

Temporal Variation in Phenotypic and Genotypic Traits in Two Sockeye Salmon Populations, Tustumena Lake, Alaska

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Abstract.—Sockeye salmon *Oncorhynchus nerka* in two tributary streams (about 20 km apart) of the same lake were compared for temporal variation in phenotypic (length, depth adjusted for length) and genotypic (six microsatellite loci) traits. Peak run time (July 16 versus 11 August) and run duration (43 versus 26 d) differed between streams. Populations were sampled twice, including an overlapping point in time. Divergence at microsatellite loci followed a temporal cline: population sample groups collected at the same time were not different ($F_{ST} = 0$), whereas those most separated in time were different ($F_{ST} = 0.011$, $P = 0.001$). Although contemporaneous sample groups did not differ significantly in microsatellite genotypes ($F_{ST} = 0$), phenotypic traits did differ significantly (MANOVA, $P < 0.001$). Fish from the larger stream were larger; fish from the smaller stream were smaller, suggesting differential fitness related to size. Results indicate run time differences among and within sockeye salmon populations may strongly influence levels of gene flow.

Anadromous sockeye salmon *Oncorhynchus nerka* exhibit precise spatial and temporal homing behavior from oceanic feeding grounds to natal habitats (Hartman and Raleigh 1966; Brannon 1987), where they spawn and then die. Progeny generally rear in a lake associated with natal habitats for one or more years before migrating to oceanic feeding grounds. Molecular genetic studies indicate significant divergence has occurred among populations originating from different lakes (Grant et al. 1980; Foote et al. 1989; Varnavskaya et al. 1994a, 1994b; Wood 1995). As a result, the nursery lake is used as the basic unit for conservation and management, although some evidence for a more complex population structure exists.

Sockeye salmon spawning in different habitats of the same lake can exhibit divergent phenotypes and genotypes. For example, smaller streams of the Wood River system in Alaska contain predominantly smaller two-ocean fish, whereas larger streams contain predominantly larger three-ocean fish (Rogers 1987); river spawning fish of the Kvi-

chak River, Alaska, are larger at the same age and older than fish that spawn along island beaches (Blair et al. 1993), and littoral spawning populations show significant molecular genetic differences compared to tributary spawning populations (Varnavskaya et al. 1994a). Consistent differences in quantitative life history traits related to fitness (e.g., age and size at maturity; Stearns 1992) and in genetic markers indicate some reproductive isolation among spawning aggregations in different habitats.

Differences in return or spawn time among spawning aggregations can also influence population structure. Breeding studies indicate these traits are heritable in salmonids. Sex-specific heritability estimates for return time range from 0.18 (males) to 0.39 (females) in *O. gorbuscha* (Smoker et al. 1998). Heritability estimates for spawn date range from 0.55 ± 0.07 (*O. mykiss*, Siitonen and Gall 1989) to 0.57 ± 0.24 (*O. kisutch*, Silverstein 1993). Therefore, if fish inherit a tendency to return and spawn at a specific time, gene flow could be temporally limited, contributing to within-lake population structure. Comparisons between early- and late-returning sockeye salmon populations sometimes indicate significant phenotypic or molecular genetic differences. Early-returning sock-

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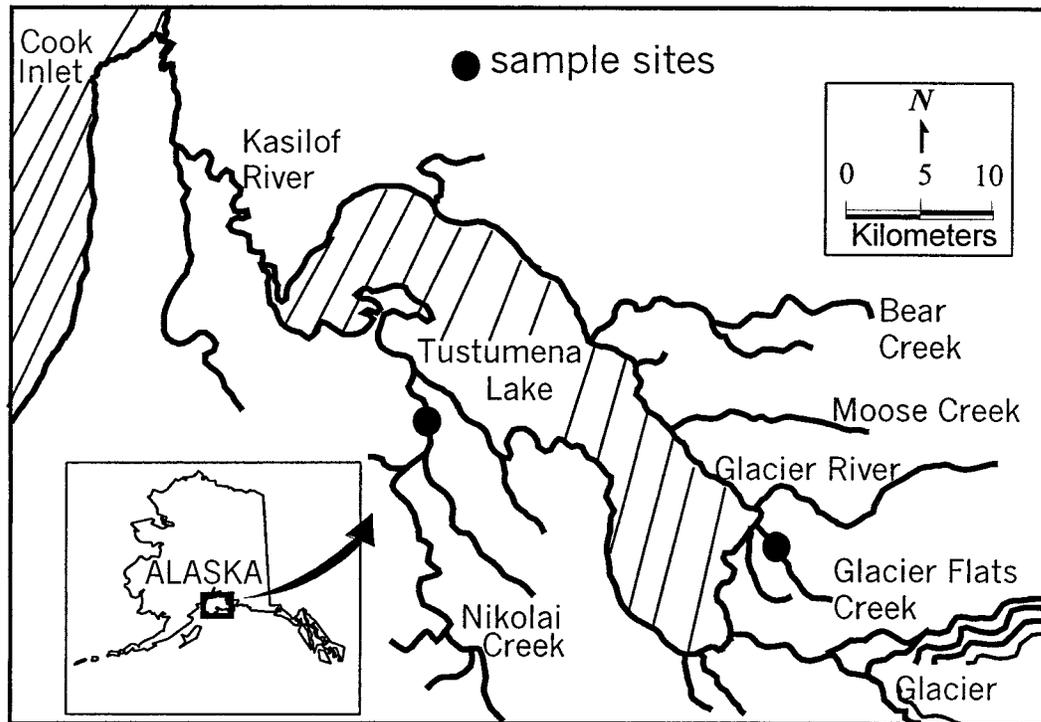


FIGURE 1.—Sample sites (weir) used for characterization of sockeye salmon populations in Nikolai and Glacier Flats creeks, Tustumena Lake, Alaska, 1994.

eye salmon populations in Karluk Lake, Alaska, are consistently smaller and less fecund than fall-returning fish (Gard et al. 1987), and some allozyme studies indicate greater divergence between early and late spawning populations than among those spawning in different habitats (Wilmot and Burger 1985; Varnavskaya et al. 1994a). The within-lake differences suggest potential for population structure at the natal stream scale: fish returning early may not have an opportunity to reproduce with fish returning late and vice versa. Some evidence for this pattern has been observed in pink salmon (Brykov et al. 1999).

