles corrected for sample size) of the Lake Clark group (5.0) was significantly lower than that of the Six-mile Lake group (6.0; $P < 0.01$). There was no mode shift in allele frequency distribution in any population surveyed or population group (Fig. 4). However, the mean proportion of rare alleles was significantly lower in the Lake Clark group (0.58) than the Six-mile Lake group (0.64) ($U'_{10,4} = 37$, $P = 0.01$) and eight of 10 Lake Clark group populations had a lower proportion of rare alleles than all four populations of the Six-mile Lake group. Similarly, eight of 10 populations in the Lake Clark group had an excess of heterozygosity relative to that expected at mutation–drift equilibrium (Fig. 5). This effect was not statistically significant within populations (*P* from 0.12 to

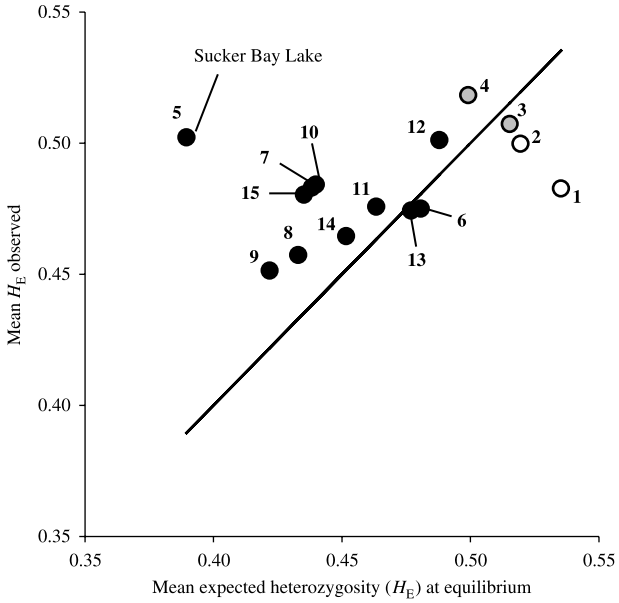

Fig. 5 Relationship between mean expected heterozygosity (H_E) observed and expected at mutation–drift equilibrium under the infinite alleles model of mutation (IAM) for Lake Clark, Six-mile Lake and Lake Iliamna sockeye salmon populations. Recently bottlenecked populations have greater heterozygosity (H_E) than expected at migration–drift equilibrium as a result of the loss of rare alleles. Nonbottleneck populations have an H_E that is equal to or less than that expected under IAM (on or below equality line). Points represent populations from Lake Clark (black), Six-mile Lake (grey), or Lake Iliamna (white). Refer to Table 1 for population numbers.

0.68) but the probability of obtaining this pattern across populations (eight of 10 exhibiting heterozygosity excess) is low ($P = 0.04$) assuming a population is equally likely to exhibit a heterozygosity excess or deficit. The statistic M suggests bottleneck effects ($P < 0.05$ and $M < 0.68$) in six populations within Lake Clark and one Six-mile Lake group population (Table 1). The smallest values were found in the Upper Tlikakila ($M = 0.595$) and Little Lake Clark Beach ($M = 0.637$) populations of Lake Clark. Currant Creek, Kijik Lake South Beach, and Priest Rock Creek also showed significant bottleneck effects ($M = 0.640–0.678$). Mean M among Lake Clark group populations (0.680) was reduced relative to populations in the Six-mile Lake group (0.742; $U'_{10,4} = 33$, $P = 0.05$).

The most severe bottleneck effect found was in Sucker Bay Lake. The Sucker Bay Lake sample had less than half the number of alleles found in the Six-mile Lake group (48 vs. 105). Mean allelic richness in Sucker Bay Lake fish (4.2) was lower than that of both the Lake Clark (5.0, $P = 0.084$) and Six-mile Lake (6.0, $P < 0.001$) groups. The Sucker Bay Lake population did not exhibit a mode shift in allele frequency distribution but had 37% fewer rare alleles than populations of the Six-mile Lake group (Fig. 4). Heterozygosity excess among Sucker Bay Lake fish was greater than all other populations surveyed (Fig. 5). This effect was present in eight of 11 surveyed loci and over all loci (0.502) where it was far in excess of that expected if the population were in mutation–drift equilibrium (0.389; $P < 0.005$). The Sucker Bay Lake population had the second lowest M of all populations surveyed ($M = 0.615$, $P < 0.05$; Table 1).

