

## Migration Timing, a Life History Trait Important in the Genetic Structure of Pink Salmon

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Timings of migration are critical events in salmon life history. The physical conditions of the stream (e.g. temperature and water flow) determine spawning success and estuarine conditions markedly influence emigrant survival. Previous studies that demonstrated the contribution of genetic components to timing related polygenic traits such as migration timing and rate of embryo development suggest that with sufficient statistical power, genetic divergence related to timing should be detectable in biochemical genetic traits in pink salmon. Here we analyzed allozyme data from pink salmon in Auke Creek and other streams near Juneau, Alaska to examine the influences of return timing, spawning site, stream, and sample year on genetic structure. Log-linear modeling and heterogeneity G-tests indicated that return timing (early or late), year of return, and stream all influenced structure. Site within stream (upstream or intertidal) had no effect. For odd-brood-year Auke Creek collections, timing had a stronger influence than year. For 1979 collections from streams in the Juneau area, timing and stream both influenced structure and timing had a stronger influence. For even-brood-year collections, timing, stream, and year influenced structure, but we could not differentiate the extent of their influence. A similar study of data from Iterup Island (Kuril Islands in Asia) pink salmon revealed only an effect of year, but the analysis was weak because data from few loci were available. Detection of biochemical genetic variation correlated to life history characters demonstrates the importance of such characteristics in determining the population structure and basis for productivity of salmon populations.



### INTRODUCTION

The time during the spawning season at which salmon return to a stream to spawn is clearly important to their survival and productivity. Because water flows and temperatures vary seasonally, the fish must time their return for when the stream provides satisfactory spawning conditions and, subsequently, water flows and temperatures favorable for incubation, development, and timely emergence of deposited embryos. The importance of timing has been documented in a variety of experiments from broad geographic scales to local. At the scale of species range, salmon spawning migrations generally occur

earlier in northern regions and later in southern regions. For example, most chum (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*) spawning in systems draining into the Bering Sea return during July and August; whereas in the Pacific Northwest and Japan, those species return from between September and January (Heard 1991; Salo 1991). In both instances, the fish return when stream and incubation conditions are generally favorable. Evidence for the importance of timing also exists at the regional level. The Fraser River system has several different lake systems that support sockeye salmon populations. The mean incubation temperature varies among those systems from a little over 2° to more than 8°C.

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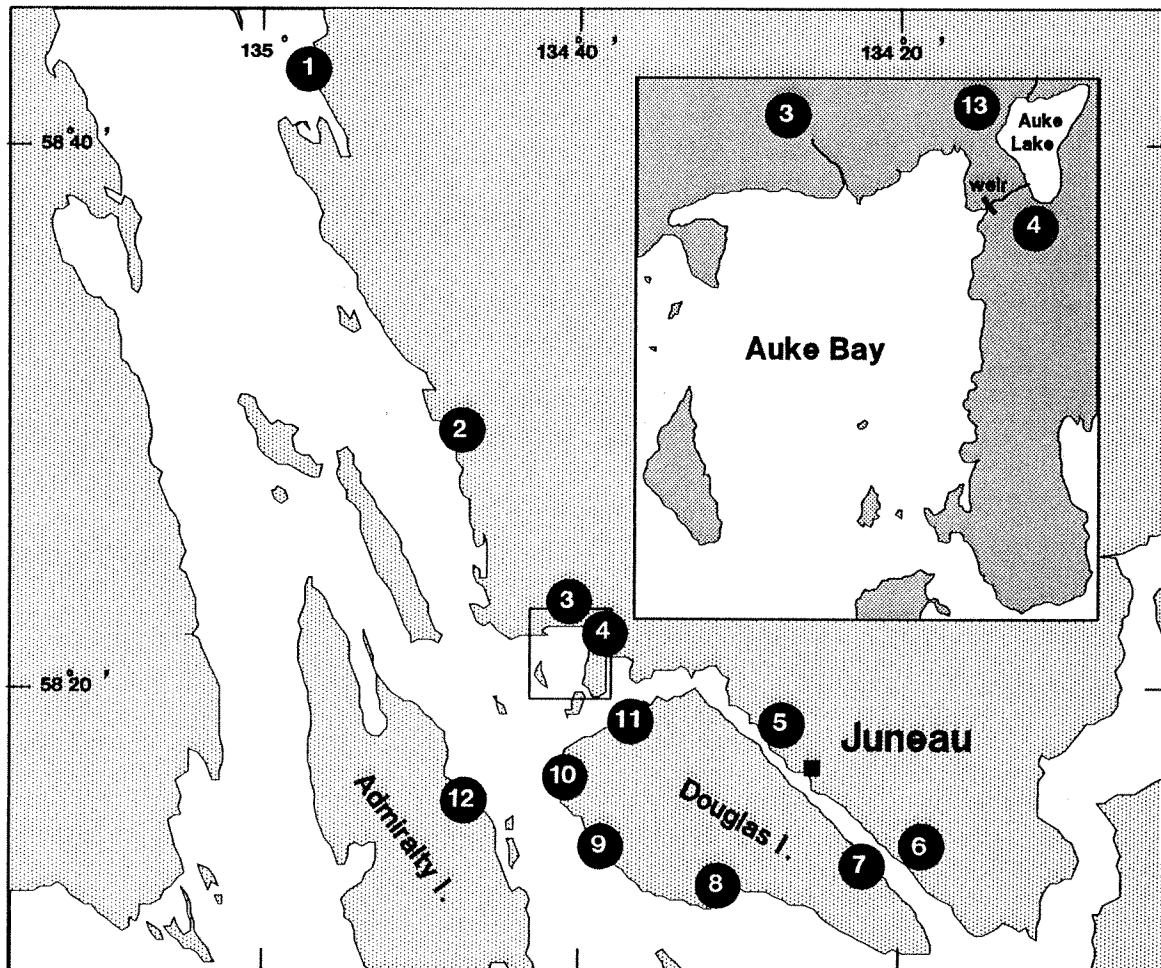
Spawning timing of populations returning to a lake is strongly correlated with the temperature of the system, populations returning to colder lakes return earlier, presumably to allow more time for embryonic development (Brannon 1987).