Studies examining within-lake population structure of sockeye salmon indicate that variation in quantitative life history traits and molecular genetic traits can exist among populations, although causal factors are not well understood. Variation among populations in life history traits can indicate adaptive differences (Stearns 1992; but see Adkison 1995), whereas variation in molecular markers can indicate levels of gene flow. How variation among populations in the two types of traits corresponds is not well understood. This study compares variation in phenotypic (size at maturity)

and molecular (microsatellite markers) traits for early- and late-returning fish to two tributary streams to the same nursery lake.

Study Site

Tustumena Lake is a turbid glacial lake located on the Kenai Peninsula of Alaska (Figure 1). Sockeye salmon primarily spawn along two lake beaches and within seven clear-water tributaries (Burger et al. 1995). Populations from two tributaries (about 20 km apart), Nikolai and Glacier Flats creeks (Figure 1), were studied because prior research indicated population differences in potentially adaptive traits (return time, age and size at maturity; Kyle 1992; Flagg 1986). Such differences indicate some degree of reproductive isolation. Prior analyses of mitochondrial DNA and allozyme data from the two populations failed to indicate molecular genetic differences between the two populations (Burger et al. 1997); therefore, microsatellite markers were used because they may provide higher resolution of genetic structure (Sanchez et al. 1995).

Nikolai Creek is a fourth-order, highly diverse, tannin-stained, valley bottom stream with an es-

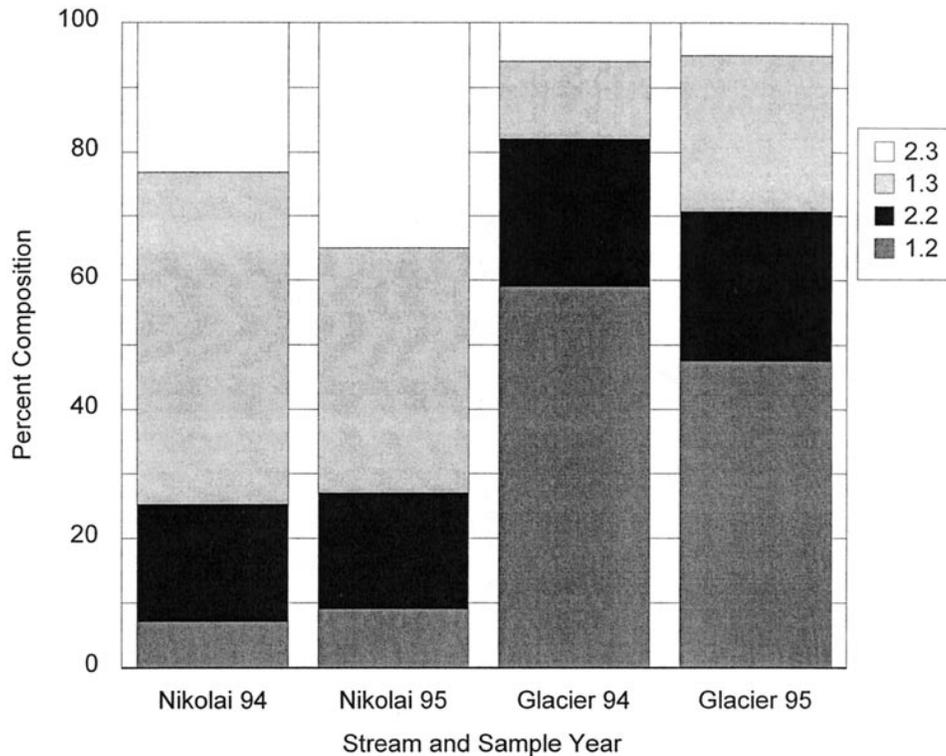


FIGURE 2.—Age at maturity for Nikolai Creek ($N = 305$) and Glacier Flats Creek ($N = 303$) sockeye salmon, 1994 and 1995, Tustumena Lake, Alaska. The first number denotes years spent in freshwater; the number after the decimal denotes years in the ocean.

timated age of 14,000 years (Reger 1993). The lower 7 km main stem of the 85-km long stream averages 7.6 m wide and 29.9 cm deep, and the first 4 km of the west fork averages 6.1 m wide and 18.4 cm (Woody 1998). It is fed by precipitation and often experiences dramatic fluctuations in flow and temperature regimes.

Sockeye salmon enter Nikolai Creek beginning in early July and continuing through August (Kyle 1992; Woody 1998). Aerial survey estimates indicate an average annual return of 16,000 fish (Kyle 1992). Age determination of 303 otoliths in 1994 indicated 25% of Nikolai Creek sockeye salmon return as two-ocean fish and 75% as three-ocean fish (Figure 2). Tagging studies in 1994 indicated about 88% of the females spawn within an average of 6 d after stream entry and 22% spawn within an average of 2.6 d (Woody 1998).

Glacier Flats Creek is a first-order, monomorphic, clear-water, relict glacial channel that was covered by the Tustumena Glacier as recently as 2,000 years ago (Karlstrom 1964). It averages 6.9 m wide and 20.6 cm deep (Woody 1998), and stream length

varies annually (2–4 km) because it is fed entirely from clear, upwelling groundwater, which tempers thermal and flow regimes.

Sockeye salmon enter the stream beginning in early August and continuing through the first week of September (Kyle 1992; Woody 1998). Aerial estimates and weir data indicate an average annual return of 32,000 fish (Kyle 1992). Age determinations of 305 otoliths in 1994 indicated 82% of the fish return as two-ocean fish and 18% as three-ocean fish (Figure 2). The majority of females (91%) in 1994 spawned in an average of 3.25 d after stream entry, and the remainder spawned in an average of 7.1 d (Woody 1998).

Methods

Sampling procedures.—Prespawning migrant sockeye salmon were counted and sampled at weirs in each stream approximately 0.5 km upstream of the lake (Figure 2). Weirs were installed prior to each run and removed after salmon stopped entering (0 fish/week—Glacier Flats Creek) or when fewer than 200 fish/week were observed (Nikolai Creek).