Factors promoting population structure

The data did not support isolation by spawning habitat type (beach vs. tributary). There was no tendency for populations spawning in a given habitat type to be genetically more similar to one another than to populations spawning in a different habitat type (Table 4). This result held across all surveyed populations (Lake Clark, Six-mile Lake, Lake Iliamna, $P = 0.97$) and among Lake Clark populations both when Sucker Bay Lake is included ($P = 0.72$) and when it is excluded (Lake Clark group, $P = 0.85$).

Table 4 Results of Mantel tests between genetic divergence (pairwise F_{ST}) and spawning habitat (HAB), geographical distance (GEODIST), difference in spawning time (SPTIME), and difference in mean allelic richness (ALLRICH) among Lake Clark, Six-mile Lake and Lake Iliamna sockeye salmon

	All populations		Lake Clark populations	
Test	*r*	*P*	*r*	*P*
HAB	−0.003	0.97	0.049	0.72
GEODIST	0.263*	< 0.01	−0.106	0.45
SPTIME	0.505*	< 0.001	0.409*	< 0.01
ALLRICH	0.515*	< 0.001	0.247	0.08
SPTIME (ALLRICH)	0.375*	< 0.001	0.393*	< 0.01
ALLRICH (SPTIME)	0.389*	< 0.001	0.218	0.11

Controlled variable in partial Mantel tests in parentheses.
*Significant at $P < 0.05$.

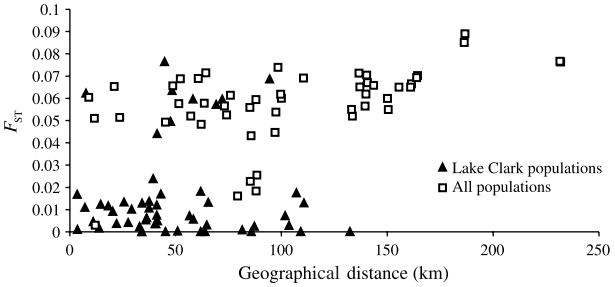

Fig. 6 Plot of F_{ST} against geographical distance among Lake Clark, Six-mile Lake and Lake Iliamna Sockeye Salmon populations.

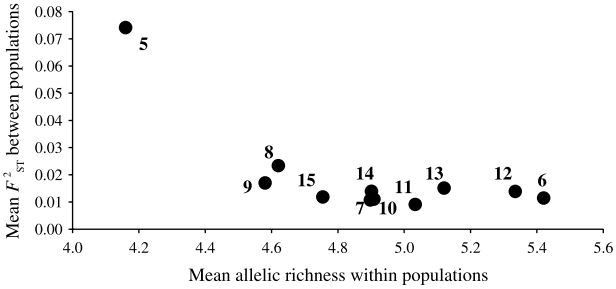

Fig. 7 Plot of mean $F2_{ST}$ between mean allelic richness within spawning populations of Lake Clark sockeye salmon (all Lake Clark populations: $P = 0.01$; excluding Sucker Bay Lake: $P = 0.11$).

Geographic distance and genetic divergence are significantly correlated among all surveyed populations ($P < 0.01$) but not among Lake Clark populations ($P = 0.45$; Table 4; Fig. 6). Partial Mantel tests reveal that the effect of geographical distance on genetic divergence among all populations is insignificant. Geographic distance is correlated with both spawning time ($P < 0.01$) and allelic richness ($P < 0.001$) among all populations. After removal of spawning time effects ($r = 0.505$, $P < 0.001$), the addition of geographical distance was not significant ($r = 0.124$, $P = 0.21$). Similarly, geographical distance was not significantly correlated with genetic divergence ($r = 0.072$, $P = 0.47$) after removal of the effect of allelic richness ($r = 0.515$, $P < 0.001$). The proportion of variation in F_{ST} explained by a model including spawning time and allelic richness was not increased with the addition of geographical distance ($R^2 = 0.41$ with and without GEODIST). Thus, geographical distance did not explain the variation in F_{ST} among all populations itself but was correlated with variables that were significantly correlated with genetic divergence (SPTIME and ALLRICH).