Genetically based temporal structure also exists within streams, even very small streams. Pink salmon in Auke Creek, 18 km north of Juneau, Alaska have been intensively studied using markers, genetic marking, and quantitative genetic analysis. Auke Creek flows approximately 350 m from Auke Lake into Auke Bay (Figure 1). The National Marine Fisheries Service operates a weir just above tidewater that enumerates returning adult salmon and emigrant fry and a small hatchery that is used for cooperative interagency research programs. The stream has natural runs of both even- and odd-year pink salmon. Fin-marking studies identified both temporal and spatial structure of the pink salmon returning (Taylor 1980); four components within Auke Creek are early-

run intertidal (below the weir), early run upstream (above the weir), late run intertidal, and late run upstream. The early run returns from late July to August; the late run returns during September. The late run is ordinarily preceded by reduced returns of early-run fish and indicated by incompletely mature, "sea-bright" fish.

The temporal structure of pink salmon returns in Auke Creek were more thoroughly examined by genetically marking the latter half of the late upstream component. In 1979, the frequencies of allozyme alleles were altered in that component by screening adults for and breeding in the hatchery only individuals possessing *MDH-3,4\*70\** and not *MDH-3,4\*130\** alleles (Lane 1984; Lane et al. 1990). These fish returned in 1981 and interbred with naturally produced fish, genetically marking the run. From samples taken throughout the run it was observed that the marked component of the run remained in the late portion of the late upstream

Fig. 1 Map of the locations of Alaskan pink salmon streams studied. An inset shows Auke Bay and the Auke Lake drainage. Streams are: 1 Sawmill Creek, 2 Peterson Creek (mainland), 3 Waydelich Creek, 4 Auke Creek, 5 Salmon Creek, 6 Sheep Creek, 7 Ready Bullion Creek, 8 Hilda Creek, 9 Middle Point Creek, 10 Peterson Creek (Douglas Island), 11 Fish Creek, 12 Bear Creek, and 13 Lake Creek.



component for several generations (Gharrett et al. in prep.). Additional evidence that timing is an important component of population structure is that emigrant fry produced by the late-run component emigrated later (on average) than fry produced by earlier returning parents (Gharrett and Smoker 1993).

The genetic marking experiment provided compelling evidence that timing was an important component of population structure and probably had a genetic basis, because the marked component produced offspring that had the same timing. For confirmation, Smoker et al. (in prep) conducted breeding experiments in both early-run and late-run Auke Creek fish and demonstrated a significant heritable component (sire effect) in return timing, not just for return to early or late run, but for timing of return within each period. This experiment shows that a fish tends to return to spawn at the same time during a run that its parents did.

With abundant evidence for the role of timing in population structure, and specific evidence that at least partial reproductive isolation exists among components of Auke Creek pink salmon, we would expect some divergence in molecular markers of those components. Temporal structure does not preclude gene flow; however, significant divergence can be detected in the presence of gene flow if sufficient power is used to detect it (Gharrett 1994).

Between 1978 and 1984, we conducted allozyme surveys of the spatial-temporal components of Auke Creek and of other systems near Juneau, Alaska (including McGregor 1983; Lane 1984). Here we analyze those data to test for heterogeneity among collections in frequencies of allozyme alleles and determine which factors have significant effects on allele frequencies. The factors we examine are timing of spawning (early versus late), site of spawning (intertidal versus upstream), the spawning stream, and the year in which data were collected (Appendix). Where sufficient data were available, we also estimated gene flow in numbers of individuals per generation.

## MATERIALS AND METHODS

### Protein electrophoresis

Protein electrophoresis (Aebersold et al. 1987) was used to estimate allozyme frequencies in collections of pink salmon from streams near Juneau, Alaska. Tissues sampled were kept at -20 °C until analysis. Loci (Shaklee et al. 1990) examined were aconitate hydratase (*mAH-3\** and *mAH-4\**), adenosine deaminase (*ADA-2\**), aspartate amino transferase (*sAAT-3\**), glycerol-3-phosphate dehydrogenase (*G3PDH-1\**), isocitrate dehydrogenase (*sIDH-1*), leucyl-glycyl-glycine peptidase (*PEPB-1\**), malate

dehydrogenase (*sMDH-B1,2\**), malic enzyme (*mMEP-1\**), mannose-6-phosphate isomerase (*PMI\**), phenylalanyl-proline peptidase (*PEPD-2\**), phosphoglucomutase (*PGM-2\**), 6-phosphogluconate isomerase (*GPIA\** and *GPI-B1,2\**), and 6-phosphogluconate dehydrogenase (*PGDH\**). Tissue specificities and buffer conditions were described previously (Gharrett and Thomason 1987) except for *sIDH-1* which we resolved in liver on a pH 7.0 tris-citrate buffer (Shaw and Prasad 1970).

### Analysis

Allele frequency analyses were made with log-likelihood ratios (Sokol and Rohlf 1995). Analyses for effects on allele frequencies were conducted using log-linear modeling which is the categorical data analog to analysis of variance. In our analyses, only models that involved interactions between effects and allele frequencies were examined because we were looking for effects of factors on allele frequencies. Without allele frequency in the model, the factors test only sample size differences among collections, which are of no interest. Log linear models generate log-likelihood ratios (*G*-statistics) for effects and interactions of effects. Balanced data of allele frequencies of odd-year pink salmon sampled from early intertidal, early upstream, late intertidal, and late upstream components of Auke Creek in each of 1979, 1981, and 1983, were analyzed using a saturated model for interactions of effects with allele frequency:

$$\ln f_{ijkl} = A_i * T_i + A_i * S_j + A_i * Y_k + A_i * T_i * S_j + A_i * T_i * Y_k + A_i * S_j * Y_k + A_i * T_i * S_j * Y_k$$

where  $T_i$  is the effect of timing (early or late run),  $S_j$  is the effect of spawning site (intertidal or upstream), and  $Y_k$  is the effect of year (1979, 1981, and 1983).  $A_i$  is allele frequency, and is tacitly assumed in subsequent models and table headings. Because *sMDH-B1,2\** was involved in the genetic mark of a component of Auke Creek, it was omitted from the analysis. Also, because an (unsuccessful) attempt was made to mark the even-year brood line with a *G3PDH-1\** allele, *G3PDH-1\** data were omitted from potentially affected Auke Creek components (1982 and 1984 late-run). Of interest in this analysis were not only the factors that significantly influenced allele frequency, but also the relative effect of some pairs of factors. Such ranking was done with an *F*-statistic estimated as:

$$F_{df(i), df(j)} = [G_i / df(i)] / [G_j / df(j)]$$

where  $G_i$  and  $G_j$  are the log-likelihood ratios for factors  $i$  and  $j$ , and  $df(i)$  and  $df(j)$  are their degrees of freedom.

