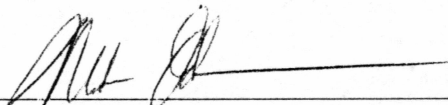# OUTBREEDING DEPRESSION IN HYBRIDS BETWEEN SPATIALLY

SEPARATED PINK SALMON (*ONCORHYNCHUS GORBUSCHA*) POPULATIONS:

MARINE SURVIVAL, HOMING ABILITY, AND VARIABILITY IN FAMILY SIZE

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OUTBREEDING DEPRESSION IN HYBRIDS BETWEEN SPATIALLY

SEPARATED PINK SALMON (*ONCORHYNCHUS GORBUSCHA*)

POPULATIONS: MARINE SURVIVAL, HOMING ABILITY,

AND VARIABILITY IN FAMILY SIZE

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

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Fairbanks, Alaska

May 2003

Abstract

Hybridization between distinct populations of salmon can cause fitness loss (outbreeding depression), and may result in reduced survival. The erosion of fitness-related traits such as homing ability and change in family size distribution may underlie reduced survival. Outbreeding depression was investigated in two independent experiments that made hybrids between geographically separated and genetically divergent pink salmon populations. Control crosses were made from male and female Auke Creek (Southeast Alaska) pink salmon and hybrid crosses were between Auke Creek females and Pillar Creek (Kodiak Island, about 1000km away) males. Parentage assignment from microsatellite analysis improved estimates of survival and straying, and was used to examine variation in family size. The return rates of even-broodyear F_1 control and hybrid fish were similar, but the odd-broodyear F_1 control returns exceeded hybrid returns. The F_2 control returns exceeded hybrid returns in both the even- and odd-broodyears. Hybridization did not impair homing ability; weekly surveys in nearby (~ 1km) Waydelich Creek revealed similar straying rates from Auke Creek by both hybrid and control fish in all years. Family data were available only for even-broodyear returns; hybridization did not increase the index of variability (ratio of variance to mean) in family size in these years. Outbreeding depression in hybrids of geographically separated populations demonstrates the potential for introgression of nonnative fish to erode natural production.

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Preface

The work in this thesis has been submitted and tentatively accepted for publication in a

dedicated issue of the journal *Environmental Biology of Fishes* (published by Kluwer

Academic Publishers) as part of the proceedings from the 20th Lowell Wakefield

Fisheries Symposium on Genetics of Subpolar Fish and Invertebrates. The authorship on

the paper is S.E. Gilk, I.A. Wang, C.H. Hoover, W.W. Smoker, S.G. Taylor, A.K. Gray,

and A.J. Gharrett. I conducted all the microsatellite and data analysis, nursed one

generation of pink salmon, directed recovery efforts for one generation of fish, and wrote

the manuscript. A.J. Gharrett and W.W. Smoker designed and set up the experiments.

I.A. Wang maintained the first three generations of fish, oversaw clipping in these years,

and directed recoveries of returning fish for the first returns. C.H. Hoover facilitated

collections of returning fish in the final returns, and will evaluate family size in the much

larger odd-broodyear returns in her thesis. S.G. Taylor is manager of the Auke Creek

weir and hatchery and is responsible for capturing the returning fish at the weir. A.K.

Gray provided laboratory guidance.

This thesis would not have been possible without the help of M. Adkison, S. Hall,

Z. Li, J. Lum, A. Matala, E. Peterson, and the many others who assisted in seining

efforts, tissue collections, and breeding experiments. Many thanks to my committee

members for their guidance and support. Thanks also to my family and friends for their

continued patience and encouragement.

The U.S. National Marine Fisheries Service Auke Bay Fisheries Laboratory, the

Alaska Department of Fish and Game, and the University of Alaska Fairbanks cooperated

General Introduction

Experiments demonstrating the genetic impact of cultured fish on wild stocks are rare

(Campton 1995). One of the possible effects is reduced survival in hybrids between

cultured and wild stocks, an effect called outbreeding depression. Relatively few studies

of outbreeding depression have been made (i.e. Philipp & Claussen 1995, Gharrett &

Smoker 1991, Gharrett et al. 1999), and little has been done to evaluate the potential of

outbreeding depression in salmonids even though management practices may put

endemic populations at risk by introducing alien stocks.

This thesis is part of an ongoing outbreeding depression study of pink salmon

returning to Auke Creek, near Juneau, Alaska that will eventually contribute to the theses

of several graduate students. A hatchery and permanent weir located just above tidewater

aids in the collection of donors and experimental fish, as well as in monitoring upstream

and downstream migrations. Previous studies conducted at Auke Creek demonstrated a

genetic component in emigration timing of pink salmon (Lane et al. 1990, McGregor et

al. 1998, Smoker et al. 1998), and that outbreeding depression occurs in hybrids between

odd- and even-broodyear pink salmon. Gharrett & Smoker (1991) observed that F_1

hybrid and control survival rates were similar but that there appeared to be a decrease in

survival and increase in bilateral asymmetry in F_2 hybrids (although no controls were

available). When these experiments were repeated to include controls, Gharrett et al.

(1999) observed significantly higher returns in F_2 controls than in F_2 hybrids, but no

differences in bilateral asymmetry. Since these hybrids did not represent crosses that

might occur naturally or as a result of management practices, experiments were initiated

in 1996 to examine the outbreeding depressive effects of hybridization of spatially separated pink salmon from Auke Creek and Pillar Creek, Kodiak Island (great circle distance 1048 km). If outbreeding depression is detectable in populations as genetically disparate as even- and odd-brood year pink salmon, subsequent experiments involving less distant populations within the same broodyear will provide insight into the extent of genetic divergence necessary to produce outbreeding depression.

Observations of survival are the most direct way to quantify outbreeding depression; however, fitness-related traits such as homing ability and changes in the distribution of family size may underlie a decrease in survival. Therefore, the experiment included the identification of families from which inbreeding coefficients can be determined and from which the distributions of family size could be described. In this thesis, survivals of both the first and second generation returns of even- and odd-broodyear experiments are reported. In addition, analysis of straying and inbreeding depression are presented. The analysis of family size is restricted to the even-broodyear; C. Hoover is examining the larger returns of the odd-broodyear.

Outbreeding depression in hybrids between spatially separated pink salmon

(*Oncorhynchus gorbuscha*) populations: Marine survival, homing ability, and variability

in family size[1]

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Synopsis

Hybridization between distinct populations and introgression of nonnative genes can erode fitness of native populations through outbreeding depression, either by producing a phenotype intermediate to that of both contributing genomes (and maladapted in either population's environment) or by disrupting distinct coadapted complexes of epistatic genes. In salmon, fitness-related traits such as homing ability or family-size distribution may be eroded. We investigated geographically separated pink salmon populations in repeated trials in independent broodyears (odd and even). Hybrids were made between female Auke Creek (Southeast Alaska) pink salmon and Pillar Creek (Kodiak Island, ~ 1000 km away) males; hybrids and their offspring were compared to offspring of control crosses of the same females with Auke Creek males. Parentage assignment from microsatellite analysis was used to improve estimates of survival and straying and to examine variation of family size. Hybridization reduced return rates of adults (a proxy for survival at sea) in the F_1 generation in the odd-year broodline ($p < 0.0001$) but not in the even-year broodline ($p = 0.678$). Hybridization reduced survival in both the odd- and even-broodyear F_2 ($p < 0.005$ and $p < 0.0001$). Hybridization did not appear to impair homing ability; weekly surveys revealed similar straying rates (~ 2%) by both hybrid and control fish into nearby (~ 1 km) Waydelich Creek in both generations in both trials. Hybridization did not increase the index of variability (σ^2/μ) in family size. Decreased survival in the hybrid F_2 generation supports an epistatic model of outbreeding depression; nonepistatic effects may have contributed to reduced survival in the odd-broodyear F_1 hybrid fish. Outbreeding depression in hybrids of geographically separated

populations demonstrates that introgression of nonnative fish can erode fitness, and should be recognized as a potential detriment of both aquaculture and management practices.

Introduction

Pacific salmon (*Oncorhynchus* spp.) have been transplanted between zoogeographic regions and between watersheds within regions and have been artificially produced and released in large numbers for over a century, generally for harvest enhancement and species restoration or mitigation for lost habitat (reviewed by Lichatowich 1999). Pacific salmon generally home to natal sites for spawning, thereby promoting reproductive isolation and exposing populations to diversifying selective pressures (Carvalho 1993). Releases of cultured or transplanted fish into environments inhabited by indigenous salmonid populations remove natural barriers to gene flow between populations. Recurrent introgression from transplanted fish or a hatchery stock derived by transplantation may alter the structure of locally-evolved genomes, thereby reducing the fitness of the local populations (Hindar et al. 1991, Campton 1995). Although introductions of hatchery-raised fish may aid in restoration of populations at risk of extinction or may increase the productivity of populations in degraded habitat, hybridization between salmon populations adapted to different local environments can also reduce the average fitness of wild salmon populations, known as outbreeding depression (Shields 1982, Hard 1995).

Outbreeding depression can result from an additive combination of average genetic values in the parental populations, disruption of an epistatic interaction among alleles at different loci, or both. A fitness loss expected from non-epistatic effects would occur in the first (F_1) and subsequent generations. Examples include intermediate resistance to *Ceratomyxa shasta* in hybrids between resistant and nonresistant coho salmon (*O. kisutch*) stocks (Hemmingson et al. 1986) and reduced tolerance to DDT in F_1 mosquitoes (King 1955).

Reproductively isolated populations may also evolve different genomes comprising coadapted, epistatic genes (Wallace 1981, Lynch 1991). Hybridization would disrupt the coadapted genomes (Dobzhansky 1950), but disruption may not appear in F_1 hybrids because each offspring possesses a complete coadapted genome from each parent. In fact, hybrid individuals may demonstrate heterosis (hybrid vigor; Emlen 1991), but reduction in fitness would be expected in the second (F_2) and later generations (Dobzhansky 1950). Outbreeding depression from a disrupted coadapted genome has been suggested in several species (e.g., Dobzhansky 1948, 1950, Brncic 1954, Gordon & Gordon 1957, Leberg 1993, Philipp & Claussen 1995), including salmonids (Gharrett & Smoker 1991, Danzmann et al. 1999, Gharrett et al. 1999).