Among all populations surveyed, both spawning time and allelic richness were significantly correlated with F_{ST} (SPTIME and ALLRICH: $P < 0.001$) and with each other ($P < 0.01$). Each of the two variables explained a significant amount of the variation in pairwise F_{ST} after the effects of the other variable had been removed (Table 4) and the best model included only these two variables ($R^2 = 0.41$). Spawning time and allelic richness had similar magnitudes of effect, as shown by their almost equal correlation coefficients both before (SPTIME $r = 0.505$; ALLRICH $r = 0.515$) and after the removal of the effects of the other variable (SPTIME corrected for ALLRICH $r = 0.375$; ALLRICH corrected for SPTIME $r = 0.389$; Table 4).

Spawning time and allelic richness explained a significant amount of the variation in genetic divergence among Lake Clark populations as well. F_{ST} was significantly correlated with spawning time ($P < 0.01$) and marginally correlated with allelic richness ($P = 0.08$) while the two factors (SPTIME, ALLRICH) were independent of each other ($P = 0.62$). The correlation coefficient of spawning time was consistently higher than that of allelic richness (Table 4). Furthermore, the smaller reduction in the correlation coefficient of spawning time (before correction for ALLRICH $r = 0.409$; after $r = 0.393$) post-removal of the effect of allelic richness suggests that it was more closely correlated with F_{ST} than allelic richness (before correction for SPTIME $r = 0.247$; after $r = 0.218$; Table 4). However, the amount of variation in F_{ST} among Lake Clark populations explained by spawning time ($R^2 = 0.17$) was increased with the addition of allelic richness to the model ($R^2 = 0.22$) and there was a strong correlation between the allelic richness of a population and its mean divergence from all other populations as measured by $F2_{ST}$ ($F_{1,9} = 10.43$; $P = 0.01$; Fig. 7). Thus, allelic richness was also highly associated with the genetic population structure of Lake Clark sockeye salmon.

Much of the correlation between spawning time, allelic richness and F_{ST} among Lake Clark sockeye salmon can be attributed to Sucker Bay Lake. Neither spawning time ($r = 0.172$, $P = 0.24$) nor allelic richness ($r = -0.087$, $P = 0.58$) explained a significant amount of variation in pairwise population F_{ST} within Lake Clark when Sucker Bay Lake was excluded (Lake Clark group). The trend for a negative correlation between allelic richness and mean $F2_{ST}$ per population was still evident but not statistically significant when Sucker Bay Lake was excluded ($F_{1,8} = 3.31$, $P = 0.11$; Fig. 7).

The lack of isolation by distance suggests nonequilibrium conditions among Lake Clark, Six-mile Lake, and Lake Iliamna populations of sockeye salmon (Table 4, Fig. 6). F_{ST} was highly variable both among all populations surveyed (0–0.089) and among Lake Clark populations (0–0.077), suggesting that historical drift has been a powerful force in shaping the genetic population structure of these populations. Much of the variation in F_{ST} among Lake Clark populations was the result of Sucker Bay Lake and F_{ST} among Lake Clark populations was consistently low (0–0.024) when this population was excluded. This pattern suggested that gene flow had historically dominated drift among most Lake Clark populations but that drift had driven the divergence between Lake Clark populations and those of Sucker Bay Lake, Six-mile Lake and Lake Iliamna.