Salmon were netted from a collection chamber in the weir and a small fin clip (approximately 5 cm²) was collected from the dorsal fin for genetic analysis. Two measurements were taken on each fish (± 2 mm): (1) midorbit of the eye to the posterior of the hypural plate (MEH) and (2) body depth, measured perpendicular from the anterior insertion of the dorsal fin to the abdomen. Size data were collected only for ripe prespawning individuals, because individual depth increases with the development of secondary sexual characteristics. Fish were released upstream of the weir after sampling. Approximately 50 males and 50 females were sampled over the course of 2–3 d at the beginning and during the latter portions of each run. Sampling dates are coded as follows: Nikolai Early = 16–19 July, Nikolai Late = 9–10 August, Glacier Early = 10–11 August, and Glacier Late = 24–25 August 1994.

Genotypic characterization of temporal sample groups.—Genomic DNA was obtained for polymerase chain reaction (PCR) using a rapid cell lysis protocol modified from Hoelzel and Green (1994). Microsatellite amplifications were carried out using primers designed by Scribner et al. (1996) and O'Reilly et al. (1996) in two multiplex combinations adapted from Olsen et al. (1996). Multiplex one contained *One μ 1*, *One μ 11*, and *One μ 14* and multiplex two *One μ 2*, *One μ 8*, and *Ssa85* (Olsen et al. 1996).

DNA samples were diluted 1:1 with lysis buffer prior to the PCR. Two PCRs were conducted for each sample. Each 10 μ L-PCR contained 1–0.5 μ L diluted DNA sample, 10 mM tris-HCL (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 0.8 mM dNTPs, 0.5–1.0 U *Taq* DNA polymerase, and 0.08–0.19 μ M forward and reverse primers for each of three microsatellite primer pairs. The following profile was used for amplification in a Perkin-Elmer 9600 thermocycler: 1 cycle of 94°C (2 min) + 7 cycles of: 94°C (1 min) \rightarrow 58°C (30 s) \rightarrow 72°C (15 s) + 18 cycles of: 94°C (30 s) \rightarrow 58°C (30 s) \rightarrow 72°C (15 s). The PCR products were stored at 4°C until use.

One microliter from each triplex PCR was combined in a vial containing 4 μ L of loading buffer (0.60 μ L 50 mM EDTA, 3.15 μ L formamide, and 0.25 μ L (1.0 fmol) Perkin-Elmer GS 350 internal size standard). Samples were then denatured at 95°C for 3 min, placed on ice, then electrophoresed on a 6% denaturing polyacrylamide gel for 9 h using an Applied Biosystems Inc. (ABI) 373A Genescanner (ABI 1993). Electrophoretic data were analyzed with Genotyper software (ABI 1994).

Analyses.—The joint distributions of length and

depth adjusted for length were compared across samples using multivariate analysis of variance (MANOVA). Males and females were analyzed separately because of dimorphism in secondary sexual characters. The allometric dependence between length and depth was removed by combining samples for a given sex and regressing $\ln(\text{depth})$ on $\ln(\text{length})$. The residual $\ln(\text{depth})$ measures were exponentiated to give a measure of depth adjusted for length (mm). The MANOVA assumptions were graphically assessed, and the canonical variates were explored to gain insight into the dominant response differences among samples (Krzanowski 1988). Bartlett's sequential χ^2 method (Bernstein 1988) was used to determine if the bivariate means differed significantly along the second canonical variate after adjusting for the first canonical variate. If the MANOVA revealed a significant difference across samples, all pairwise MANOVAs were conducted, and their results were assessed using the sequential Bonferroni correction with a familywise error rate of $\alpha = 0.05$ (Rice 1989). All MANOVAs were assessed using the Pillai trace statistic, which is known to be robust to departures from the assumption of homogeneity of variance-covariance matrices (Tabachnick and Fidell 1996). Analyses were conducted in S-Plus 2000 (Mathsoft 1999).

Hardy-Weinburg equilibrium and heterogeneity in allele frequencies among genetic samples were tested with the program GENEPOP 3.1 (Raymond and Rousset 1995). Unbiased *P* value estimates were derived with a modified Markov chain method (Dememorization = 1,000; Batches = 300; Iterations = 1,000/batch) (Guo and Thompson 1992).

Genetic divergence between samples was measured by F_{ST} (Wright 1969; Weir and Cockerham 1984), which was estimated with the program FSTAT (Goudet 1995). Tests of the significance of F_{ST} over all loci were calculated by permuting alleles within totals (1,000 iterations). A sequential Bonferroni correction with a familywise error rate of $\alpha = 0.05$ (Rice 1989) was used for all pairwise comparisons.

Results

Phenotypic Comparisons

The 1994 Nikolai Creek sockeye salmon return began approximately 25 d earlier and lasted twice as long as the return to Glacier Flats Creek (Figure 3). Fish may have tried to ascend Glacier Flats up to 3 d prior to 9 August; however foul weather prevented access to the study site. Because the

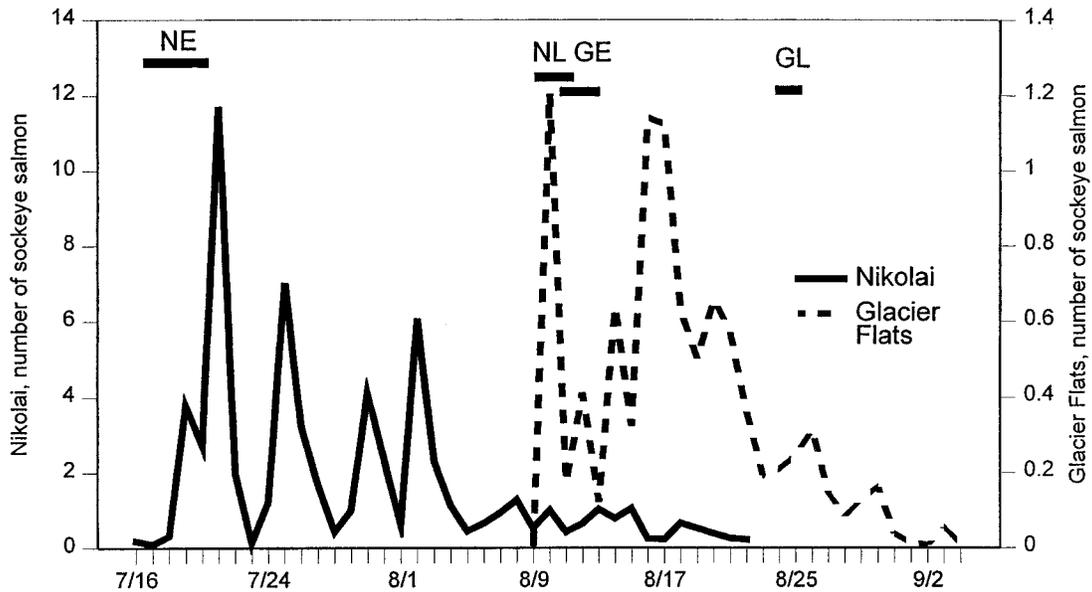


FIGURE 3.—Sockeye salmon return (in thousands), 1994, to Nikolai and Glacier Flats creeks, Tustumena Lake watershed, Alaska. Sampling dates are indicated for each stream by horizontal lines above return data. Sample dates are coded as follows: NE = Nikolai Creek early return, 16–19 July; NL = Nikolai Creek late, 9–10 August; GE = Glacier Flats Creek early, 10–11 August; GL = Glacier Flats Creek late 24–25 August.