Outbreeding depression may be directly observed as reduced survival. It may also be observed in the degradation of individual traits related to fitness such as impaired homing ability or of population traits such as a change in the distribution of family size distribution. Homing ability has been suggested as an important adaptive trait that responds to local selective pressures such as stream stability (Quinn 1993). Obvious

changes in homing ability may reflect changes in population fitness. In a previous transplantation and hybridization study on pink salmon, both hybrids and transplants had similar survivals; however, many fewer transplants than hybrid native/transplanted salmon negotiated their way to the release site, suggesting that terminal homing may have a genetic basis (Bams 1976). However, transplantation/hybridization studies have not followed hybrids into the F_2 generation where outbreeding depression would be expected (reviewed by Quinn 1993).

Changes in family size distribution may also reflect fitness loss. Genetic differences between families may result in different responses (survival) to variable marine conditions. Consequently, some families of a particular generation are better adapted to particular environmental conditions, but the most favored genotypes may change from generation to generation (Geiger et al. 1997) as conditions fluctuate. Hybridization might reduce the number of contributing families such that a small number of parents contribute disproportionately to subsequent generations. Coupled with genetic drift, this may increase inbreeding and decrease the effective population size. A smaller effective population size would tend to reduce genetic variability and most likely the average fitness of a population (Falconer & Mackay 1996) and could exacerbate outbreeding depression due to stochastic losses of essential alleles (Nevo et al. 1986, Emlen 1991). Observations of nonrandom variation of marine survival between families of Pacific salmon populations (Simon et al. 1986, Geiger et al. 1997, Hard et al. 2000) underscore the importance to population fitness of maintaining genetic variation in a population.

Molecular markers make it possible to reconstruct pedigrees. The parental sources of returning fish can be confirmed, and family size distributions can be determined from these data. Polymorphic microsatellite loci provide information on parent-offspring relationships and have been used to study parentage in many species, including fishes (e.g. Estoup et al. 1998, Waldbieser & Wolters 1999).

Previous work at Auke Creek studied the survivals of hybrids between odd- and even-broodyear pink salmon. Gharrett & Smoker (1991) observed that F_1 hybrid and control survival rates were similar, and observations of F_2 hybrids suggested a decrease in survival, although controls were not possible. When these experiments were repeated to include controls, returns of F_2 controls exceeded returns of F_2 hybrids (Gharrett et al. 1999). Hybrids between broodyears do not represent crosses that are likely to occur naturally or as the result of management practices; therefore, this study was begun in 1996 to examine the outbreeding depressive effects of hybridization of spatially separated pink salmon from the same broodyear.

Our objective was to examine the effects of hybridization between pink salmon from widely separated populations. To accomplish this objective, hybrids were produced between pink salmon from Auke Creek near Juneau, Alaska and Pillar Creek on Kodiak Island and controls that had only Auke Creek parents; marine survivals were observed in both F_1 and F_2 generations. The effects of hybridization on two fitness related traits, homing ability and the distribution of family size, are examined here. Parentage determined from microsatellite inheritance was used to improve estimates of survival and straying and to examine family size distributions. Evidence of outbreeding depressive

effects on survival suggests additive effects on one of the F_1 hybrids and epistatic effects in both F_2 hybrids. There was no evidence of an effect on either homing ability or family size distribution, which suggests that these mechanisms may not be important contributors to decreased survival.

Materials and methods

Crosses and incubation procedures

The experiments evaluated hybrids between pink salmon from two Alaskan populations: Auke Creek near Juneau, Alaska (58°23'N, 134°37'W) and Pillar Creek on Kodiak Island (57°47'N, 152°28'W). Auke Creek, a lake-fed stream of moderate grade, is about 350m long and has spawning populations that vary between 2,000 and 20,000[2]. Pillar Creek is a reservoir-fed stream of shallow gradient, runs about 1800m, and has spawning populations that vary between 1,000 and 40,000[3]. The distance between these streams (great circle distance 1048 km; the continental shelf distance is considerably greater) prevents direct gene flow between the populations, even though they are at the same latitude (Appendix A).

The first generation hybrids were made at Auke Creek in 1996 and 1997 in an experiment that modeled Auke Creek as the recipient of a transplantation and Pillar Creek as the donor population. The mating scheme, a blocked incomplete-factorial

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[2] Taylor, S.G. & J.L. Lum. 2002. Annual Report Auke Creek Weir 2001: Operations, Fish Counts, and Historical Summaries. Unpublished Report 37 p. National Marine Fisheries Service. Auke Bay Laboratory, 11305 Glacier Highway, Juneau, Alaska 99801-8626.

[3] Alaska Department of Fish and Game, Kodiak. 2000. Stream survey data of Pillar Creek, Kodiak, AK from 1968 to 2000. Unpublished data.

design (Appendix B), was intended for quantitative genetic analyses not reported here. Eggs of each of 40 Auke Creek females were divided between hybrid and control lines. Two Auke Creek males and two Pillar Creek males were crossed with each of two females (hereafter referred to as a "block") to make control and hybrid crosses. Twenty different blocks were produced by mating 40 Auke Creek males, 40 Auke Creek females, and 40 Pillar Creek males.

Each family was subdivided into two portions that were randomly assigned to incubation cells in vertical FAL^{TM} incubators partitioned with dividers (MariSource, Milton, WA). Hybrid families were incubated in different cabinets than control families; however, high flow from a common water source maintained very similar environmental conditions in the two incubators; temperature records of each incubator document virtually identical temperature regimes. Embryos were reared until about 5% of their yolk remained, when they were differentially marked by mirror image pelvic fin excisions and an experiment-identifying adipose fin excision. The polarity of the marks was alternated between hybrid and control groups over the years of the experiment. About 20,000 hybrid and 20,000 control fish were released each spring, and equal numbers of each family were released when possible. The fry were released at the peak of emigration of the wild fish in Auke Creek.

Returning F_1 individuals were bred in 1998 and 1999. To produce each of 20 blocks of F_2 control and 20 blocks of hybrid progeny, two control males were crossed with each of two control females, and two hybrid males were crossed with each of two hybrid females. The control blocks included 40 F_1 Auke Creek males and 40 F_1 Auke

Creek females; the hybrid blocks had 40 F_1 hybrid males and 40 F_1 hybrid females. About 20,000 F_2 hybrid and 20,000 F_2 control fish were differentially clipped and released at the peak of the emigration of the wild fish in Auke Creek in mid-April of 1999 and 2000.

Survival and straying in even- and odd-broodyears

Returning F_1 and F_2 adults were recovered in August and September 1998-2001 at a permanent weir located just above the high tideline at Auke Creek. During the return, intertidal Auke Creek and all of nearby Waydelich Creek were surveyed weekly with beach seines and dipnets during the spawning period in order to collect straying experimental fish. Survival was defined as return to the weir or recovery in a survey. Fish recovered at the weir were held in pens until they could be used for crosses or processed for data. The carcass of each returning fish was tagged, its fin mark (designating hybrid or control) and sex noted, and heart or muscle tissue samples were taken for genetic analysis. The equality of rates of survival and straying between groups was tested using log-likelihood ratios, (*G*-tests; Sokal & Rohlf 1995). Parentage information from microsatellite analysis was used to improve estimates of survival and straying made from observation of marks by confirming the parental origin (control or hybrid) of returning fish, including those that had indistinct fin excisions.

Microsatellite and parentage analysis

Tissues taken for DNA analysis were placed in preservative (Seutin et al. 1991) and

stored at -20°C prior to DNA extraction. Total DNA was isolated with Puregene DNA™

isolation kits (Gentra Systems, Inc., Minneapolis, MN). Polymerase chain reaction

(PCR) amplification was done in 96-well microtitre plates in a Stratagene 96 Robocycler.

Reactions were performed in 10 µL volumes [10 mM Tris-HCl at pH 8.3, 50 mM KCl,

25 mM $MgCl_2$, 2.5 mM each deoxyribonucleotide triphosphate (dNTP), 0.5 units *Taq*

polymerase, 0.1-0.5 µM each primer, and 50-100 ng DNA template] overlaid with

mineral oil. In addition to unlabeled forward and reverse primers for each locus, each

mixture included a forward primer labeled with an infrared-sensitive dye, IRDye™ (LI-

COR, Inc., Lincoln, NE). After evaluating several microsatellite loci, six loci were

chosen (Appendix C): *Ssa*197 (O'Reilly et al. 1996), *Ots*1 (Banks et al. 1999), *Ots*103

(Small et al. 1998), *Ogo*1a (Olsen et al. 1998), and *Oki*10 and *Oki*11 (Smith et al. 1998).

In general, PCR conditions were as follows: 1 cycle at 95°C for 3 minutes; 30 cycles at

95°C for 1 minute, x°C for 1 minute, and 72°C for 1 minute; and finally 1 cycle at 72°C

for 5 minutes; where x is the annealing temperature (52°C for *Ssa*197, 49°C for *Ots*1,

47°C for *Ots*103, 59°C for *Ogo*1a, 55°C for *Oki*10, and 53°C for *Oki*11). After

amplification, DNA products were denatured by adding an equal volume of stop buffer

(95% formamide, 0.1% Bromophenol Blue) and heating for 3 minutes at 95°C. Finally,

1-1.5µL of PCR product was loaded onto polyacrylamide denaturing gels composed of

6% PAGE-PLUS™ 40% Concentrate (AMRESCO, Inc., Solon, OH), 7M urea, and 5X

TBE (TBE is 90mM tris-boric acid and 2mM EDTA, pH 7.5) in a reaction catalyzed by

ammonium persulfate and TEMED (N,N,N',N' tetramethylethylenediamine).