Other data sets were unbalanced and a saturated model could not be analyzed. Therefore, we estimated first order effects on allele frequency but not higher level interactions. We used only data for which there averaged at least one allele of each type per collection used in the analysis. Because infrequent observations may occasionally produce  $G$ -statistics that are not asymptotic to the  $\chi^2$ -distribution and exaggerate departure from expectations, the significance of all "significant" single factor tests were confirmed by comparison of the test statistic to a distribution of 2000 statistics generated by Monte-Carlo resampling assuming the null hypothesis, homogeneity. All results of this compared well with significance deduced from the  $\chi^2$ -distribution.

When the structural hierarchy of populations had been determined, we used analysis of variance of allele frequencies (assuming Hardy-Weinberg equilibrium) to estimate components of variation attributable to each factor in the hierarchy (Weir and Cockerham 1984; Weir 1996). These components are analogous to estimates of gene diversity (Nei 1973; Chakraborty and Leimar 1987) and can be used to estimate the number of immigrants ( $N_e m$ ) per generation from:

$$\theta = \{4N_e m [n / (n-1)]^2 + 1\}^{-1}$$

where  $\theta$  is the component of variance estimated from the analysis of variance,  $m$  is the rate of immigration into a component of the population structure,  $N_e$  is the effective size of the component ( $N_e m$  is the number of individuals), and  $n$  is the number of components in a particular level of the hierarchical model. For example, if one considers timing to be either early or late, there are only 2 components, so the  $[n/(n-1)]^2$  term is 4, whereas if one considers that there are a large number of streams in the area ( $n$  is large), even though we only sampled a subset of the total,  $(n/(n-1))^2$  approaches 1 (Zhivotovskiy et al. 1994).

## RESULTS

We approached our questions about the hierarchical structure of pink salmon populations by starting at the finest scale, the within population structure of Auke Creek. Results obtained at the finest level should be pertinent to coarser scales, but the reverse may not be true. Data for odd-year Auke Creek pink salmon were available for three brood years, 1979, 1981, and 1983. For each year we had data from samples of all four Auke Creek spatially and temporally defined components for nearly every allozyme locus we examined. These data permitted us to examine the effects of **timing**, **site**, and **year** on allele frequency using a saturated model for these factors and their interactions (Table 1). The question

posed here involves the combined analysis of several loci to detect influences on the overall genetic structure of populations. The power in the analysis comes from the number of loci involved; significant divergence at particular loci is of lesser interest, especially if divergence is attributed to isolation and is random. In this analysis both **timing** ( $P < < 0.001$ ) and **year** ( $P < 0.01$ ) significantly affected allele frequencies, whereas spawning **site** did not. Comparisons of the variability due to **timing** with that due to spawning **site** indicates that **timing** is more important than **site** ( $P < 0.01$ ). Similarly, although both **timing** and **year** affect frequencies, **timing** appears to be a stronger influence.

From these results, we can deduce that population structure within Auke Creek can be conceptually structured as spawning **site** nested within **timing**. In this hierarchy, we consider **year** as replication of a particular spawning time and place within Auke Creek. Presumably returns to a time and place in Auke Creek derive primarily from parents spawning at the same place during the same time of the spawning season the previous generation. We used that hierarchy as the basis of an analysis of variance to partition genetic variation into components attributable to each level of hierarchy, analogous to gene diversity analysis. Although the components were small relative to the within-collection genetic variation, those attributable to differences in run timing and to differences between sites within run timing were much larger than their standard errors. Estimates (95% confidence interval) of gene flow ( $N_e m$ ) were between 11 and 37.4 for the effective number of fish moving between early and late run times within Auke Creek and 41.7 to 165.8 for the number moving between intertidal and upstream components within a run time.

We extended our analysis of the genetic structure of odd-year pink salmon to additional streams in the area. Because **year** had a significant effect on allele frequencies and not all streams were sampled in every year, we restricted this analysis to collections sampled in 1979, before any genetically marked fish returned. Not all streams had both intertidal and upstream components, and we did not obtain samples for both early- and late-run fish for every stream. Therefore, it was not possible to analyze the complete model for all possible effects and interactions of effects on allele frequency. Our analysis examined the primary factors **timing**, **site**, and **stream** (Table 2). Both **timing** ( $P < < 0.001$ ) and **stream** ( $P < 0.01$ ) significantly influenced allele frequencies; **timing** had a larger effect than either **stream** ( $P < 0.001$ ) or **site** ( $P < 0.05$ ).

We nested stream within run time and used analysis of variance to partition the genetic variation. The result was small components for both variation due to differences in run timing and to differences in

**Table 1. A.** Analysis of within stream population structure of odd-year pink salmon for Auke Creek, Alaska. Contingency analyses (log-linear modeling) partitioned the effects of spawning location (upstream or intertidal), spawning time (early or late), and year (1979, 1981, 1983) on allozyme variation. Tabled are G-statistics and degrees of freedom for each effect and interaction of effects. **B.** Gene flow ( $N_e m$ ) between components of odd-year pink salmon in Auke Creek, Alaska. Gene diversity was estimated using ANOVA of allele frequencies (Weir 1996). The population's structural hierarchy is spawning site (upstream or intertidal) within spawning time (early or late); years (1979, 1981, 1983) are replicates within sites. Estimates were based on 14 variable allozyme loci. <sup>a</sup>  $P < 0.05$ ; <sup>b</sup>  $P < 0.01$ ; <sup>c</sup>  $P < 0.001$ ; <sup>d</sup>  $P < 0.001$ .