Nikolai run lasted over 35 d, there was a temporal overlap with the Glacier Flats return from 9–22 August. A total of 63,732 sockeye salmon passed the Nikolai weir, and 10,347 passed the Glacier Flats weir.

Significant differences were detected in the joint distributions of fish length and depth adjusted for length across the four samples of each sex (females—Pillai trace = 0.5901, approximate *F* statistic = 24.14, 6, 346 df, *P* < 0.0001; males—Pillai trace = 0.5522, approximate *F* statistic =

23.39, 6, 368 df, *P* < 0.0001). Investigation of residuals and within-population variance-covariance matrices showed no departures from the MANOVA assumptions. The two canonical variates associated with each MANOVA revealed similar patterns of phenotypic divergence among the samples within a sex. The first canonical variate captured the greatest separation among means and was driven slightly more by length than depth adjusted for length (see standardized coefficients, Table 1); this variate appeared to separate out Nikolai

TABLE 1.—Canonical variate analysis associated with the multivariate analysis of variance for each sex. Coefficients have been standardized (adjusted for differences in variances between length and depth adjusted for length) to give relative measures of each response dimension to the canonical variate. For example, for canonical variate I of the females, a change of one standard deviation in length produces approximately 10 times the effect of a change of one standard deviation in depth adjusted for length (0.0834/0.0088 = 9.477). Squared canonical correlation measures the amount of total variation in the responses explained by variation in the given canonical variate. The last column gives the amount of variation among sample group means explained by variation in the given canonical variate. See Krzanowski (1988) and Scheiner (1993) for more details.

Sex	Canonical variate	Standardized length coefficient	Standardized depth adjusted for length coefficient	Squared canonical correlation (%)	Variation among groups means explained by this canonical variate (%)
♀	I	0.0834	0.0088	50.4	91.5
	II	-0.0119	0.0185	8.6	8.5
♂	I	0.0781	0.0470	50.0	94.8
	II	-0.0319	0.0774	5.2	5.2

TABLE 2.—Pairwise multivariate analysis of variance results for phenotypic trait comparisons of sockeye salmon populations originating from Nikolai and Glacier Flats creeks, Tustumena Lake, Alaska. Values in the upper right section of the table are *P* values for the female comparisons; the lower left section are *P* values for the male comparisons. For each gender, all comparisons are significant using a sequential Bonferroni familywise error rate of $\alpha = 0.05$.

Origin	Nikolai Creek		Glacier Flats Creek	
	Early	Late	Early	Late
Nikolai Creek early		0.00074	<0.00001	<0.00001
Nikolai Creek late	<0.00005		<0.00001	<0.00001
Glacier Flats Creek early	<0.00001	<0.00001		0.00061
Glacier Flats Creek late	<0.00001	<0.00001	0.04343	

Early, Nikolai Late, and the Glacier Flats samples (Figure 4). In both sexes, the second canonical variate captured a significant difference among sample means that remained after adjusting for differences in the first canonical variate (Bartlett's sequential χ^2 : females—approximate $P = 0.0013$; males—approximate $P = 0.02$). The second canonical variate was driven slightly more by depth adjusted for length than by length (standardized coefficients, Table 1) and appeared to separate out early and late components within each creek (Figure 4). The samples of each sex differed in all pairwise MANOVAs (Table 2).

Genotypic Comparisons

The six microsatellite loci surveyed were highly polymorphic. The mean number of alleles per microsatellite locus (\pm SE) was 11.3 ± 2.4 , and ranged from 4 (*One μ 11*) to 20 (*Ssa85*) (Appendix

I). Expected heterozygosity (H_E) among loci (populations combined) ranged from 0.24 (*One μ 1*) to 0.83 (*One μ 2*) and averaged 0.61. Locus *One μ 14* was not included in the average heterozygosity estimates because it departed significantly from the Hardy-Weinburg equilibrium assumption of the statistical comparisons (Goudet 1995). This locus showed heterozygote deficiency relative to expected in both Glacier Flats female sample groups and in the early-run males of both streams (Table 3). Excluding *One μ 14*, average expected heterozygosities were similar between streams and run components within streams (Table 3). The mean expected heterozygosity among all Glacier Flats and Nikolai subgroups (early female, early male, late female, late male) combined, was 0.57 ± 0.018 and 0.58 ± 0.28 , respectively.

Genic tests revealed no significant difference in

TABLE 3.—Hardy-Weinberg probability test results for early- and late-returning sockeye salmon to Nikolai and Glacier Flats creeks. Sampled run components are: Nikolai Creek early = 16–19 July and late = 9–10 August; Glacier Flats Creek early = 10–11 August and late = 24–25 August 1994. H_E = expected heterozygosity; parenthetical values are *P* values for test that the subgroup is in Hardy-Weinburg equilibrium. Microsatellite multiplexes are indicated by line joining loci names. Locus *One μ 14* was part of the *One μ 1* triplex but was not used in the mean heterozygosity analysis because frequency data did not conform to Hardy-Weinberg. Asterisks indicate significant deviations from expected equilibrium proportions after application of a sequential Bonferroni with familywise error rate of $\alpha = 0.05$.