Discussion

Reproductive isolation and genetic population structure

The Six-mile Lake populations (Lake Clark Outlet, Tazimina River) are more similar genetically to the Lake Iliamna populations than to the Lake Clark populations. The genetic similarity among fish spawning in Six-mile Lake and Lake Iliamna is surprising because Six-mile Lake is closer to Lake Clark than to Lake Iliamna and has therefore historically been grouped with Lake Clark for management purposes. In addition, the Newhalen River (~39 km long) between Six-mile Lake and Lake Iliamna is a barrier to fish migration at high water velocities (Poe & Mathisen 1981, 1982). However, no current or recent barrier to fish migration between Lake Clark and Six-mile Lake is known. Satellite imagery documents the presence of a major outwash fan from the Tazimina Valley (Dr Patricia Heiser, University of Alaska, Anchorage, personal communication) that could have blocked fish entry to Lake Clark in the past. A barrier of this kind could have caused either isolation between sockeye salmon already present in Lake Clark and Six-mile Lake or delayed colonization of Lake Clark by sockeye salmon.

Reproductive isolation and genetic divergence among the populations of Lake Clark, Six-mile Lake and Lake Iliamna sockeye salmon are probably not driven by differences in spawning habitat type or geographical distance. Differences in spawning time, however, are highly correlated with genetic divergence among populations. There is little difference in peak spawning time between populations of Sucker Bay Lake (~August 30), Six-mile Lake (August 25 to September 13), and Lake Iliamna (August 20–30). However, mean peak spawning time in populations of the Lake Clark group (September 18 to October 11) varies from other populations by 1 week (Kijik system) to 4 weeks (Hatchet Point Beach, Priest Rock Creek, Little Lake Clark Beach, Lower Tlikakila River). Therefore, temporal isolation contributes to the genetic divergence between the Lake Clark group and other populations but cannot explain the high level of genetic divergence between Sucker Bay Lake and the Six-mile Lake group.

There is significant genetic population structure within the Lake Clark group (all populations spawning within Lake Clark excepting Sucker Bay Lake) though many populations are genetically similar. Fish spawning in Kijik Lake (South Beach and the outlet Little Kijik River) do not differ from one another in allele frequencies but are significantly differentiated from all other populations of the Lake Clark group. Priest Rock Creek fish are also highly differentiated from all other Lake Clark populations. Within the Lake Clark group, pairwise population genetic divergence is not correlated with differences in spawning habitat, geographical distance between populations, or differences in spawning time. Thus, the pattern of genetic population structuring among sockeye salmon of the Lake Clark group is not one of simple ecological, geographical, or temporal isolation.

Bottleneck effects and genetic population structure

Evidence for genetic bottleneck effects. The data suggest a bottleneck effect in Lake Clark sockeye salmon relative to Six-mile Lake and Lake Iliamna populations. The prevalence of a reduced proportion of rare alleles (nine of 11 populations), heterozygosity excess (nine of 11 populations, statistically insignificant except in Sucker Bay Lake), and reduced *M* (seven of 11 populations) among populations within Lake Clark relative to the Six-mile Lake group suggest a bottleneck event that reduced the genetic diversity of Lake Clark sockeye salmon over all. The most extreme bottleneck signal was found in the Sucker Bay Lake population of Lake Clark. The strong genetic divergence between most Lake Clark populations, the Sucker Bay Lake population, and populations spawning in Six-mile Lake and Lake Iliamna also suggest significant bottleneck effects.

Genetic drift changes allele frequencies and decreases genetic variation within populations. Therefore, if bottleneck effects have influenced the genetic population structure of Lake Clark sockeye salmon, we would expect a negative correlation between genetic divergence among and genetic variation within populations. We found a negative correlation between mean genetic divergence ($F2_{ST}$) and mean allelic richness among Lake Clark populations. Furthermore, there is a significant, positive correlation between pairwise F_{ST} and the difference in mean allelic richness among both all populations surveyed and Lake Clark populations, suggesting that difference in allelic diversity is coupled with degree of genetic divergence between populations. Taken together, these results suggest that genetic population structure among the sockeye salmon of Lake Clark, Six-mile Lake and Lake Iliamna has been significantly influenced by genetic drift that is probably the result of bottleneck effects.