Electrophoresis and detection were performed on a LI-COR automated sequencer

(LongReadIR 4200^{TM}, LI-COR, Inc., Lincoln, NE) in 1X TBE running buffer, with

running parameters 31.5 W, 1500 V, 35 mA, and 50°C plate temperature. GeneImagIR

RFLPscan software (Version 3.52, Scanalytics, LI-COR, Inc., Lincoln, NE) was used to

estimate the sizes of microsatellite alleles. Allele sizes were determined by comparing

allele band patterns with IRD700^{TM} or IRD800^{TM} standard ladders (LI-COR, Inc.,

Lincoln, NE) and standardized using reference alleles.

Allele frequencies were estimated for the Auke and Pillar Creek populations for

both even- and odd-broodline crosses. GenePop version 3.2a (Raymond & Rousset

1995) was used to test conformance to Hardy-Weinberg expectation using the following

parameters: 10,000 dememorization steps, 1000 batches, and 1000 iterations per batch.

Excess homozygosity can be an indication of null alleles, which complicate parentage

analysis.

To demonstrate genetic differences between the Auke and Pillar Creek

populations, allele frequencies were estimated for both the even- and odd-broodline

parents. Homogeneity of allele frequencies between populations at all six loci was tested

using a G-test for which the probability levels were estimated by 20,000 iterations in a

Monte-Carlo simulation (e.g. Roff & Bentzen 1989). Probabilities were combined to

examine overall heterogeneity for each broodyear using Fisher's method (Winer 1971).

Parentage of each returning fish was deduced using PROBMAX with default

tolerance levels and allowing mis-scored loci (Version 1.2, Danzmann 1997). Parentage

information was used to confirm parental origins of well-marked fish, to determine parental origins (control, hybrid, or not experimental) of fish returning with indistinct marks, and to estimate family size of the even-broodyear returns. The parentage information also made it possible to estimate inbreeding coefficients (F; defined as the probability that alleles at a locus in an individual are identical by descent) in each segment of the experiment to determine if inbreeding had been imposed as a result of the limited sample of parents in the F_2 crosses (Falconer & Mackay 1996). The inbreeding coefficient was calculated assuming that $1/2N$ is the probability that uniting gametes have identical genes.

Variance in family size of even-broodyears

The underlying model for the estimation of effective population size is that the family size is approximately distributed as a Poisson. An increase in variance in a population (for a given mean) reduces the effective population size (Crow & Kimura 1970), a possible consequence of outbreeding depression that could occur if only a few families produced most of the offspring.

The observed family sizes in the even-broodyear F_1 and F_2 hybrid and control returns were compared to expected Poisson distributions of average family size with a goodness of fit *G*-test (Sokal & Rohlf 1995). Then, to avoid effects of correlations between families, the data blocks were randomly reduced to single full-sib families and bootstrapped. The bootstrap analysis randomly reduced the data so that each female in the analysis was assigned to a single control male and a single hybrid male.

Consequently, the program randomly chose one control male and one hybrid male for the first female in each block and assigned the other males to the second female in the block. These bootstrap values were used to compare the means and variances of the distribution of family sizes of F_1 and F_2 returns, and to estimate the index of variability in family size. The index of variability contrasts the variance and mean in family size (σ^2/μ), and tends to 1 under random survival (Crow & Morton 1955), which is the Poisson expectation. A larger index of variability in hybrids than controls may indicate that increased variability in family size contributed to a reduction in survival.

Results

Microsatellite variation in Auke and Pillar creek, and parentage analysis

Homogeneity tests showed significant divergence between 40 Pillar Creek pink salmon and 80 Auke Creek pink salmon used for parentage in each F_1 experiment (Appendix D). Two loci in the even-broodyear (1996) samples showed significant heterogeneity between the populations (*Ogo*1a, $p < 0.001$; *Oki*11, $p < 0.001$) and four loci in the odd-broodyear (1997) samples showed significant heterogeneity (*Oki*10, $p < 0.0001$; *Ogo*1a, $p < 0.01$; *Ots*103, $p < 0.01$; *Oki*11, $p < 0.01$). The probabilities combined across loci demonstrated overall heterogeneity between the Auke Creek and Pillar Creek pink salmon populations used in breeding experiments in both the even- and odd-broodyears (both $p < 0.0001$). Homogeneity tests based on genotype frequencies had similar results.

Not all loci conformed to expected Hardy-Weinberg frequencies. Tests of Hardy-Weinberg suggested the presence of null alleles at two loci: *Ssa*197 and *Ots*103.

Pedigree information from progeny was used to reconstruct *Ssa*197 genotypes and identify null alleles. There was insufficient pedigree information available to reconstruct all of the *Ots*103 genotypes. However, the high variability at most of the loci made all of the loci useful in contributing to parentage analyses. (See Appendix E for allele frequencies.)

Microsatellite markers were used to determine parentage of all even-broodyear fish, the odd-broodyear fish used for breeding experiments, and the returning odd-broodyear fish that had indistinct marks. Of 355 fish carrying indistinct fin marks, microsatellites revealed the parentage of 327 fish. The remaining 28 fish to which parents could not be assigned were included in hybrid counts to provide conservative hybrid survival estimates. Of the 1266 individual progeny with distinct fin excisions that were genotyped, 59 did not correspond to combinations of parents that were recorded when the matings were conducted; these fish were excluded from subsequent analyses. The seven fish with undetermined parentage that contributed to the odd-broodyear F_1 broodstock probably resulted from misreading fin marks when matings were conducted. These seven fish and their progeny were not included in estimates of the inbreeding coefficient of odd-broodyear fish. In both the even- and odd-broodyear F_1 breeding experiments, one individual was bred as a hybrid even though subsequent microsatellite genotyping indicated it was a control fish. Blocks including these eight fish were removed from analyses.

Survival and straying in even- and odd-broodyears

The F_1 generation fish from even-broodyear experiments returned to Auke Creek in 1998 (Table 1); returns (survival rates of fry to adulthood) of F_1 control (1.43%) and hybrid (1.41%) fish were nearly identical. F_1 odd-broodyear fish returned to Auke Creek in 1999; the F_1 control return (5.14%) exceeded ($p < 0.0001$) that of F_1 hybrid fish (3.67%). In 2000, returns of even-broodyear F_2 fish (0.88%) were higher ($p < 0.0001$) than F_2 hybrid returns (0.54%). Similarly, odd-broodyear F_2 control returns (1.27%) exceeded F_2 hybrid returns (0.96%) in 2001 ($p < 0.005$).

Survival rates[4] of cultured fish in these experiments were lower than those of wild fish returning to Auke Creek in all years. In the even-broodyears, returns of wild fish (7.29% and 4.07%, respectively) exceeded those of cultured fish. In odd-broodyears, survival of wild fish (46.3% and 5.95%, respectively) was also higher than that of cultured fish.

Weekly recovery efforts in nearby (about 1 km) Waydelich Creek revealed similar straying rates (about 2% or less) of both hybrid and control fish in all years (Table 2). Although the straying rates of hybrid fish were higher than those of the control fish, the straying rates were too low to detect statistical differences. The levels of straying do not appear to compensate for the much larger differences in return rate.

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[4] Survival rates were estimated from counts of fry passing the weir in spring and adults passing the weir in summer of the following year. Data can be found in: Taylor, S.G. & J.L. Lum. 2002. Annual Report Auke Creek Weir 2001: Operations, Fish Counts, and Historical Summaries. Unpublished Report 37 p. National Marine Fisheries Service. Auke Bay Laboratory, 11305 Glacier Highway, Juneau, Alaska 99801-8626.

Inbreeding in even- and odd-broodyears

Inbreeding in the hybrid F_2 generations did not result from the choice of parents in either the even- or odd-broodyear F_1 generations. The even-broodline F_2 inbreeding coefficient for controls (F = 0.034) exceeded that of F_2 hybrids (F = 0.003). Similarly, in the odd-broodline, the inbreeding coefficient for F_2 controls (F = 0.013) was higher than that of F_2 hybrids (F = 0.006). Both hybrid broodlines had low inbreeding coefficients that were similar to those expected theoretically (F = 0.003).

Variance in family size of even-broodyears

Parentage data of the F_2 generation were available only for the even-broodyear returns. Distributions of the F_1 control and hybrid offspring were not Poisson (Figure 1; p < 0.001 and p < 0.01 respectively, *G*-test). Removing an outlier in the F_1 control group did not alter the results. The distribution of F_2 control family size was not Poisson (p < 0.001). However, the distribution observed for the F_2 hybrid family size was weakly consistent with a Poisson distribution (p = 0.051).

The index of variability did not increase in either the controls (2.91 and 1.78 for the F_1 and F_2 generations, respectively) or the hybrids (1.76 and 1.30 for the F_1 and F_2 generations, respectively). In addition, the index of variability in the F_2 hybrids did not exceed that of controls in either the F_1 or F_2 generation. Removing an outlier in the F_1 control group did not change these results.

Discussion

Microsatellite variability in Auke and Pillar creek, and parentage analysis

Outbreeding depression is most likely to occur in hybrids of geographically separated

populations if there is no direct gene flow and they have evolved separately for a

considerable time. By choosing populations from approximately the same latitude, we

attempted to match environments, though the match was not perfect. These two

populations were expected to have distinct coadapted gene complexes, and the

microsatellite analyses confirmed that the Auke Creek and Pillar Creek pink salmon

populations differ genetically. These differences are consistent with previous

observations that populations of pink salmon diverge over large geographic distances

(e.g., Gharrett et al. 1988, Noll et al. 2001, Hawkins et al. 2002).