Creek	Sample group	Sex	<i>N</i>	Expected heterozygosity (H_E)						Mean
				<i>Oneμ1</i>	<i>Oneμ11</i>	<i>Oneμ14</i>	<i>Oneμ2</i>	<i>Oneμ8</i>	<i>SSa85</i>	
Nikolai	Early	♀	53	0.21 (0.504)	0.51 (0.951)	0.64 (0.017)	0.81 (0.015)	0.68 (0.991)	0.78 (0.014)	0.60
	Early	♂	52	0.21 (1)	0.64 (0.194)	0.67 (0.001)*	0.85 (0.609)	0.73 (0.342)	0.77 (0.086)	0.64
	Late	♀	51	0.13 (1)	0.51 (0.054)	0.80 (0.069)	0.77 (0.472)	0.60 (0.648)	0.53 (0.087)	0.51
	Late	♂	49	0.26 (0.098)	0.50 (0.686)	0.69 (0.098)	0.78 (0.947)	0.68 (0.576)	0.72 (0.374)	0.59
Glacier Flats	Early	♀	48	0.15 (0.023)	0.41 (1)	0.81 (0.000)*	0.80 (0.615)	0.67 (0.013)	0.66 (0.836)	0.54
	Early	♂	43	0.34 (0.211)	0.53 (0.495)	0.79 (0.008)*	0.81 (0.279)	0.74 (0.929)	0.70 (0.002)*	0.62
	Late	♀	49	0.31 (0.491)	0.37 (1)	0.80 (0.000)*	0.79 (0.327)	0.61 (0.459)	0.67 (0.558)	0.55
	Late	♂	46	0.30 (0.465)	0.50 (0.139)	0.77 (0.020)	0.80 (0.024)	0.67 (0.025)	0.57 (0.518)	0.57

TABLE 4.—Genic differentiation P values and average F_{ST} among sample groups from Nikolai and Glacier Flats creeks, Tustumena Lake, Alaska, 1994. Sample groups are: Nikolai Creek early = 16–19 July; Nikolai Creek late = 9–10 August; Glacier Flats Creek early = 10–11 August; Glacier Flats Creek late = 24–25 August. Significant genic differences are indicated by asterisks following P values from Fisher’s exact test, computed with a modified Markov Chain method ($SE < 0.01$); sequential Bonferroni used with familywise error rate of $\alpha = 0.05$.

Pairwise comparison	<i>Oneμ1</i>	<i>Oneμ11</i>	<i>Oneμ2</i>	<i>Oneμ8</i>	<i>Ssa85</i>	F_{ST} (P)
	Within stream					
Nikolai early × Nikolai late	0.264	0.632	0.113	0.639	0.043	0.006 (0.007*)
Glacier Flats early × Glacier Flats late	0.221	0.904	0.000*	0.889	0.399	0.003 (0.114)
	Between stream					
Nikolai early × Glacier Flats early	0.295	0.835	0.008*	0.809	0.001*	0.006 (0.01)
Nikolai early × Glacier Flats late	0.003*	0.445	0.021	0.412	0.000*	0.011 (0.001*)
Nikolai late × Glacier Flats early	0.717	0.293	0.401	0.715	0.250	0
Nikolai late × Glacier Flats late	0.170	0.080	0.000*	0.639	0.216	0.009 (0.004*)
Nikolai × Glacier Flats	0.048	0.154	0.110	0.377	0.000*	0.005 (0.001*)

allele frequencies between sexes comprising early- or late-run components within a stream. When sexes were pooled within temporal samples, four of six pairwise temporal comparisons indicated significant differences at one or more loci (Table 4, columns 2–6). The two exceptions were Nikolai Early and Late, and Nikolai Late and Glacier Early. When samples within a stream were combined, significant differences between Nikolai and Glacier Flats remained detectable only at locus *Ssa85* (Table 4).

Genetic divergence measured by F_{ST} generally paralleled the genic differentiation results, except for the within-stream comparisons (Table 4). Estimates of F_{ST} were significant between Nikolai Early and Late ($F_{ST} = 0.006$, $P = 0.007$; Figure 3; Table 4), but not between Glacier Early and Late ($F_{ST} = 0.003$, $P = 0.114$). No difference ($F_{ST} = 0$) was observed between the contemporaneous Nikolai Late and Glacier Early samples (Table 4). In general, F_{ST} estimates tended to increase with increasing temporal separation (Figure 3; Table 4).

Discussion

Run time in sockeye salmon is a precise behavior and is inversely related to natal incubation regimes in a particular region (Brannon 1987). Return and spawn time in salmonids has a demonstrated genetic component (Siitonen and Gall 1989; Silverstein 1993; Smoker et al. 1998), and the two traits are related in the Tustumena study populations: Nikolai fish (88%) spawn in an average of 6 d after stream entry, whereas Glacier fish (91%) spawn in an average of 3.25 d (Woody

1998). Nikolai fish returning at the peak of the run (19–21 July) would complete their spawning cycle before both the Nikolai Late and Glacier fish entered their natal streams to spawn. If return time is precise within an individual run, and our results indicate this possibility, then the degree of divergence observed in both phenotypic and genotypic traits would be influenced by reproductive isolation among temporal components.

Phenotypic Analysis

Nikolai Creek sockeye salmon originate in a larger system, are larger sized (Figure 3), larger at the same age (Woody 1998), and generally older than Glacier Flats fish (Figure 2; also see Kyle 1992). This pattern fits the previously observed positive association of natal tributary size with fish age and size at maturity (Beacham and Murray 1987; Rogers 1987; Blair et al. 1993). The positive correlation of size with age at maturity, combined with the demonstrated genetic component of the latter factor (Gjerde 1984; Hankin et al. 1993), suggests a fitness advantage of larger size in large systems—smaller size in small systems.