A.																
Locus	Time		Site		Year		T*S		T*Y		S*Y		T*S*Y		Total for locus	
<i>mAH-3*</i>	31.33 <sup>d</sup>	1	3.01	1	4.36	2	0.87	1	2.88	2	4.93	2	0.01	2	47.39 <sup>d</sup>	11
<i>mAH-4*</i>	13.89 <sup>c</sup>	1	0.34	1	12.1	2	2.63	1	3.95	2	14.94 <sup>c</sup>	2	0.01	2	47.86 <sup>d</sup>	11
<i>SAAT-3*</i>	0.08	1	2.38	1	6.55 <sup>a</sup>	2	0.05	1	4.27	2	2.20	2	3.31	2	18.84	11
<i>PGDH*</i>	0.22	1	0.84	1	0.65	2	0.36	1	1.98	2	2.01	2	3.15	2	9.21	11
<i>GPIA*</i>	0.01	1	0.28	1	3.62	2	0.34	1	9.48 <sup>b</sup>	2	1.18	2	0.21	2	15.12	11
<i>PEPD-2*</i>	8.04 <sup>a</sup>	2	0.84	2	6.15	4	1.25	2	4.69	4	2.47	4	1.52	1	24.96	19
<i>PGM-2*</i>	7.86 <sup>b</sup>	1	4.34 <sup>a</sup>	1	3.84	2	4.09 <sup>a</sup>	1	1.84	2	0.01	2	2.00	2	23.98 <sup>a</sup>	11
<i>G3PDH-1*</i>	4.66 <sup>a</sup>	1	0.40	1	0.05	2	1.13	1	0.05	2	2.08	2	1.27	2	9.64	11
<i>mMEP-1*</i>	0.16	1	1.58	1	6.11 <sup>a</sup>	2	3.83	1	0.62	2	2.40	2	5.51	2	20.21 <sup>a</sup>	11
<i>MPI*</i>	0.30	1	0.76	1	3.68	2	1.09	1	3.43	2	1.93	2	1.69	2	12.88	11
<i>sIDHP-1*</i>	11.03 <sup>c</sup>	1	1.48	1	0.33	2	3.37	1	1.03	2	2.09	2	3.32	2	22.65 <sup>a</sup>	11
<i>ADA-2*</i>	0.14	1	0.29	1	4.92	2	1.00	1	0.11	2	1.28	2	--	--	7.74	9
Total	77.70 <sup>d</sup>	13	16.54	13	52.36 <sup>b</sup>	26	20.01	13	34.33	26	37.52	26	22.00	21	260.48 <sup>d</sup>	140

F-test for run timing (Time) versus spawning location (Site):  $F_{13,13} = 4.70$ :  $P < 0.01$ .

F-test for run timing (Time) versus brood year spawning location (Year):  $F_{13,26} = 2.97$ :  $P < 0.05$ .

B.			
Source	Parameter	Value $\pm$ S.E.	$N_e m$ (95% C.I.)
Total for collections	$\theta_{ss}$	0.00504 $\pm$ 0.00071	
Among years within sites	$\theta_{ss} - \theta_s$	0.00042 $\pm$ 0.00060	includes $\infty$
Between sites within run time	$\theta_s - \theta_p$	0.00096 $\pm$ 0.00030	41.7 - 165.8
Between run times	$\theta_p$	0.00367 $\pm$ 0.00102	11.0 - 37.4

**Table 2. A. Analysis of population structure of odd-year pink salmon streams near Juneau, Alaska. Contingency analyses partitioned the effects of spawning time (early or late), site (upstream or intertidal), and stream (12 local streams) on allozyme variation of 1979 returns. Tabled are G-statistics and degrees of freedom for each effect. Data are unbalanced, so no interactions were estimated. B. Gene flow ( $N_e m$ ) between components of odd-year pink salmon near Juneau, Alaska. Gene diversity was estimated using ANOVA of allele frequencies (Weir 1996). The population's structural hierarchy is stream within spawning time (early or late). Estimates were based on 16 variable allozyme loci. \*  $P < 0.05$ ; <sup>b</sup>  $P < 0.01$ ; <sup>c</sup>  $P < 0.001$ ; <sup>d</sup>  $P < 0.001$ .**

A. Locus	Stream		Timing		Site	
SAAT-3*	18.88	11	0.32	1	0.01	1
PGDH*	20.33 <sup>a</sup>	11	5.87 <sup>a</sup>	1	0.51	1
mAH-3*	12.09	11	17.66 <sup>c</sup>	1	1.59	1
G3PDH-1*	9.83	11	1.12	1	0.00	1
GPIA*	15.05	11	0.22	1	0.35	1
PEPD-2*	28.22	22	0.29	2	0.39	2
PGM-2*	11.73	11	12.46 <sup>c</sup>	1	4.68 <sup>a</sup>	1
mMEP-1*	11.04	11	0.02	1	7.29 <sup>b</sup>	1
MPI*	18.99	11	0.00	1	0.34	1
SIDHP-1*	15.64	10	3.84 <sup>a</sup>	1	1.10	1
ADA-2*	17.22	11	15.39 <sup>d</sup>	1	0.32	1
Total for factor	179.02 <sup>b</sup>	131	57.19 <sup>d</sup>	12	16.58	12

F-test for run timing (Timing) versus stream (Stream):  $F_{12,131} = 3.48$ ;  $P < 0.001$

F-test for run timing (Timing) versus stream (Site):  $F_{12,12} = 3.45$ ;  $P < 0.05$

B. Source	Parameter	Value $\pm$ S.E.	$N_e m$ (95% C.I.)
Total for collections	$\theta_s$	0.00286 $\pm$ 0.00062	
Among streams for a run time	$\theta_s - \theta_p$	0.00193 $\pm$ 0.00055	83.1 -292.5
Between run times	$\theta_p$	0.00086 $\pm$ 0.00017	41.0 -182.7

stream. Estimates of gene flow ( $N_e m$ ) were 41.0 to 182.7 (95% confidence interval) as the effective number of fish moving between early and late runs and between 83.1 to 292.5 fish immigrating into each stream from the combination of other streams.

Data from even-year pink salmon in the area were not as abundant and many of the systems were not sampled. Combining all the even-year data permitted us to examine the effect of single factors on allele frequencies, but not interactions of more than one factor. In this analysis, **timing** ( $P < 0.001$ ), **stream** ( $P < 0.001$ ), and **year** ( $P < 0.001$ ) significantly influenced allele frequency, but **site** ( $P > 0.05$ ) did not (Table 3). Comparisons of the effect of **timing** to the effects of **site**, **year**, and **stream** were not significant, which means that it is not possible to infer a hierarchy of effects, although it is probably safe to conclude that site is at the bottom of the hierarchy. Because the data were sparse and a clear hierarchy could not be deduced, no further analysis was conducted on these data.