Survival and Straying in even- and odd-broodyears

A reduction in survival is the most direct indication of outbreeding depressive effects of

hybridization. By examining the returns in both the F_1 and F_2 generations, it is possible

to distinguish between epistatic or non-epistatic effects on outbreeding depression. The

return rates for hybrids and controls were similar for the even-broodyear F_1 fish,

consistent with the survival reported for F_1 hybrids between even- and odd-broodyear

pink salmon (Gharrett & Smoker 1991, Gharrett et al. 1999). In a somewhat similar

experiment, Bams (1976) released first-generation hybrid and transplanted (from the

Kakweieken River in B.C., Canada) pink salmon fry from Tsolum River/Headquarters

Creek, about 146 km away; no control release was possible. Both the hybrids and

transplants had similar survivals, as measured by recoveries in the commercial fishery. However, many fewer transplants than hybrid native/transplanted salmon negotiated their way about 24 km up the Tsolum River to the release site, suggesting that terminal homing may have a genetic basis.

In contrast to the even-broodyear, the return rate of F_1 control fish exceeded that of the hybrids in the odd-broodyear. This is the first of the Auke Creek pink salmon outbreeding depression experiments that demonstrated a reduced survival in F_1 hybrids and suggests that non-epistatic effects can contribute to overall survival. For the crosses reported here, developmental characters were also examined (Wang[5], in preparation). In those studies, timing of mid-hatch was influenced by non-epistatic effects in the first generation. The idea that different genetic mechanisms may have evolved in the two broodyears is consistent with large genetic differences observed between odd- and even-broodlines of pink salmon (e.g. Aspinwall 1974, McGregor 1982). In fact, little similarity in the distribution of mtDNA variable sites was observed between the even- and odd-broodlines of pink salmon (Churikov et al. 2001), which may reflect differences in postglacial recolonizations of the two broodyears (Churikov & Gharrett 2002).

Both the even- and odd-broodline F_2 hybrids between Pillar Creek and Auke Creek pink salmon had reduced survivals relative to control fish, suggesting that outbreeding depression results from the disruption of coadapted, epistatically-acting gene complexes. These results are consistent with the reduced survival of F_2 pink salmon

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[5] Wang, I.A., W.W. Smoker & A.J. Gharrett. Evidence of outbreeding depression through differences in development rates of pink salmon hybrids. Proceedings of the Northeast Pacific Pink and Chum Salmon Workshop, March 3-5, 1999, Juneau, Alaska. National Marine Fisheries Service, 11305 Glacier Hwy., Juneau, AK 99802. Cited by permission.

hybrids between even- and odd-broodyear pink salmon at Auke Creek (Gharrett & Smoker 1991, Gharrett et al. 1999).

Other factors that could have influenced these results include interannual variation and incomplete control over culture conditions. Natural populations experience year-to-year environmental variation. These experiments sampled only two environmental sequences and it is possible that the environmental conditions sampled in our experiments fortuitously favored one group over the other. Fluctuating environmental differences are difficult to assess because only one set of environmental experiences can be observed at a time. In addition, it is possible that subtle incubation differences or marking effects may have influenced the differences in survival. It is unlikely, however, that either of these factors produced the primary effects observed as outbreeding depression because every pink salmon hybridization experiment performed at Auke Creek has yielded similar results (Gharrett & Smoker 1991, Gharrett et al. 1999).

The differences observed in the survivals of wild and cultured fish are not uncommon (e.g. Miller 1953, Bachman 1984). The reasons for the differences may include the effects of fin excisions, the contribution of uncounted fry derived from intertidal spawning to returns of wild salmon, or delayed mortality in cultured fish resulting from culture conditions.

The small number of experimental fish recovered at Waydelich Creek suggests that, at least in some instances, the straying rate of pink salmon is low. The number of hybrid strays is higher than the number of control strays, but the total number of strays each year was less than 5 and is not statistically significant. Local straying does not

account for survival differences. The straying rates observed in experimental fish were all less than a point estimate (2.8%) of straying of native Auke Creek fish within 10km made from observations of otolith-marked fish[6], but were similar to estimates (less than 1%) made using a genetic marker (Gharrett et al. 2001).

Failure to observe differences in straying rates of F_1 hybrids and controls is consistent with Smoker & Thrower (1995), who did not observe increased straying for either transplanted or hybrid chum salmon compared to native fish. However, in a transplantation study (Bams 1976), more F_1 pink salmon hybrids between native and transplanted fish were able to navigate 24km up the natal river to the release site than transplanted fish. It is difficult to compare these experiments because they used different kinds of crosses and evaluated homing ability differently. Moreover, neither Bams (1976) nor Smoker & Thrower (1995) followed the fish into the F_2 generation where outbreeding depression might be expected.

Inbreeding in even- and odd-broodyears

Because the broodstock for the F_2 generation derived from a limited number of parents, the possibility existed that a disproportionate number of full- or half-sib matings might have been included in the experiment, which would increase inbreeding. A loss of genetic diversity due to inbreeding can decrease fitness if essential alleles are lost and deleterious recessive alleles are expressed (Nevo et al 1986, Emlen 1991) and might

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[6] Mortensen, D., A. Wertheimer, J. Maselko & S. Taylor. 2000. Survival and straying of pink salmon using recoveries of coded-wire tags and thermally induced otolith marks. Feature article, AFSC Research Activities quarterly report for July-Aug-Sept 2000. Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA.

mask depressive effects due to outbreeding. The inbreeding coefficients for the F_2 experiments were of the same order of magnitude as would be expected from random breeding; and fortuitously, the hybrid broodstocks had slightly lower inbreeding coefficients than the control broodstocks. Inbreeding was not increased by our choice of broodstock for this study, and it is unlikely that the reduced survival in the F_2 hybrids resulted from inbreeding.

Variance in family size of even-broodyears

Observations of variability in family size and nonrandom marine survival in Pacific salmon populations (Simon et al. 1986, Geiger et al. 1997, Hard et al. 2000) suggest that certain combinations of genotypes resulting from epistatic relationships among genes may cause some individuals to have higher fitness in some environments, but these same combinations may not be favorable as conditions change. If, by chance, an F_2 family receives a favorable set of alleles and has a greater return, its offspring may contribute disproportionately to the return and subsequent production. If family size variability increases, the few successful families may not be the most fit in subsequent generations, thereby extending the effects of outbreeding depression to additional generations. In this experiment, more families completely failed and a few families produced more offspring than predicted by the Poisson frequency distribution for both hybrids and controls in the even-broodyear returns; in aggregate, the overall distribution was overdispersed. However, our results suggest that an increase in variability of family size does not account for the decrease in survival seen in the hybrid fish. The relatively small number

of F_2 returns in the even-broodyears of this study may have provided insufficient power to detect small differences in the variability of family size.

Conclusion

Hybrids between two spatially separated Pacific salmon populations exhibited reduced survival due to outbreeding depression, and evidence of both epistatic and non-epistatic effects was observed. It does not appear that a decreased local homing ability or increased variability in family size accounted for the decrease in hybrid survival. Whether due to non-epistatic or epistatic effects, the decrease in hybrid survival could jeopardize biodiversity of wild stocks if repeated introductions are made. Small, one-time introductions may be of minor concern because natural selection might be expected to remove maladapted genotypes rapidly from a population; however, repeated introductions or development of non-local hatchery brood stocks have the potential to decrease productivity in local wild streams. The possibility of outbreeding depression suggests that broodstocks should be developed from locally adapted natural populations and that salmon managers should consider the potentially detrimental effects of transplanting stocks from one hatchery to another.
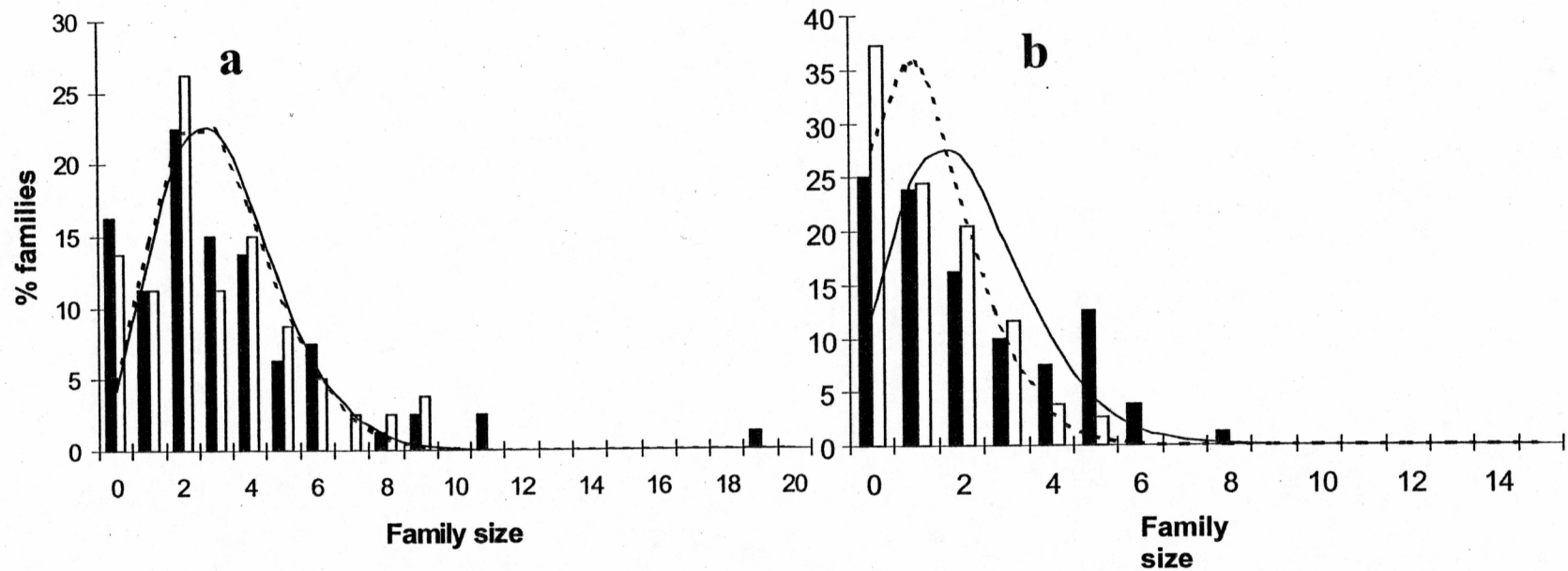
Figure 1. Individual family sizes for even-broodyear F_1 and F_2 pink salmon returns to Auke Creek. Vertical bars show the observed distribution of offspring; filled bars are control distributions and open bars are hybrid distributions. The curves show the Poisson distribution with the same mean as the observed family size; solid lines are control distributions and dashed lines are hybrid distributions. (a) 1998 F_1 returns. Neither the distribution of the control ($p < 0.001$) nor the hybrid ($p < 0.01$) samples fit a Poisson distribution (William's corrected *G*-test; Sokal and Rohlf 1995). (b) 2000 F_2 returns. The distribution of the control samples did not fit a Poisson distribution ($p < 0.0001$). The hybrid fit to a Poisson distribution was nearly significant ($p = 0.0505$)