Alternatively, the observed size difference between Nikolai and Glacier fish may be due not to habitat-specific fitness, but rather random genetic events (drift, mutation) or environmentally induced variation (phenotypic plasticity) (Stearns 1992). It seems unlikely that random aberrations would cause persistent life history trait differences among populations; such traits are related to fitness (e.g., the larger Nikolai fish produce more and

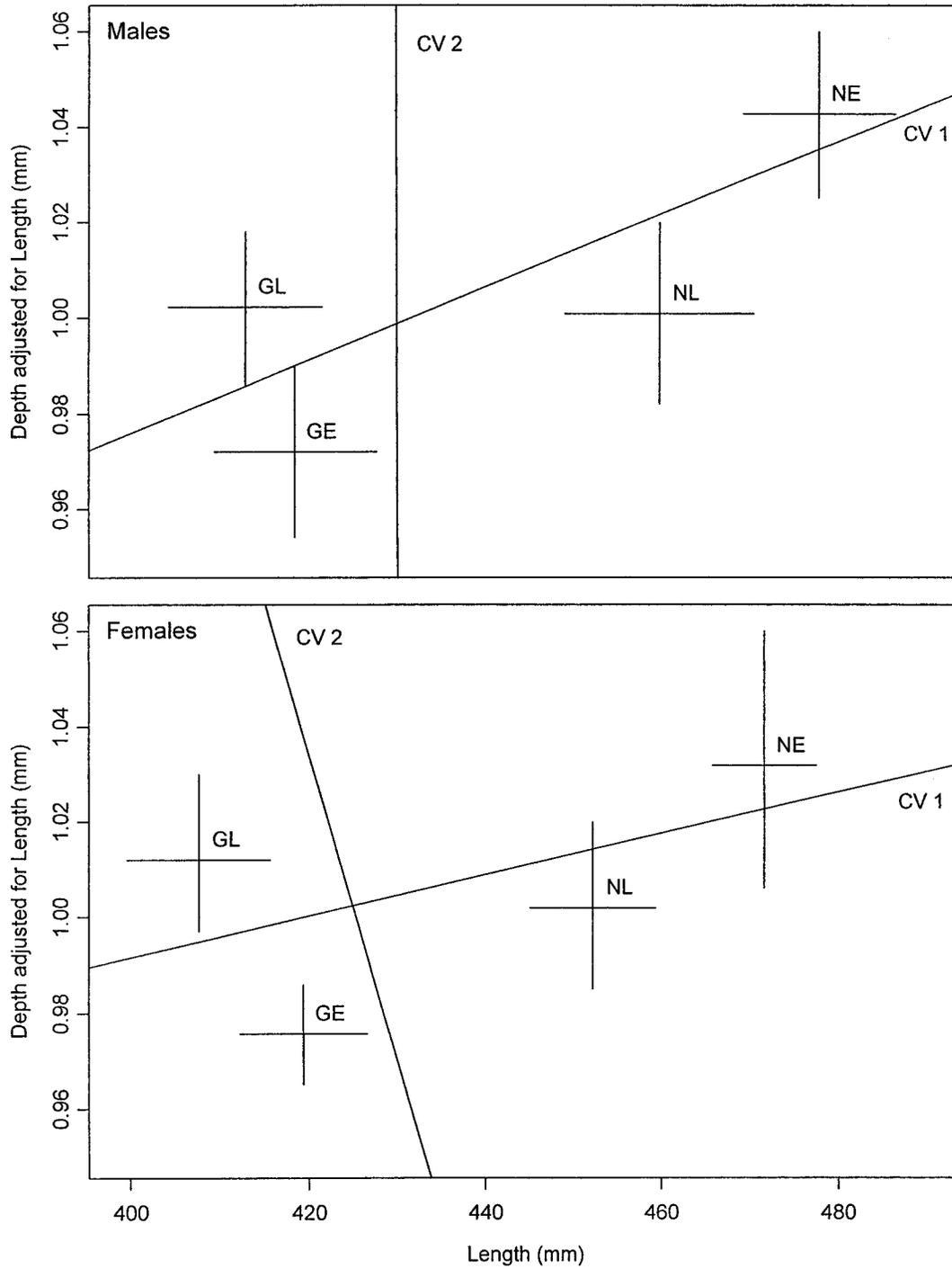


FIGURE 4.—Sample bivariate means (x -coordinate = length, y -coordinate = depth adjusted for length) and 95% normal univariate confidence intervals. Each multivariate analysis of variance produces two canonical variates: linear combinations of response variables that maximize group separation (see Krzanowski 1988); these lines are denoted by CV1 and CV2.

larger eggs; Woody 1998) and are sensitive to selection (Stearns 1992). Because the size difference persists even in the homogenizing presence of apparent gene flow ($F_{ST} = 0$; Nikolai Late versus Glacier Early; Table 4; Figure 4), a fitness advantage related to size is indicated (Endler 1986).

Phenotypic plasticity induced by heterogeneous growing environments is a plausible explanation for some of the observed size differences. Some environments may provide the opportunity for a population to grow faster or larger than another, which may explain why Nikolai fish are larger at the same age than Glacier fish (Woody 1998). However, the consistent difference in average age and size at maturity (Kyle 1992; Woody 1998) suggests adaptive differences maintained by differential selection pressures in the natal environments.

Nikolai Early fish are larger than Nikolai Late fish, which are larger than Glacier Early fish (Figure 4). One factor contributing to the size differential between Nikolai Early and Late fish may be straying of smaller fish into Nikolai from other streams in the system. Since Nikolai Creek fish exhibit early peak return time relative to other Tustumena spawning aggregations (Barrett 1972), the early-returning and larger sized run component may be less susceptible to genetic "swamping" by other Tustumena populations; however, straying into the Nikolai Late samples is possible. Straying from Glacier into Nikolai Late is specifically suggested by the lack of divergence in microsatellites between the Nikolai Late and Glacier Early ($F_{ST} = 0$; Table 4) and between Glacier Early and Late samples ($F_{ST} = 0.003$, $P = 0.114$; Table 4); the smaller size of the Glacier Early fish (Figure 4); and the overlap in return time. Other populations may contribute, as mark-recapture studies by Flagg et al. (1986) indicate a 0.09% stray rate between Glacier Flats and the Bear Creek populations (Figure 1), both of which dominate Nikolai proportionally in the Tustumena escapement (relative percent 1984–1991: Glacier 27.6, Bear 49.8, Nikolai 7.2; Kyle 1992). Some fitness advantage of larger size in Nikolai, combined with the isolating effect of run time, could maintain the size difference observed among samples, despite the homogenizing effects of gene flow between Nikolai Late and Glacier Early (Endler 1986).

The size difference between Nikolai Early and Nikolai Late fish could also be related to temporal variation in habitat use within a population. Large streams may comprise a variety of habitats, and different portions of a salmon run may use different portions of a stream (Tallman and Healey 1991).

For example, larger fish tended to spawn in deeper water of lower Nikolai Creek while smaller fish tended to spawn in the shallower upper tributary (Woody 1998). However, whether more small late-run fish spawned in the upper tributary is currently unverified.