In a parallel experiment conducted in the same years, Zhivotovsky et al. (1989) sampled streams on Iterup Island in the Kuril Islands throughout the spawning period. We analyzed the effects of **stream** and **timing** on allele frequencies of even-year collections made in 1982 and of **stream**, **timing**, and **year** on frequencies in collections taken in 1979, 1981, and 1983 (Table 4). Of these effects, only **year**

had a significant influence on allele frequencies, and that factor was examined only for odd-year samples. These analyses had relatively little power; data from only 3 loci were available for even-year collections, and data from 4 loci were available from odd-year collections.

## DISCUSSION

Spawning time was previously demonstrated in several ways as an important component of population structure. Our prediction was that, **with sufficient statistical power**, we should be able to detect the influence of spawning time on allele frequencies. Results presented herein demonstrate those effects on different components of Auke Creek odd-year pink salmon returns. We also observed spawning timing effects on allele frequencies of both odd-year and even-year pink salmon in streams near Juneau, Alaska. For odd-year pink salmon we also detected a significant influence of year of return within Auke Creek and for stream in the survey of 1979 returns. In both analyses, spawning time had a stronger effect. Spawning site within the stream had no significant influence. The even-year pink salmon returns to the Juneau area were not as completely sampled (and therefore a weaker test), but exhibited effects of spawning time, year of return, and stream on allele frequencies. In that experiment, spawning time was

**Table 3.** Analysis of population structure of even-year pink salmon streams near Juneau, Alaska. Contingency analyses partitioned the effects of spawning time (early or late), site (upstream or intertidal), stream (6 local streams), and year (1978, 1980, 1982, and 1984) of sample on allozyme variation. Tabled are G-statistics and degrees of freedom for each effect. Data are unbalanced, no interactions were estimated. <sup>a</sup> P < 0.05; <sup>b</sup> P < 0.01; <sup>c</sup> P < 0.001; <sup>d</sup> P << 0.001.

Locus	Timing		Site		Year		Stream	
SAAT-3*	5.08 <sup>a</sup>	1	6.07 <sup>a</sup>	1	0.38	2	3.33	5
PGDH*	0.44	1	5.26 <sup>a</sup>	1	8.92 <sup>a</sup>	3	18.99 <sup>b</sup>	5
mAH-4*	0.37	1	0.58	1	7.31	3	5.74	4
G3PDH-1*	0.25	1	0.07	1	5.36	3	28.26 <sup>d</sup>	5
PEPD-2*	8.03 <sup>a</sup>	2	0.76	2	14.86 <sup>a</sup>	6	27.52	10
PGM-2*	8.70 <sup>b</sup>	1	0.75	1	0.74	3	12.57	5
PEPB-1*	0.31	2	2.60	2	0.51	2	18.30	10
sMDH-B2*	1.40	2	1.94	2	19.61 <sup>b</sup>	6	14.07	10
mMEP-1*	4.45 <sup>a</sup>	1	2.10	1	8.18 <sup>b</sup>	3	6.45	5
ADA-2*	5.76	2	0.84	2	6.93	4	5.12	10
sIDHP-1*	2.42	1	1.45	1	1.04	1	8.29	4
Total	37.21 <sup>d</sup>	15	22.42	15	73.84 <sup>c</sup>	36	148.64 <sup>d</sup>	73

F-test for Timing versus Site:  $F_{15,15} = 1.66$ : P > 0.05

F-test for Timing versus Year:  $F_{15,36} = 1.21$ : P > 0.05

F-test for Timing versus Stream:  $F_{15,73} = 1.22$ : P > 0.05

**Table 4.** Analysis of genetic structure of Iterup Island (Kuril) pink salmon. Log-likelihood ratios partitioned the effects of spawning time (early or late) and stream on allozyme variation. These are 1979, 1981, 1982, and 1983 returns. Tabled are G-statistics and degrees of freedom for each effect. Estimates of interactions of effects were confounded by the unbalanced data, but all were very small. <sup>a</sup> P < 0.05; <sup>b</sup> P < 0.01.

Even year return (1982)				
Locus	Stream		Timing	
G3PDH-1*	1.16	4	0.07	1
PGDH*	1.95	8	0.00	2
sMDH-B1,2*	7.56	4	0.80	1
Total	10.67	16	0.87	4

F-test for Stream versus Timing:  $F_{16,4} = 1.50$ : P > 0.05.

Odd year returns (1979, 1981, 1983)						
Locus	Stream		Timing		Year	
G3PDH-1*	7.63	8	0.01	1	1.74	2
PGDH*	23.04	16	1.42	2	5.17	4
sMDH-B1,2*	13.92	8	1.31	1	10.59 <sup>a</sup>	2
PGM-2*	9.23	8	0.06	1	6.84 <sup>a</sup>	2
Total	53.82	40	2.80	5	24.34 <sup>b</sup>	10

F-test for Stream versus Timing:  $F_{10,5} = 4.35$ : P > 0.05

F-test for Stream versus Year:  $F_{10,40} = 1.81$ : P > 0.05

not a significantly stronger influence than year of return, spawning site, or stream. Tests of the effect of spawning time on Kuril Island pink salmon returns were quite weak because they involved only 3 or 4 loci. Only an effect of year of return was detected.

Failure to detect an effect or to be able to determine a hierarchy of effects in the latter analyses does not necessarily mean that there is no effect or hierarchy. Failure could also mean that with the number of loci, collections, and sample sizes used (i.e. the statistical power) the effects and structure could not be resolved, taking us back to our original prediction which required **sufficient power**. We are aware of two other studies that attempted to resolve an effect of return timing on allele frequencies. Whereas nine collections of even-year pink salmon from the Znamenka River and six collections from the

Ochepucha River on Sakhalin Island revealed no heterogeneity (Noll et al. In prep.), more powerful tests involving pools of collections indicated temporal structure of both even- and odd-year pink salmon returns to the Poronaisk River and odd-year returns to the Firsovka and Lesnaya rivers on Sakhalin Island (Altukhov et al. 1983).