Table 1. Number of fry and returning adults for F_1 and F_2 pink salmon controls (Auke Creek parentage) and hybrids (Auke Creek by Pillar Creek). If their origin could not be determined with microsatellite allele analysis, returning fish with indistinct marks were counted as hybrids. P-values for testing differences in return rates (survival at sea) between hybrids and controls are from log-likelihood ratio tests (Sokal and Rohlf 1995). Estimates of wild releases and returns (Auke Creek) are from annual counts of fry and adults[d].

Brood-year		Control			Hybrid			p	Wild		
		Fry released	Adult returns	% returns	Fry released	Adult returns	% returns	(G-test)	Fry released	Adult returns	% returns
1996	F_1	19912	284	1.43	20852	294[a]	1.41	0.6774	31092	2267	7.29
1997	F_1	19918	1023	5.14	19916	731[b]	3.67	<0.0001	60785	28128	46.3
1998	F_2	20050	176	0.88	20024	108	0.54	<0.0001	53535	2181	4.07
1999	F_2	20000	254	1.27	20000	192[c]	0.96	0.0033	132075	7859	5.95

[a]Includes 2 fish with indistinct marks.
[b]Includes 25 fish with indistinct marks.
[c]Includes 18 fish with indistinct marks.
[d]Taylor, S.G. & J.L. Lum. 2002. Annual report Auke creek weir 2001: Operations, fish counts, and historical summaries. Unpublished report 37 p. National Marine Fisheries Service. Auke Bay Laboratory, 11305 Glacier Highway, Juneau, Alaska 99801-8626.

Table 2. Number of adult pink salmon recovered at Waydelich Creek. If their origin could not be determined with microsatellite parentage analysis, returning fish with incomplete fin excisions were counted as hybrids.

Brood-year		Control			Hybrid		
		Total returns	Number captured	% captured	Total returns	Number captured	% Captured
1996	F_1	284	1	0.35	292	3	1.03
1997	F_1	1023	0	0	706	1	0.14
1998	F_2	172	1	0.58	104	2	1.92
1999	F_2	254	1	0.39	192	4[a]	2.08

[a]Includes 2 fish with indistinct marks.

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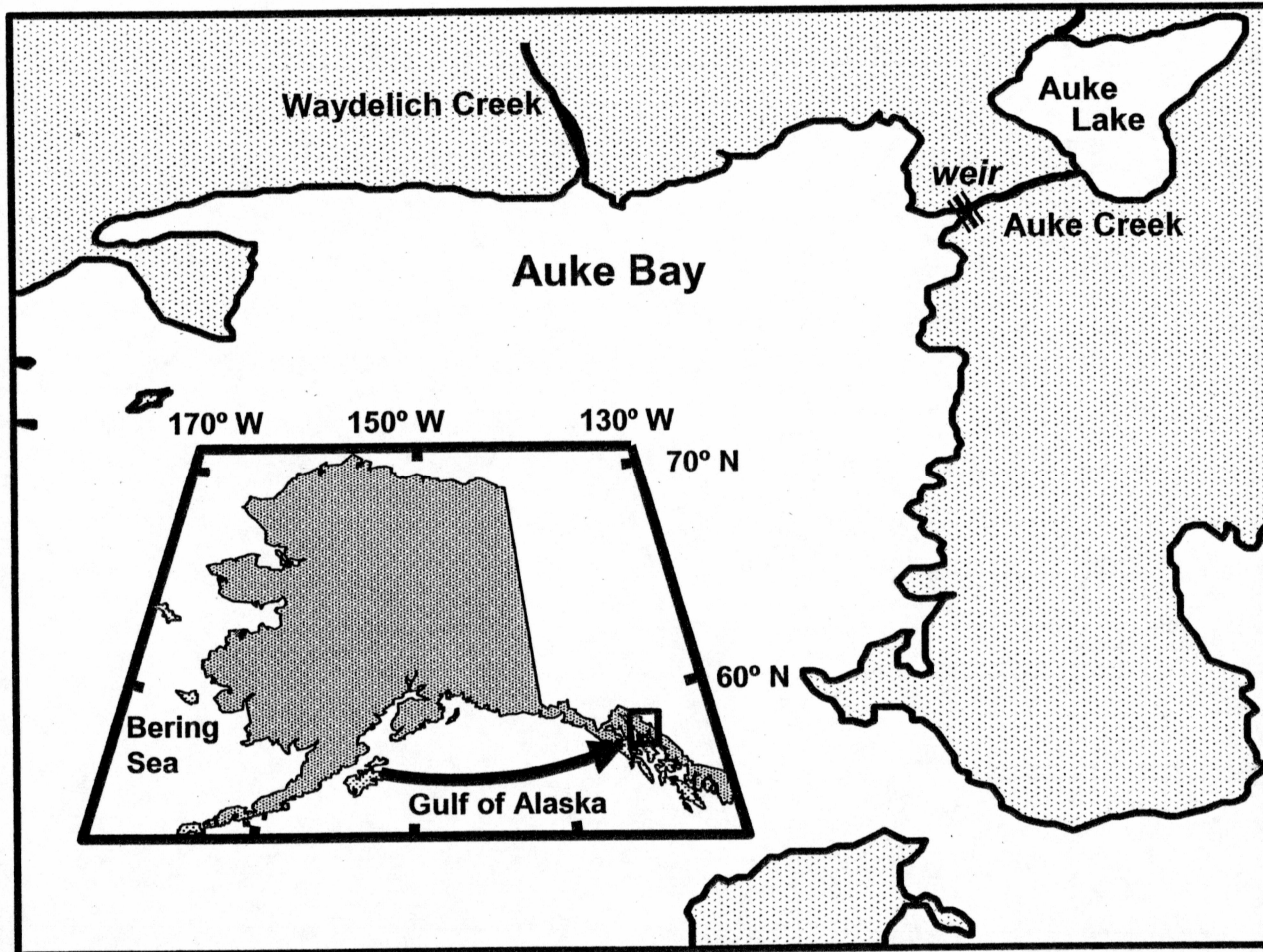
Figure A.1. Map of study area. Salmon returning to Auke Creek, near Juneau, Alaska were the model native population. Males from Pillar Creek, Kodiak, Alaska (great circle distance 1048 km away) were the model donor population. Nearby (about 1 km) Waydelich Creek was routinely seined to collect any possible strays.

Even-year broodline

Controls   **Hybrids**

1996 Auke Ck **X** 1996 Auke Ck **X** 1996 Pillar Ck   **Parental generation**

1998 controls → back crosses ← 1998 hybrids   **F₁ generation**

2000 controls → back crosses ← 2000 hybrids   **F₂ generation**

Odd-year broodline

Controls   **Hybrids**

1997 Auke Ck **X** 1997 Auke Ck **X** 1997 Pillar Ck

1999 controls → back crosses ← 1999 hybrids

2001 controls → back crosses ← 2001 hybrids

Figure B.1. Replicated incomplete-factorial mating design used for experiments. Though originally intended for other quantitative genetic analyses, the data was adequate to examine family size variability.

Table C.1. Microsatellite loci used for pink salmon parentage analysis. Samples are Pillar Creek and Auke Creek fish used for breeding experiments in 1996 and 1997.

Locus	Reference	Primer sequence (5'-3')	Size (bp)	No. of alleles	Sample size (N)
*Ogo*1a	Olsen et al. 1998	F: GATCTGGGCCTAAGGGAAAC	181- >400[a]	>84	500
		R: ACTAGCGGTTGGAGAACCC			
*Oki*10	Smith et al. 1998	F: GGAGTGCTGGACAGATTGG	135-325	52	502
		R: CAGCTTTTTACAAATCCTCCTG			
*Oki*11	Smith et al. 1998	F: TCTGAGACAGGCAAATGCAC	76-252	72	436
		R: GTTTTAAACCTCACCATTGAGT			
*Ots*1	Banks et al. 1999	F: GGAAAGAGCAGATGTTGTT	218-256	20	478
		R: TGAAGCAGCAGATAAAGCA			
*Ots*103	Small et al. 1998	F: AGGCTCTGGGTCCGTG	208-497	59	500
		R: GACATAGCGTTCAGCACAG			
*Ssa*197	O'Reilly et al. 1996	F: GGGTTGAGTAGGGAGGCTTG	116 -208	25	477
		R: TGGCAGGGATTTGACATAAC			