Are the bottleneck effects also founder effects? It is important to identify populations that have undergone recent and acute bottlenecks (within the past 100 generations, $\leq 20\ N_e$) because they may be affected by problems due to small population size (demographic stochasticity, inbreeding, fixation of deleterious alleles, reduced evolutionary potential, increased probability of extinction) and may respond to mitigative management (Cornuet & Luikart 1996; Luikart *et al*. 1998). Gametic disequilibrium, allele frequency distribution mode shifts, and heterozygosity excess are highly transient effects and are only expected in populations that have experienced recent and acute genetic bottlenecks (Waples 1991; Cornuet & Luikart 1996; Luikart & Cornuet 1998; Luikart *et al*. 1998). In contrast,

reduced allelic richness, and possibly reduced *M*, will persist much longer and may be present in populations that experienced bottlenecks in the more distant past (age > 100 generations; Garza & Williamson 2001; Spong & Hellborg 2002).

Bottleneck effects within Lake Clark could be the result of several factors, including recent declines in the number of sockeye salmon returning to the Kvichak system, an historic and cyclical pattern of low returns (Regnart 1998; Faire 2000), a velocity barrier at the Newhalen River (Poe & Mathisen 1981, 1982), reduction in spawning habitat of established populations, and colonization of newly created habitats by a few individuals (founder effects). Recent reductions in numbers of spawning fish are an unlikely cause of these bottlenecks because typically five to 10 generations must pass before these measures are effective (Luikart *et al*. 1998; Storz *et al*. 2002) and recent declines have only occurred since 1999 (Faire 2000), a single generation of sockeye salmon. Cyclic reductions in the numbers of spawners and the sporadic presence of a velocity barrier to fish passage at the Newhalen River are also unlikely causes of these bottlenecks because these events are typically short lived and are not known to have persisted for even a single generation of sockeye salmon (4–5 years). It is similarly unlikely that reductions in spawning habitat caused these bottlenecks because known habitat loss and disturbance within the system are not as widespread as the bottleneck signals and there is only weak evidence of bottleneck effects in populations known to be affected by recent habitat loss (e.g. Priest Rock Creek). The presence of both significantly reduced allelic richness and *M* in most Lake Clark populations suggests that the putative bottleneck occurred within the last 100–400 sockeye salmon generations (~400–1400 years for Lake Clark sockeye salmon) depending on the demographic recovery of the population (Garza & Williamson 2001). The prevalence of reduced allelic richness (the longest lasting effect) but the lack of consistent significant effects for the most transient bottleneck measures (gametic disequilibrium, allele frequency distribution mode shifts, heterozygosity excess) suggests older bottleneck effects, perhaps associated with the colonization of Lake Clark after the last ice age.

The lack of isolation by distance and the high variability of F_{ST} over geographical distance also support a strong, historical effect of genetic drift among these populations. For all Lake Clark populations excepting Sucker Bay Lake, historical gene flow predominates, suggesting that the genetic similarity found among most Lake Clark sockeye salmon is the result of a common founding event. This historical pattern may persist today because high contemporary gene flow resists its erosion by drift. For example, high gene flow among most Lake Clark populations but restricted gene flow because of differences in spawning time between these and populations of Sucker Bay Lake, Six-mile Lake and Lake Iliamna would help maintain this pattern. This historical pattern may also persist because insufficient time has elapsed since colonization to reach equilibrium. The rate of approach to migration–drift equilibrium depends on N_e or the inverse of the migration rate ($1/m$), whichever is greater (Slatkin 1994). Following a founder event, reaching equilibrium can take tens to hundreds of generations (Crow & Aoki 1984; Waples 1998; Kinnison *et al*. 2002).