In our interpretation of the results, we must consider the possibility that our marking experiments of 1979 and 1980 influenced our results. In order to minimize any influence we removed *sMDH-B1,2\** entirely from the analysis of odd-year fish and removed *G3PDH-1\** data from 1982 and 1984 late-run collections which might have been influenced by the less successful even-year marking experiment. It should be noted that the 1978, 1979, and 1980 brood years could not have been influenced by the

experiments, and most of the 1981 and 1982 Auke Creek returns revealed no effect. The number of parents used as breeders for the marked fish was large (about 200 males and 200 females) and their returns contributed at most one-half of the late returns. We detected no gametic disequilibrium in returning genetically marked fish (identified by a fin mark) and saw heterogeneity between years only at the *PEPD-2\** locus of 1979 and 1981 late returns. *PEPD-2\** does not contribute to the significance of interannual variability in analyses of odd-year collections. Finally, and most compelling, the analysis of the 1979 data for streams near Juneau, Alaska produced similar results.

The basis of the influences of spawning timing and site on genetic composition are easy to understand; interannual variation is not as straightforward. An obvious explanation is that differences between generations reflect random genetic drift, errors in sampling gametes from a finite population. An alternative explanation is that the difference attributed to year results in part from the spawning time effect. Most collections of samples were made on one or a few days during the early or the late returns, not throughout the run. Because return timing has a heritable component, temporal genetic structure exists beyond "early-" and "late-run" (Smoker et al. in prep.). Our inability to sample exactly the same portion of the run in several years may mean that some of the statistical effect of year of return reflects temporal genetic structure within a return.

For two of our odd-year analyses of Auke Creek and Juneau-area pink salmon, we estimated average gene flow between levels of hierarchy in genetically effective fish per generation ( $N_e m$ ). Within odd-year Auke Creek returns, we estimated that 11.0 to 37.4 fish moved between spawning times and 41.7 to 165.8 moved between intertidal and upstream spawning areas. The size of both these estimates seems reasonable. Early and late runs have overlapping tails which might make the estimated number of migrants seem small; however, heritable adaptive differences (e.g. Hebert et al. accepted) between the runs may reduce fitness of hybrids and there is some evidence that fish spawning during the overlap of early and late returns have lower survivals (Joyce 1986). In Auke Creek, gene flow between intertidal and upstream spawners is enhanced by the weir which does not allow fish that move past it to return down stream. In light of this and the extensive movements in which pink salmon engage prior to spawning (Jones and Thomason 1984; Thrower 1988), it is surprising that the number is not larger.

Among odd-year populations in the Juneau area, we estimated that 41.0 to 182.7 fish moved between spawning times and that each stream received between

83.1 and 292.5 immigrants from other local systems in each of the early and late run components. The size of these estimates appears reasonable. But, intuitively, the estimate of movement between early and late components seems low whereas the movement among streams seems high based on our study of genetically marked Auke Creek pink salmon (Gharrett et al. in prep.).

It is important to realize that the model used to estimate  $N_e m$  assumes no selection and that equilibrium has obtained (Zhivotovsky et al. 1994). Moreover, these estimates reflect long-term averages, not necessarily constant generation by generation movements. If equilibrium has not obtained or there is convergent (homogenizing) selection, the estimates of gene flow will be high. If there is divergent (heterogeneous) selection and equilibrium has obtained, the estimates of gene flow will be low (e.g. Gharrett 1994). Although equilibrium for gene diversity estimates ( $F_{ST}$ ,  $G_{ST}$ , and  $\theta_s$ ) is relatively rapid on an evolutionary time scale (Chakraborty and Leimar 1987), the key phrase is **evolutionary time** which is measured in hundreds or more of generations. Much of the habitat pink salmon occupy is more transient than that. Watershed altering cataclysms such as land and snow slides and earthquakes occur frequently. In Southeast Alaska, many new streams appeared (and are still appearing) in recent centuries following glacial recession which began about 10,000 years ago and continues today. What is surprising is that given the dynamic nature of their habitat and of necessity the populations, there is any detectable structure. Our ability to attribute that structure to spawning time and stream suggest that both are critical components in the genetic structure of pink salmon populations and critical elements in their productivity. Although there is gene flow among population components, the estimates are relatively small, such numbers would be inadequate to ensure short-term maintenance of productivity if some of the components in the system were severely depleted. Over long time periods, however, the movements should be sufficient to colonize (or restore) available habitat. The question we can not address using molecular markers alone is the extent of divergence among population components that is adaptively important and that may be necessary for restoration after a natural or anthropogenic cataclysm.

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## Appendix

Frequencies of allozyme alleles in collections of pink salmon near Juneau, Alaska. E designates early run, L late run, I intertidal spawners, and U upstream spawners. Common allele frequencies (100\*) are omitted.