Genotypic Analysis

Gene flow among sample groups diminishes on an apparent temporal cline as small, but increasing, levels of divergence are observed with increased temporal separation of samples (Table 4; Figure 3). There is no difference ($F_{ST} = 0$) in marker frequencies where the sample groups overlap in time (Nikolai Late and Glacier Early), whereas the largest difference is between the most extreme temporal samples (Nikolai Early and Glacier Late; $F_{ST} = 0.011$; Table 4; Figure 3). Divergence may be due to the increased reproductive isolation caused by differences in return (and spawn) times as, for example, early returning salmon to Nikolai Creek spawn and die before Glacier Flats Creek salmon spawn (Figure 3). Since return time is a precise (Brannon 1987), heritable (Smoker et al. 1998) behavior in salmonids, it can contribute to reproductive isolation among sockeye salmon populations within a watershed.

Reproductively isolated populations evolve differences in neutral markers (microsatellites) through random genetic events (mutation and genetic drift), whereas gene flow among populations (e.g., salmon straying) acts as a homogenizing force. Mutation and genetic drift are opposing diversifying forces: mutation increases genetic variation and drift decreases genetic variation within populations. Because the genetic profiles of the study groups are similar (average heterozygosities range = 0.50–0.60; Table 3) and there are few alleles unique to each population (private alleles) (Appendix I), it is reasonable to assume mutation rates are similar among the study groups. Furthermore, mutation is likely to be a weak diversifying force on time scales relevant to this study. The recent founding of Glacier Flats [approximately 2,000 years ago (ya)] and Nikolai (approximately 10,000 ya) creeks, combined with evidence of gene flow, where Nikolai and Glacier runs overlap in return time (Figure 3; Table 4), discounts mutation and implicates genetic drift as causal in the observed divergence.

The diversifying evolutionary force of genetic drift is a function of population size. Our microsatellite results show none of the signals associated with a previous period of low effective population

size: low heterozygosity, heterozygote excess, a difference in heterozygosity among populations, a difference in the number of alleles among populations, or gametic disequilibrium within populations (Hartl and Clark 1997). Historic demographic data also indicates large effective population sizes (ground and aerial spawner counts; Kyle 1992). Therefore, genetic drift not influenced by an abrupt decrease in effective population size best explains the statistically significant F_{ST} estimates for temporally distinct segments within and among samples.

The results indicate that within-lake population structure of sockeye salmon may be strongly influenced by return time. Conclusions about within-lake population structure based on samples collected at the same time may differ from those collected at different times. Ideally, sockeye salmon researchers would have prior knowledge regarding peak return times of their study populations and could plan sampling regimes accordingly. Unfortunately, such population-specific information is often lacking because it is expensive and difficult to obtain, particularly in remote areas such as Alaska. In such cases, sampling populations through time, versus sampling at one point in time, may provide a more accurate characterization of population structure.

Inferences based on just the phenotypic (size at maturity) or genotypic (microsatellite markers) data would likely differ from inferences drawn using both. The two types of data are complementary; each adds information not revealed by the other. Variation among populations in heritable phenotypic traits, such as run time and life history traits, can indicate the presence of adaptive differences; variation among populations in microsatellite markers can indicate the level of gene flow. Both types of information are important to consider, particularly in regards to developing conservation and management plans.

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Appendix: Allele Frequencies

TABLE A.1.—Allele frequencies for early- and late-run, male and female, tributary spawning sockeye salmon originating from Glacier Flats and Nikolai creeks, Tustumena Lake, Alaska, 1994.

Locus allele (bp)	Glacier Flats Creek				Nikolai Creek				Early run		Late run	
	♀ Early	♀ Late	♂ Early	♂ Late	♀ Early	♀ Late	♂ Early	♂ Late	♀ ♂ Glacier	♀ ♂ Nikolai	♀ ♂ Glacier	♀ ♂ Nikolai
<i>Oncp.1</i>												
(N)	(48)	(47)	(43)	(46)	(53)	(50)	(52)	(48)	(91)	(105)	(93)	(98)
110	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.000
112	0.083	0.106	0.151	0.120	0.066	0.060	0.115	0.104	0.115	0.090	0.113	0.082
114	0.917	0.819	0.802	0.826	0.887	0.930	0.885	0.854	0.863	0.886	0.823	0.893
116	0.000	0.021	0.023	0.000	0.047	0.010	0.000	0.010	0.011	0.024	0.011	0.010
118	0.000	0.053	0.023	0.043	0.000	0.000	0.000	0.031	0.011	0.000	0.048	0.015
<i>Oncp.11</i>												
(N)	(46)	(46)	(43)	(45)	(53)	(50)	(51)	(48)	(88)	(104)	(91)	(98)
146	0.054	0.043	0.081	0.089	0.094	0.090	0.069	0.042	0.067	0.082	0.066	0.066
150	0.728	0.750	0.616	0.656	0.623	0.610	0.637	0.604	0.674	0.630	0.703	0.607
156	0.207	0.207	0.302	0.244	0.283	0.300	0.284	0.354	0.253	0.284	0.225	0.327
158	0.011	0.000	0.000	0.011	0.000	0.000	0.010	0.000	0.006	0.005	0.005	0.000
<i>Oncp.14</i>												
(N)	(47)	(49)	(43)	(42)	(53)	(50)	(52)	(48)	(90)	(105)	(101)	(98)
129	0.106	0.041	0.198	0.107	0.066	0.070	0.096	0.073	0.150	0.071	0.081	0.071
133	0.000	0.020	0.000	0.024	0.000	0.020	0.019	0.010	0.000	0.022	0.010	0.015
135	0.149	0.173	0.174	0.190	0.170	0.190	0.154	0.135	0.161	0.181	0.162	0.163
137	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000
139	0.000	0.000	0.023	0.000	0.009	0.010	0.000	0.000	0.011	0.000	0.005	0.005
141	0.043	0.061	0.023	0.012	0.009	0.000	0.000	0.021	0.033	0.038	0.005	0.010
143	0.000	0.031	0.012	0.000	0.028	0.010	0.019	0.010	0.006	0.016	0.024	0.010
145	0.213	0.153	0.035	0.202	0.358	0.210	0.183	0.010	0.128	0.176	0.271	0.112
147	0.287	0.347	0.337	0.214	0.104	0.230	0.250	0.500	0.311	0.286	0.176	0.362
149	0.011	0.000	0.012	0.024	0.028	0.020	0.019	0.021	0.011	0.011	0.024	0.020
151	0.128	0.163	0.186	0.179	0.198	0.210	0.173	0.177	0.156	0.170	0.186	0.194
153	0.000	0.000	0.000	0.000	0.009	0.000	0.019	0.000	0.000	0.000	0.014	0.000
155	0.011	0.000	0.000	0.012	0.009	0.020	0.019	0.031	0.006	0.005	0.014	0.026
161	0.043	0.010	0.000	0.036	0.009	0.010	0.048	0.010	0.022	0.022	0.029	0.010
<i>Oncp.2</i>												
(N)	(47)	(45)	(43)	(45)	(50)	(48)	(51)	(47)	(90)	(101)	(90)	(95)
256	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.005	0.000	0.000
262	0.032	0.000	0.000	0.000	0.010	0.000	0.010	0.011	0.017	0.010	0.000	0.005
264	0.064	0.178	0.035	0.178	0.090	0.094	0.127	0.064	0.050	0.109	0.178	0.079
266	0.085	0.089	0.174	0.044	0.060	0.125	0.059	0.085	0.128	0.059	0.067	0.105
268	0.096	0.100	0.047	0.111	0.050	0.073	0.039	0.074	0.072	0.045	0.106	0.074
270	0.309	0.278	0.267	0.300	0.190	0.250	0.304	0.202	0.289	0.248	0.289	0.226
272	0.043	0.067	0.151	0.122	0.090	0.104	0.098	0.085	0.094	0.094	0.094	0.095
274	0.000	0.022	0.000	0.022	0.020	0.000	0.029	0.032	0.000	0.025	0.022	0.016
276	0.064	0.033	0.035	0.011	0.070	0.031	0.029	0.021	0.050	0.050	0.022	0.026
278	0.266	0.156	0.267	0.133	0.280	0.302	0.206	0.351	0.267	0.243	0.144	0.326
280	0.043	0.067	0.012	0.056	0.090	0.000	0.059	0.043	0.028	0.074	0.061	0.021
282	0.000	0.011	0.012	0.000	0.030	0.021	0.039	0.021	0.006	0.035	0.006	0.021