[a]Alleles over 400 bp were binned for ease of scoring.
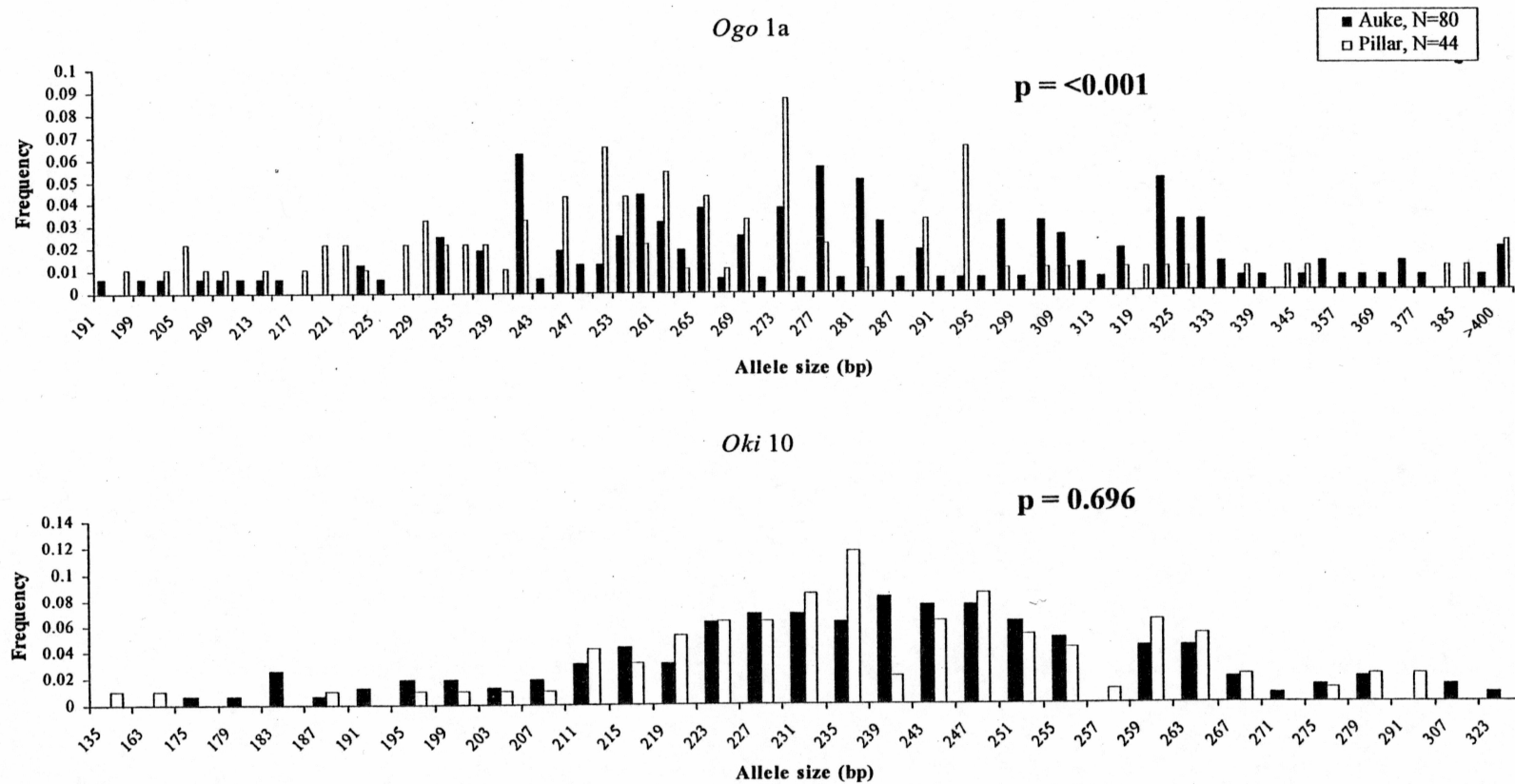
Figure D.1. Homogeneity of allele frequencies at the *Ogo*1a and *Oki*10 microsatellite loci between Auke Creek and Pillar Creek pink salmon used for breeding experiments in 1996. P-values were found using *G*-test statistics and a permutation test (20,000 iterations) similar to Roff & Bentzen (1989). N is the number of fish sampled. Homogeneity tests across all loci revealed significant ($p < 0.0001$) divergence between Auke and Pillar Creek fish used in 1996.
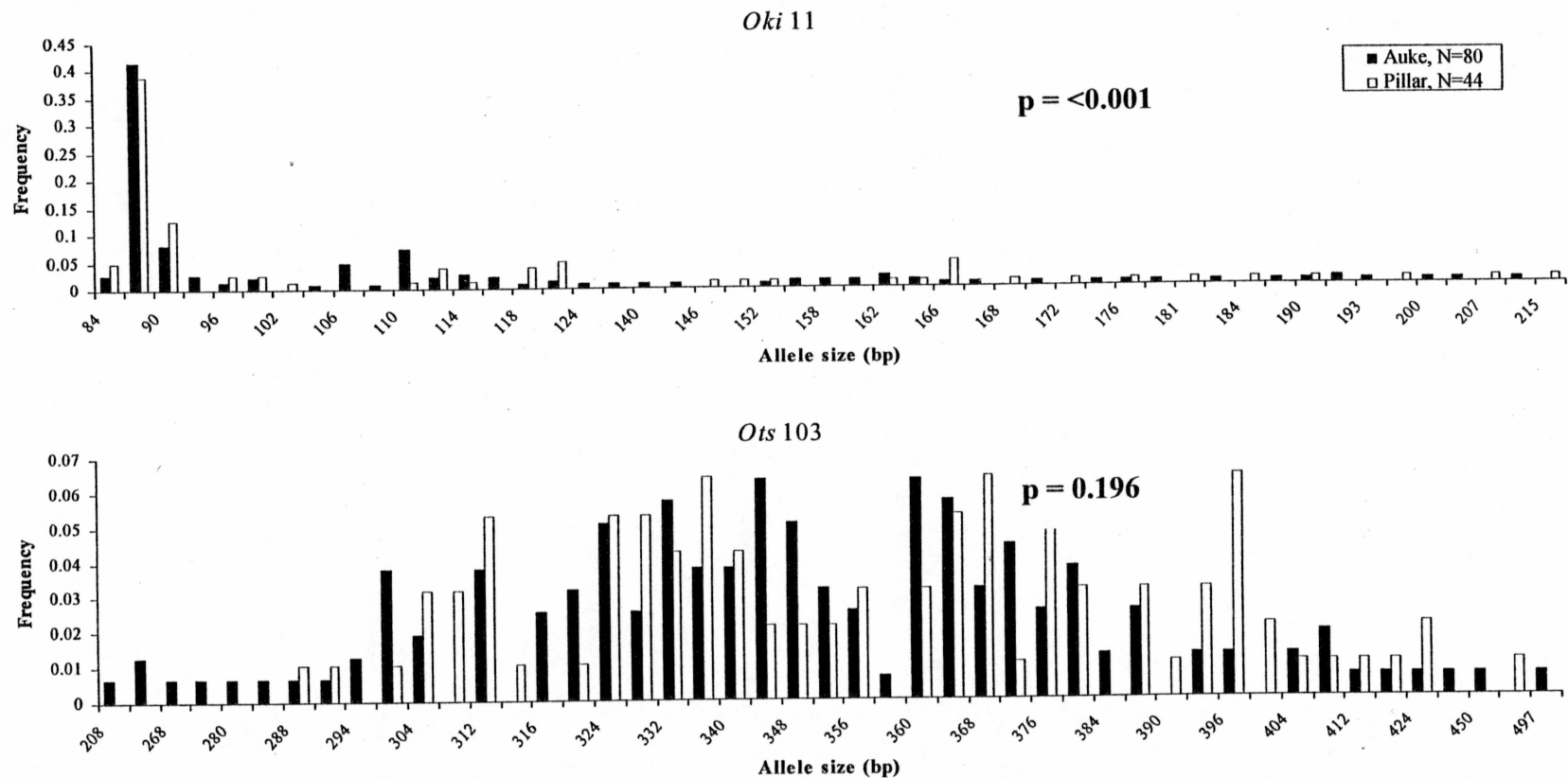
Figure D.2. Homogeneity of allele frequencies at the *Oki*11 and *Ots*103 microsatellite loci between Auke Creek and Pillar Creek pink salmon used for breeding experiments in 1996. P-values were found using *G*-test statistics and a permutation test (20,000 iterations) similar to Roff & Bentzen (1989). N is the number of fish sampled. Homogeneity tests across all loci revealed significant (p < 0.0001) divergence between Auke and Pillar Creek fish used in 1996.
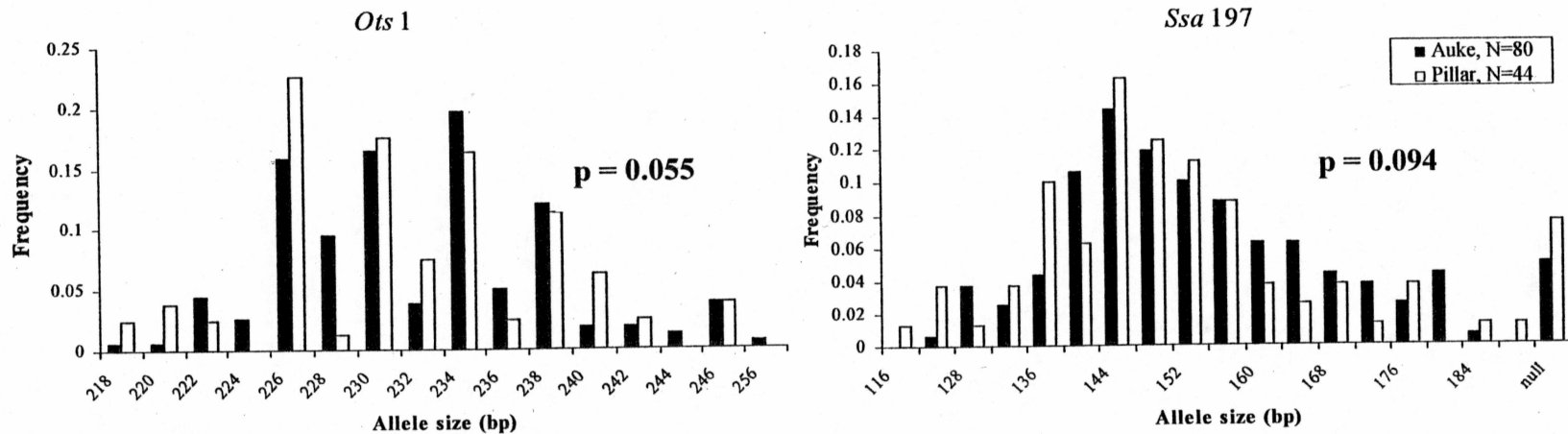
Figure D.3. Homogeneity of allele frequencies at the *Ots*1 and *Ssa*197 microsatellite loci between Auke Creek and Pillar Creek pink salmon used for breeding experiments in 1996. P-values were found using *G*-test statistics and a permutation test (20,000 iterations) similar to Roff & Bentzen (1989). N is the number of fish sampled. Homogeneity tests across all loci revealed significant (p < 0.0001) divergence between Auke and Pillar Creek fish used in 1996.