Creek	Year	Date	Time	Site	SAAT-3*		PGDH*		mAH-4*		Ada-2*		
					n	85*	n	90*	n	85* &115*	n	87*	113*
<b>Odd brood-year</b>													
1	Sawmill	1979	12/VIII	E	I	98	0.21	100	0.02	101	0	101	0.05
2	Peterson (mainland)	1979	14/VIII	E	U	49	0.18	48	0.06	48	0	48	0.03
3a	Waydelich	1979	14/VIII-29/VIII	E	I&U	43	0.27	43	0.02	43	0	43	0.08
b		1979	12/IX	L	I&U	31	0.31	31	0	31	0	31	0.13
4a	Auke	1979	15/VIII-27/VIII	E	I	91	0.24	89	0.01	90	0.02	90	0.07
b		1979	27/VIII-30/VIII	E	U	80	0.18	80	0.01	80	0	81	0.08
c		1979	19/IX-27/IX	L	I	97	0.24	100	0.02	100	0	100	0.08
d		1979	5/IX-9/X	L	U	454	0.24	506	0.01	460	0	456	0.09
e		1981	13/VIII-1/IX	E	I	99	0.23	100	0.02	100	0.02	100	0.13
f		1981	13/VIII-25/VIII	E	U	101	0.24	101	0.02	101	0.02	101	0.07
g		1981	1/IX-18/IX	L	I	98	0.29	98	0.02	98	0	98	0.10
h		1981	15/IX-28/IX	L	U	101	0.23	99	0.01	102	0.01	102	0.12
i		1983	14/VIII	E	I	32	0.25	33	0.02	33	0	0	
j		1983	12/VIII	E	U	62	0.22	94	0.02	65	0.03	28	0.05
k		1983	1/IX-15/IX	L	I	71	0.23	88	0	88	0	0	
l		1983	14/IX	L	U	126	0.14	154	0.02	154	0.01	114	0.07
5	Salmon	1979	20/VIII	E	I&U	89	0.25	45	0.01	45	0	45	0.02
6	Sheep	1979	20/VIII-27/VIII	E	I	102	0.25	43	0.01	43	0	43	0.06
7	Bullion	1979	16/VIII-22/VIII	E	I	63	0.21	63	0.02	63	0.01	63	0.04
8a	Hilda	1979	19/VIII	E	I	30	0.32	33	0.02	33	0.02	32	0.06
b		1979	7/XI	L	I	58	0.21	44	0	44	0.01	44	0.13
9a	Middle Point	1979	19/VIII	E	I	47	0.26	54	0.02	54	0.01	54	0.04
b		1979	7/IX	L	I	76	0.20	67	0	76	0.01	75	0.11
10a	Peterson (Douglas I.)	1979	20/VIII	E	I	41	0.24	40	0.03	40	0	40	0.08
b		1979	29/VIII	E	U	41	0.26	42	0.04	42	0	42	0.07
c		1979	14/IX-18/IX	L	I	52	0.28	51	0.02	51	0	51	0.08
11a	Fish	1979	7/VIII-13/VIII	E	I	83	0.23	83	0.05	83	0.01	83	0.06
b		1979	13/VIII	E	U	87	0.34	87	0.03	87	0.01	66	0.04
c		1979	18/IX-21/IX	L	I	88	0.29	89	0.02	89	0.01	89	0.10
12	Bear	1979	25/VIII	E	I&U	80	0.16	80	0.01	80	0.01	79	0.07
<b>Even brood-year</b>													
2a	Peterson (mainland)	1978	25/VIII	E	U	0		41	0.06	0		0	
b		1978	13/IX	L	U	0		45	0.04	0		0	
c		1982	3/IX-5/IX	E	U	85	0.31	97	0.04	50	0.03	97	0.05 0.03
3a	Waydelich	1980	20/VIII-25/VIII	E	I&U	100	0.27	104	0.04	103	0.04	103	0.05 0.02
b		1980	8/IX-19/IX	L	I&U	100	0.31	78	0.04	102	0.03	102	0.01 0.01
c		1982	2/IX	E	I&U	95	0.23	100	0.07	100	0.04	100	0.05 0.03
d		1982	24/IX	L	I&U	98	0.28	100	0.04	101	0.01	101	0.07 0.02
e		1984	28/VIII	E	I&U	71	0.25	78	0	78	0.04	40	0.11 0.04
f		1984	19/IX-26/IX	L	I&U	28	0.29	28	0.05	26	0.08	28	0.07 0.04
4a	Auke	1978	22/VIII-3/IX	E	U	0		128	0.06	64	0.06	0	
b		1978	9/X	L	I	0		63	0.08	15	0.17	0	
c		1978	5/IX-9/X	L	U	0		133	0.04	33	0.06	0	
d		1980	24/VIII	E	I	88	0.22	87	0.07	98	0.04	98	0.07 0.01
e		1980	24/VIII	E	U	92	0.26	90	0.04	103	0.03	103	0.11 0.01
f		1980	6/IX-28/IX	L	U	173	0.30	206	0.06	214	0.05	98	0.04 0.01
g		1982	pre 6/IX	E	I	63	0.27	67	0.05	67	0.05	67	0.05 0.04
h		1982	1/IX	E	U	100	0.29	100	0.06	100	0.03	100	0.02 0.01
i		1982	12/IX-26/IX	L	U	115	0.26	119	0.05	119	0.04	119	0.05 0.03
j		1984	30/VIII-6/IV	E	I	80	0.19	80	0.03	80	0.06	80	0.06 0.04
k		1984	9/IX-22/IX	E	U	89	0.30	90	0.04	90	0.07	0	
l		1984	18/IX-19/IX	L	I	80	0.29	80	0.07	80	0.06	80	0.04 0.01
m		1984	21/VIII-25/VIII	L	U	90	0.32	90	0.04	90	0.06	0	
5	Salmon	1982	7/IX	E	I&U	49	0.21	50	0.03	0		50	0.05 0.01
10	Peterson (Douglas I.)	1982	14/IX	L	I&U	100	0.25	100	0.03	100	0.07	50	0.04 0.01
11a	Fish	1978	27/VIII-31/VIII	E	I	0		183	0.09	120	0.05	0	
b		1978	27/VIII-31/VIII	E	U	0		78	0.06	51	0.04	0	
c		1982	12/IX	L	I&U	82	0.28	80	0.08	100	0.05	100	0.04 0.02