TABLE A.1.—Continued.

Locus allele (bp)	Glacier Flats Creek				Nikolai Creek				Early run		Late run	
	♀ Early	♀ Late	♂ Early	♂ Late	♀ Early	♀ Late	♂ Early	♂ Late	♀ ♂ Glacier	♀ ♂ Nikolai	♀ ♂ Glacier	♀ ♂ Nikolai
<i>Onc8</i>												
(N)	(45)	(42)	(43)	(41)	(50)	(40)	(51)	(43)	(88)	(101)	(83)	(83)
194	0.011	0.024	0.047	0.000	0.020	0.038	0.020	0.035	0.028	0.020	0.012	0.036
196	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.012	0.000	0.000	0.000	0.018
198	0.133	0.143	0.163	0.195	0.100	0.100	0.118	0.174	0.148	0.109	0.169	0.139
200	0.244	0.357	0.233	0.207	0.230	0.262	0.206	0.256	0.239	0.218	0.283	0.259
202	0.000	0.000	0.000	0.000	0.000	0.013	0.010	0.000	0.000	0.005	0.000	0.006
204	0.456	0.381	0.419	0.402	0.460	0.400	0.490	0.349	0.438	0.475	0.392	0.373
206	0.089	0.071	0.070	0.061	0.100	0.063	0.078	0.105	0.080	0.089	0.066	0.084
208	0.011	0.000	0.023	0.024	0.010	0.013	0.000	0.000	0.017	0.005	0.012	0.006
210	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000
212	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.012	0.000	0.005	0.000	0.006
214	0.022	0.024	0.047	0.049	0.060	0.063	0.029	0.035	0.034	0.045	0.036	0.048
224	0.033	0.000	0.000	0.049	0.020	0.025	0.039	0.023	0.017	0.030	0.024	0.024
<i>Ssa85</i>												
(N)	(45)	(44)	(41)	(37)	(48)	(37)	(51)	(44)	(86)	(99)	(81)	(81)
121	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.010	0.000	0.000
129	0.033	0.068	0.061	0.041	0.083	0.027	0.029	0.045	0.047	0.056	0.056	0.037
133	0.000	0.000	0.000	0.000	0.010	0.014	0.010	0.000	0.000	0.010	0.000	0.006
135	0.167	0.159	0.220	0.189	0.198	0.108	0.206	0.136	0.192	0.202	0.173	0.123
137	0.500	0.466	0.439	0.486	0.229	0.500	0.324	0.386	0.471	0.278	0.475	0.438
139	0.111	0.091	0.159	0.122	0.156	0.122	0.157	0.148	0.134	0.157	0.105	0.136
141	0.000	0.023	0.000	0.000	0.125	0.027	0.010	0.023	0.000	0.066	0.012	0.025
143	0.078	0.068	0.037	0.054	0.063	0.027	0.088	0.080	0.058	0.076	0.062	0.056
145	0.033	0.034	0.037	0.000	0.031	0.041	0.029	0.034	0.035	0.030	0.019	0.037
147	0.011	0.011	0.000	0.054	0.000	0.000	0.010	0.011	0.006	0.005	0.031	0.006
149	0.022	0.034	0.000	0.000	0.010	0.027	0.010	0.000	0.012	0.010	0.019	0.012
151	0.000	0.000	0.000	0.041	0.000	0.000	0.010	0.011	0.000	0.005	0.019	0.006
159	0.022	0.000	0.000	0.000	0.031	0.014	0.029	0.045	0.012	0.030	0.000	0.031
165	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.006
171	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.011	0.000	0.000	0.000	0.012
173	0.022	0.023	0.037	0.014	0.010	0.027	0.049	0.045	0.029	0.030	0.019	0.037
175	0.000	0.000	0.000	0.000	0.042	0.000	0.000	0.000	0.000	0.020	0.000	0.000
177	0.000	0.011	0.000	0.000	0.010	0.014	0.010	0.011	0.000	0.010	0.006	0.012
179	0.000	0.011	0.000	0.000	0.000	0.027	0.000	0.011	0.000	0.000	0.006	0.019
181	0.000	0.000	0.012	0.000	0.000	0.000	0.010	0.000	0.006	0.005	0.000	0.000