Why does the bottleneck signal intensity vary among Lake Clark sockeye salmon populations? The differences in magnitude of bottleneck signals may reflect differences in the state of recovery of genetic variation or the age of the bottlenecks. Once genetic variation is reduced in a population it can only be regained by mutation or immigration. The number of migrants exchanged among populations, and the difference in allele frequencies between immigrants and the population receiving them, will have a strong effect on the rate of recovery of populations (Cornuet & Luikart 1996; Waples 2002). Populations within Lake Clark that have weaker bottleneck signals may receive greater numbers of genetically diverse immigrants and/or may have been established longer allowing more time for mutation to restore genetic diversity. Conversely, Lake Clark populations exhibiting strong bottleneck signals (e.g. Sucker Bay Lake) may be highly isolated or recently founded. A founder effect associated with Lake Clark need not apply to all populations within the basin equally. Time since colonization probably varies among Lake Clark populations because of the slow retreat of glaciers and the creation of spawning habitat at the northeast end of the system. Thus, populations at the northeast end of Lake Clark that exhibit strong bottleneck signals (Upper Tlikakila River, Little Lake Clark Beach) may have been more recently founded than other Lake Clark sockeye salmon populations. For example, receding glaciers uncovered the Upper Tlikakila River within the last 200 years (unpublished data, Patricia Heiser, University of Alaska, Anchorage). The most transient of bottleneck signals may not be found in these populations because some recovery has already occurred or because the signals were never generated. The latter might be expected with a bottleneck among individuals that have already experienced a previous bottleneck from the colonization of Lake Clark.

Implications

Genetic population structure is often interpreted with the assumption of equilibrium without consideration for bottleneck or founder events. Here we provide an empirical example of the influence of founder effects, both historical and ongoing, on contemporary genetic population structure of Lake Clark sockeye salmon. Nonequilibrium

conditions and strong historical drift among these populations has implications both for management of these fish and our understanding of the genetic and phenotypic variation among sockeye salmon populations.

The detection of a common bottleneck effect among Lake Clark sockeye salmon will affect the interpretation of studies of selection and phenotypic variation among populations of Lake Clark sockeye salmon (Gould & Lewontin 1979; Adkison 1995). For example, phenotypic divergence between Six-mile Lake and Lake Clark sockeye salmon may be the result of colonization by phenotypically different founders rather than divergent selection since colonization. In contrast, phenotypic divergence among most Lake Clark populations would require divergent selection since their common founding.

The divergence between sockeye salmon in Lake Clark and those in Six-mile Lake and Lake Iliamna will allow fishery managers to identify Lake Clark fish in the Bristol Bay mixed stock fishery. For this application, it does not matter why populations differ, only that they differ enough to allow assignment of individuals to their lake of origin with confidence. The lack of migration–drift equilibrium among these populations suggests that observed genetic divergence does not reflect current levels of gene flow but rather reflects the ancestral association of the founders. Estimates of gene flow among most Lake Clark populations based on F_{ST} will be overestimates because much of the genetic similarity among populations is the result of common ancestry. For example, where F_{ST} is low (e.g. 0.01), we would estimate high gene flow (Nm ~25 migrants per population, per generation) when in fact the populations may exchange few individuals and have large effective population sizes that maintain similar allele frequencies since a common founding event. In contrast, the higher divergence observed among some populations (e.g. $F_{ST} = 0.8$) will underestimate contemporary gene flow (Nm ~ three migrants per population, per generation) between populations that are exchanging individuals since a recent founding by colonizers with different allele frequencies.

Conclusions

The magnitude of genetic differentiation among spawning populations of Lake Clark sockeye salmon is larger than that typically found between populations within the same lake (Wood 1995). The reduced allelic diversity and strong divergence of most Lake Clark populations relative to populations of Six-mile Lake and Lake Iliamna suggest a bottleneck associated with the colonization of Lake Clark by sockeye salmon. The greatest bottleneck effect detected and the most genetically distinct population was Sucker Bay Lake in Lake Clark. There is also significant genetic divergence between populations of Lake Clark and Six-mile Lake, the latter being more similar to fish of Lake Iliamna. Isolation by distance and differences in spawning habitat type apparently do not contribute to isolation and divergence among major population groups. However, temporal isolation based on spawning time and reduced allelic diversity as a result of bottleneck effects are closely correlated with the pattern of genetic population structure of Lake Clark sockeye salmon. The correlation between differences in genetic variation (allelic richness) and genetic divergence between populations suggests that founder effects have deeply affected the genetic population structure of Lake Clark sockeye salmon. The lack of correlation between genetic divergence and geographical distance among populations further suggests that these populations are not at migration–drift equilibrium and patterns of population divergence are the result of historical events. Nonequilibrium conditions and the strong influence of genetic drift caution against using estimates of genetic divergence to estimate gene flow among populations of Lake Clark sockeye salmon.

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