## Appendix (continued)

Creek	G3PDH-1*		PEPD-2*		PGM-2*		mMEP-1*		sIDHP-1*		mAH-3*		GPIA*		MPI*		
	n	200* & others	n	109* 93*	n	150*	n	130*	n	120* & 130*	n	78*	n	90* 110*	n	85* 115*	
<b>Odd brood-year</b>																	
1	101	0.12	101	0.21	0.14	101	0.06	100	0.03	101	0.59	101	0.01	101	0.01	101	0
2	47	0.06	48	0.17	0.10	48	0.04	48	0.02	47	0.78	48	0	48	0.01	48	0.02
3a	43	0.12	43	0.17	0.09	43	0.05	43	0.03	43	0.66	43	0.02	43	0	43	0
b	3	0.06	31	0.19	0.10	31	0.06	31	0.02	31	0.71	30	0	31	0	31	0
4a	90	0.11	88	0.21	0.11	90	0.05	90	0.04	86	0.77	90	0.01	90	0	90	0.01
b	81	0.07	81	0.20	0.14	81	0.07	81	0.05	80	0.62	80	0.03	81	0	81	0.01
c	100	0.09	100	0.21	0.11	100	0.06	100	0.03	85	0.59	100	0	100	0.01	100	0.01
d	460	0.12	459	0.21	0.10	460	0.03	461	0.06	90	0.62	460	0.00	459	0.00	457	0.00
e	100	0.09	99	0.24	0.11	100	0.06	100	0.05	100	0.72	100	0.01	100	0.01	98	0.02
f	101	0.10	101	0.24	0.10	101	0.05	101	0.06	98	0.68	101	0.04	101	0.02	100	0.01
g	98	0.13	97	0.21	0.11	98	0.05	98	0.06	95	0.62	98	0	98	0.01	98	0.01
h	102	0.13	102	0.14	0.08	102	0.01	102	0.03	101	0.62	102	0.01	102	0	101	0.01
i	33	0.13	33	0.24	0.17	33	0.09	33	0.02	33	0.65	33	0.03	33	0	33	0
j	572	0.10	423	0.20	0.13	507	0.05	158	0.09	65	0.67	65	0.02	539	0.00	159	0
k	88	0.13	83	0.20	0.11	88	0.07	68	0.09	86	0.63	88	0	88	0.01	88	0
l	154	0.11	0			154	0.04	120	0.06	53	0.63	154	0	154	0.01	153	0.01
5	45	0.17	45	0.27	0.02	45	0.06	45	0.06	45	0.66	45	0.02	45	0	45	0
6	43	0.09	43	0.22	0.13	43	0.07	43	0.06	43	0.69	43	0.03	43	0	43	0
7	63	0.12	63	0.18	0.14	63	0.09	63	0.06	0		63	0.01	63	0	63	0.02
8a	33	0.11	33	0.24	0.09	33	0.06	33	0.03	32	0.53	33	0	33	0	33	0.01
b	44	0.10	44	0.14	0.13	44	0.03	44	0.02	0		44	0.01	44	0	44	0
9a	54	0.13	54	0.20	0.06	54	0.06	54	0.04	51	0.66	54	0.02	54	0	54	0.02
b	76	0.13	76	0.16	0.11	76	0.08	76	0.02	70	0.67	76	0.01	76	0.01	76	0.01
10a	40	0.13	40	0.20	0.13	40	0.10	40	0.04	40	0.66	40	0	40	0	40	0
b	42	0.18	42	0.15	0.12	42	0.08	42	0.06	40	0.65	42	0.01	42	0.01	42	0
c	51	0.09	51	0.18	0.20	51	0.01	51	0.03	51	0.60	51	0.01	51	0	51	0.02
11a	83	0.07	82	0.16	0.07	83	0.05	83	0.04	83	0.66	83	0.03	83	0	83	0
b	87	0.07	62	0.14	0.15	87	0.06	86	0.05	85	0.71	87	0.01	87	0	64	0
c	89	0.13	89	0.20	0.11	89	0.04	89	0.02	89	0.61	89	0	89	0	89	0.01
12	80	0.09	79	0.19	0.13	80	0.06	80	0.03	80	0.65	80	0.04	80	0	80	0
<b>Even brood-year</b>																	
Creek	G3PDH-1*		PEPD-2*		PGM-2*		mMEP-1*		sIDHP-1*		PEPB-1*		MDH-B1,2*				
	n	200* & others	n	109* 93*	n	150*	n	130*	n	120* & 130*	n	130*	140*	n	130*	70*	
2a	47	0.20	36	0.24	0.22	47	0	43	0.19	0		0		47	0.01	0	
b	46	0.17	37	0.23	0.20	45	0	38	0.21	0		0		46	0	0	
c	96	0.15	97	0.22	0.21	97	0	77	0.19	0		95	0.17	0.01	97	0	
3a	104	0.13	104	0.24	0.21	104	0.01	104	0.27	0		0		104	0.00	0	
b	102	0.19	102	0.19	0.25	102	0.00	102	0.26	0		0		102	0.01	0.02	
c	100	0.14	98	0.22	0.22	100	0	99	0.19	98	0.37	100	0.15	0.01	100	0.02	0.01
d	100	0.18	97	0.19	0.23	101	0.03	101	0.23	84	0.27	50	0.10	0	101	0.02	0
e	78	0.13	78	0.21	0.16	78	0	78	0.24	78	0.35	78	0.12	0.01	78	0.01	0.02
f	28	0.18	28	0.29	0.30	28	0	27	0.28	23	0.33	28	0.14	0.02	28	0	0
4a	132	0.22	74	0.22	0.28	131	0.01	123	0.22	0	0			133	0.02	0.00	
b	64	0.17	58	0.16	0.33	64	0.02	61	0.29	0		0		64	0	0	
c	244	0.19	31	0.23	0.26	220	0.01	236	0.26	0		0		235	0.01	0.00	
d	98	0.22	98	0.18	0.27	98	0	98	0.24	0		0		98	0	0	
e	104	0.21	103	0.16	0.26	102	0.01	104	0.25	0		0		104	0.00	0.00	
f	214	0.17	214	0.14	0.32	214	0.01	213	0.21	0		0		214	0.01	0	
g	67	0.13	67	0.19	0.28	67	0	67	0.26	51	0.25	67	0.09	0.02	67	0	0
h	99	0.31	98	0.14	0.22	100	0	99	0.18	100	0.25	90	0.11	0.03	100	0	0
i	0		118	0.20	0.27	119	0	119	0.19	103	0.25	119	0.11	0.03	119	0	0
j	80	0.17	80	0.19	0.23	80	0	80	0.21	78	0.35	80	0.14	0.03	80	0.02	0.01
k	703	0.19	763	0.22	0.27	90	0	753	0.19	0		90	0.09	0.02	90	0.03	0
l	0		80	0.15	0.35	80	0.01	80	0.21	79	0.25	80	0.12	0.05	80	0.03	0
m	0		90	0.20	0.31	90	0.02	90	0.23	0		90	0.07	0.01	90	0.00	0.04
5	50	0.15	50	0.22	0.27	50	0	48	0.22	45	0.29	48	0.07	0.01	50	0.01	0.01
10	100	0.27	100	0.22	0.27	100	0.02	100	0.23	100	0.34	99	0.11	0.02	100	0.02	0.00
11a	195	0.23	154	0.23	0.19	187	0	162	0.20	0		0		194	0.01	0	
b	79	0.25	57	0.19	0.25	76	0.01	62	0.15	0		0		79	0.01	0	
c	100	0.24	97	0.20	0.25	100	0	99	0.22	86	0.28	99	0.09	0.03	100	0.02	0.01