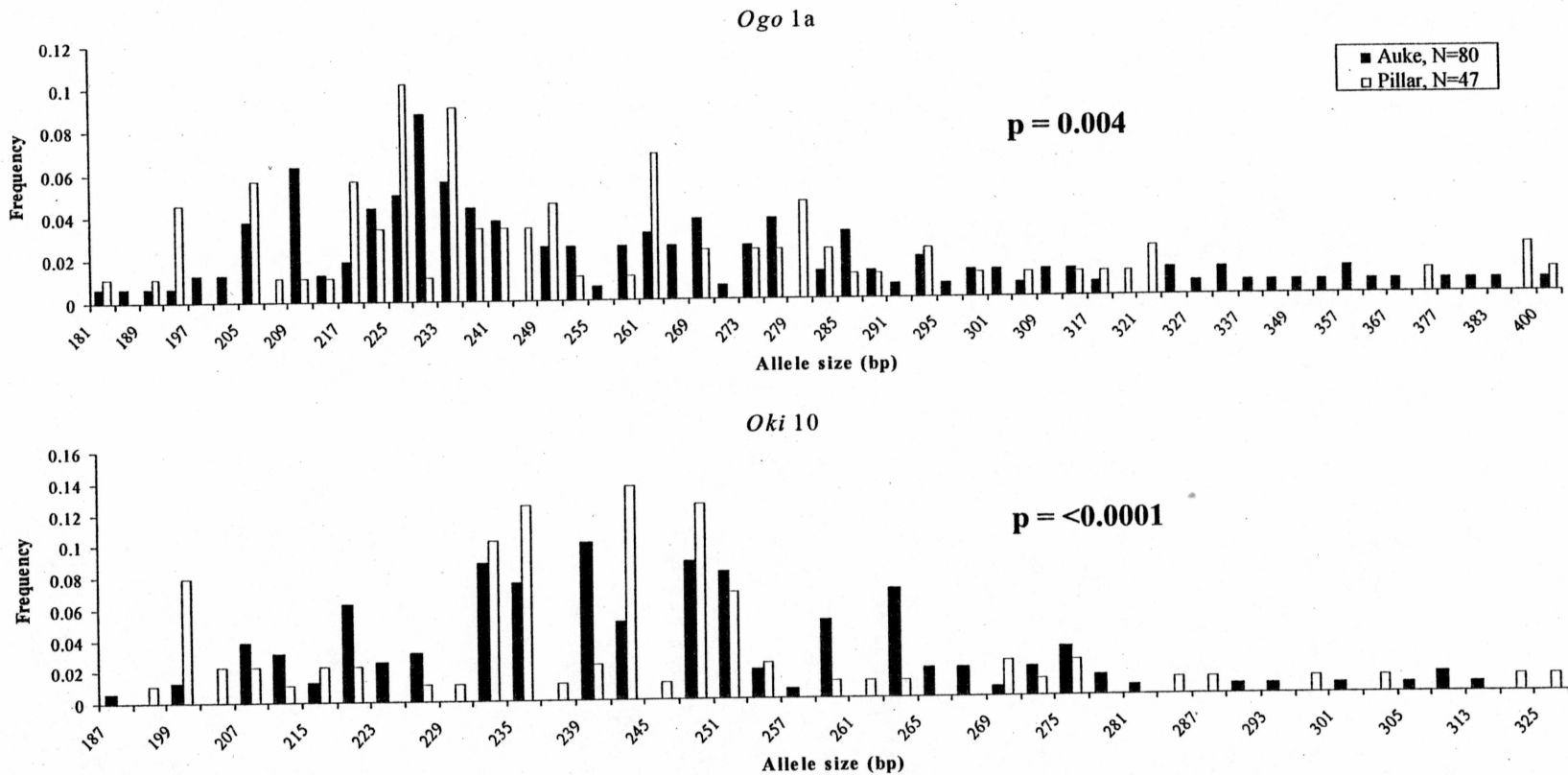
Figure D.4. Homogeneity of allele frequencies at the *Ogo*1a and *Oki*10 microsatellite loci between Auke Creek and Pillar Creek pink salmon used for breeding experiments in 1997. P-values were found using *G*-test statistics and a permutation test (20,000 iterations) similar to Roff & Bentzen (1989). N is the number of fish sampled. Homogeneity tests across all loci revealed significant (p < 0.0001) divergence between Auke and Pillar Creek fish used in 1997.
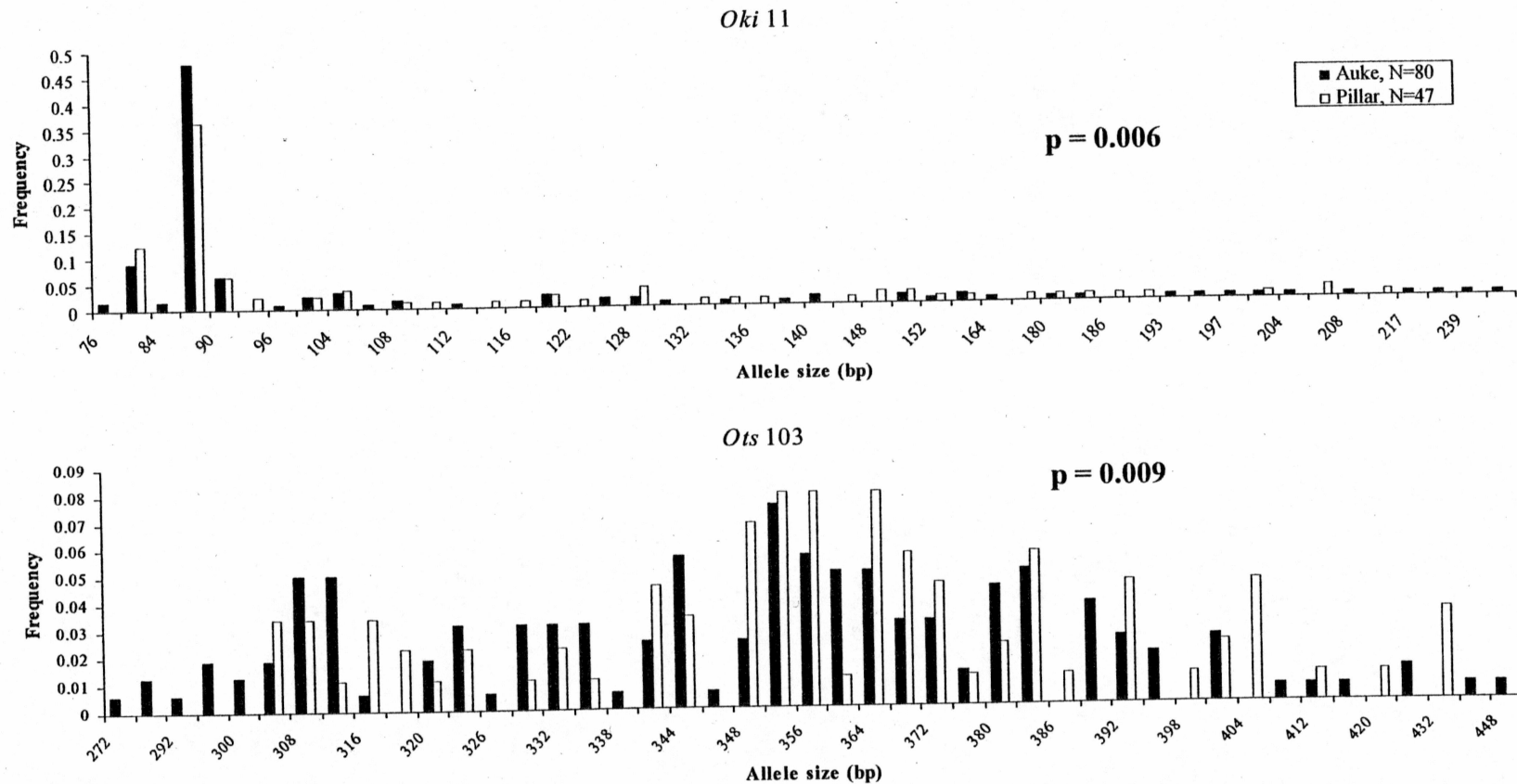
Figure D.5. Homogeneity of allele frequencies at the *Oki*11 and *Ots*103 microsatellite loci between Auke Creek and Pillar Creek pink salmon used for breeding experiments in 1997. P-values were found using *G*-test statistics and a permutation test (20,000 iterations) similar to Roff & Bentzen (1989). N is the number of fish sampled. Homogeneity tests across all loci revealed significant ($p < 0.0001$) divergence between Auke and Pillar Creek fish used in 1997.
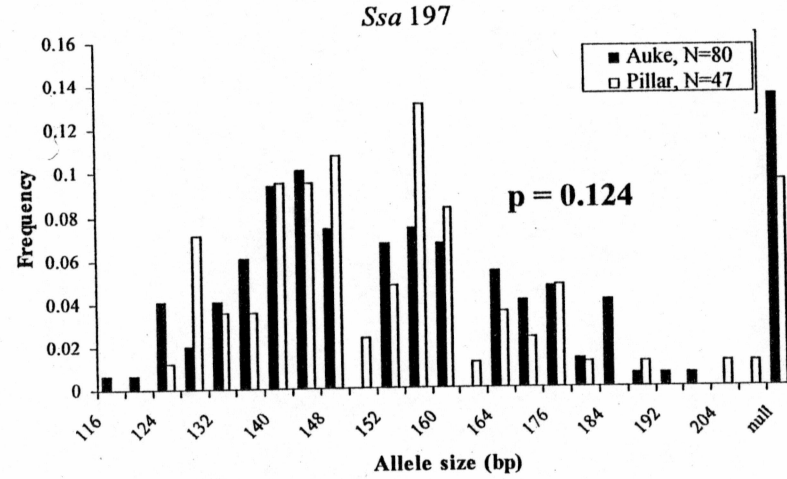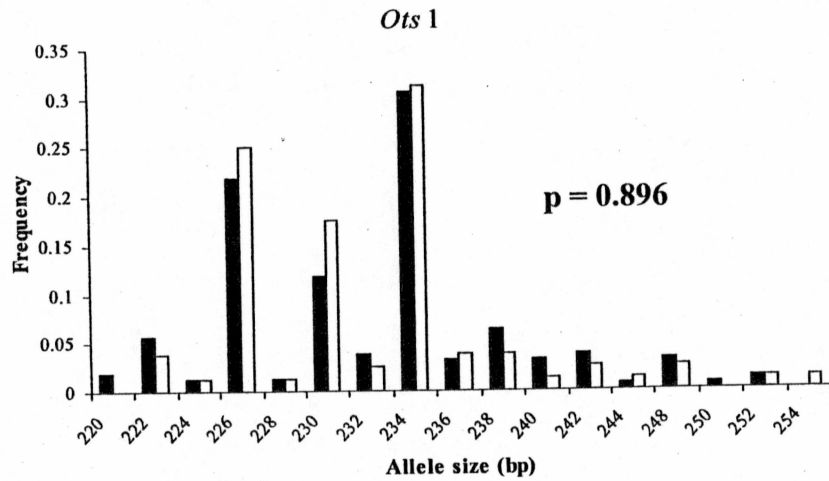
Figure D.6. Homogeneity of allele frequencies at the *Ots*1 and *Ssa*197 microsatellite loci between Auke Creek and Pillar Creek pink salmon used for breeding experiments in 1997. P-values were found using *G*-test statistics and a permutation test (20,000 iterations) similar to Roff & Bentzen (1989). N is the number of fish sampled. Homogeneity tests across all loci revealed significant (p < 0.0001) divergence between Auke and Pillar Creek fish used in 1997.

Appendix E: Allele frequencies

Table E.1. Observed allele frequencies for the *Ogo*1a
microsatellite locus in Auke and Pillar Creek pink salmon used
for breeding experiments in 1996 and 1997.

	1996			1997		
	Auke	Pillar	Total	Auke	Pillar	Total
No. of fish	80	46	126	80	44	124
*Ogo*1a alleles						
181	0	0	0	0.006	0.011	0.008
185	0	0	0	0.006	0	0.004
189	0	0	0	0.006	0.011	0.008
191	0.006	0	0.004	0	0	0
193	0	0	0	0.006	0.045	0.020
195	0	0.011	0.004	0	0	0
197	0	0	0	0.013	0	0.008
199	0.006	0	0.004	0	0	0
201	0	0	0	0.013	0	0.008
203	0.006	0.011	0.008	0	0	0
205	0	0.022	0.008	0.038	0.057	0.044
207	0.006	0.011	0.008	0	0.011	0.004
209	0.006	0.011	0.008	0.063	0.011	0.044
211	0.006	0	0.004	0	0	0
213	0.006	0.011	0.008	0.013	0.011	0.012
215	0.006	0	0.004	0	0	0
217	0	0.011	0.004	0.019	0.057	0.032
219	0	0.022	0.008	0	0	0
221	0	0.022	0.008	0.044	0.034	0.040
223	0.013	0.011	0.012	0	0	0
225	0.006	0	0.004	0.050	0.102	0.069
227	0	0.022	0.008	0	0	0
229	0	0.033	0.012	0.088	0.011	0.060
233	0.025	0.022	0.024	0.056	0.091	0.069
235	0	0.022	0.008	0	0	0
237	0.019	0.022	0.020	0.044	0.034	0.040
239	0	0.011	0.004	0	0	0
241	0.063	0.033	0.052	0.038	0.034	0.036
243	0.006	0	0.004	0	0	0
245	0.019	0.043	0.028	0	0.034	0.012
247	0.013	0	0.008	0	0	0
249	0.013	0.065	0.032	0.025	0.045	0.032
253	0.025	0.043	0.032	0.025	0.011	0.020
255	0	0	0	0.006	0	0.004

Table E.1. Continued

| | 1996 | | | 1997 | | |
	Auke	Pillar	Total	Auke	Pillar	Total
257	0.044	0.022	0.036	0.025	0.011	0.020
261	0.031	0.054	0.040	0.031	0.068	0.044
263	0.019	0.011	0.016	0	0	0
265	0.038	0.043	0.040	0.025	0	0.016
267	0.006	0.011	0.008	0	0	0
269	0.025	0.033	0.028	0.038	0.023	0.032
271	0.006	0	0.004	0.006	0	0.004
273	0.038	0.087	0.056	0.025	0.023	0.024
275	0.006	0	0.004	0	0	0
277	0.056	0.022	0.044	0.038	0.023	0.032
279	0.006	0	0.004	0	0.045	0.016
281	0.050	0.011	0.036	0.013	0.023	0.016
285	0.031	0	0.020	0.031	0.011	0.024
287	0.006	0	0.004	0	0	0
289	0.019	0.033	0.024	0.013	0.011	0.012
291	0.006	0	0.004	0.006	0	0.004
293	0.006	0.065	0.028	0.019	0.023	0.020
295	0.006	0	0.004	0.006	0	0.004
297	0.031	0.011	0.024	0.013	0.011	0.012
299	0.006	0	0.004	0	0	0
301	0	0	0	0.013	0	0.008
305	0.031	0.011	0.024	0.006	0.011	0.008
309	0.025	0.011	0.020	0.013	0	0.008
311	0.013	0	0.008	0	0	0
313	0.006	0	0.004	0.013	0.011	0.012
317	0.019	0.011	0.016	0.006	0.011	0.008
319	0	0.011	0.004	0	0.011	0.004
321	0.050	0.011	0.036	0	0.023	0.008
325	0.031	0.011	0.024	0.013	0	0.008
327	0	0	0	0.006	0	0.004
329	0.031	0	0.020	0.013	0	0.008
333	0.013	0	0.008	0	0	0
337	0.006	0.011	0.008	0.006	0	0.004
339	0.006	0	0.004	0	0	0
341	0	0.011	0.004	0	0	0
345	0.006	0.011	0.008	0.006	0	0.004
349	0.013	0	0.008	0.006	0	0.004
353	0	0	0	0.006	0	0.004
357	0.006	0	0.004	0.013	0	0.008
361	0.006	0	0.004	0.006	0	0.004
367	0	0	0	0.006	0	0.004

Table E.1. Continued

	1996			1997		
	Auke	Pillar	Total	Auke	Pillar	Total
369	0.006	0	0.004	0	0.011	0.004
373	0.013	0	0.008	0	0	0
377	0.006	0	0.004	0.006	0	0.004
381	0	0.011	0.004	0.006	0	0.004
383	0	0	0	0.006	0	0.004
385	0	0.011	0.004	0	0	0
395	0.006	0	0.004	0	0	0
399	0	0	0	0	0.023	0.008
>400	0.019	0.022	0.020	0.006	0.011	0.008

Table E.2. Observed allele frequencies for the *Oki*10 microsatellite locus in Auke and Pillar Creek pink salmon used for breeding experiments in 1996 and 1997.

	1996			1997		
	Auke	Pillar	Total	Auke	Pillar	Total
No. of fish	80	47	127	80	44	124
*Oki*10 alleles						
135	0	0.011	0.004	0	0	0
163	0	0.011	0.004	0	0	0
175	0.006	0	0.004	0	0	0
179	0.006	0	0.004	0	0	0
183	0.025	0	0.016	0	0	0
187	0.006	0.011	0.008	0.006	0	0.004
191	0.013	0	0.008	0	0	0
195	0.019	0.011	0.016	0	0.011	0.004
199	0.019	0.011	0.016	0.013	0.080	0.036
203	0.013	0.011	0.012	0	0.023	0.008
207	0.019	0.011	0.016	0.038	0.023	0.032
211	0.031	0.043	0.035	0.031	0.011	0.024
215	0.044	0.032	0.039	0.013	0.023	0.016
219	0.031	0.053	0.039	0.063	0.023	0.048
223	0.063	0.064	0.063	0.025	0	0.016
227	0.069	0.064	0.067	0.031	0.011	0.024
229	0	0	0	0	0.011	0.004
231	0.069	0.085	0.075	0.088	0.102	0.093
235	0.063	0.117	0.083	0.075	0.125	0.093
237	0	0	0	0	0.011	0.004
239	0.081	0.021	0.059	0.100	0.023	0.073
243	0.075	0.064	0.071	0.050	0.136	0.081
245	0	0	0	0	0.011	0.004
247	0.075	0.085	0.079	0.088	0.125	0.101
251	0.063	0.053	0.059	0.081	0.068	0.077
255	0.050	0.043	0.047	0.019	0.023	0.020
257	0	0.011	0.004	0.006	0	0.004
259	0.044	0.064	0.051	0.050	0.011	0.036
261	0	0	0	0	0.011	0.004
263	0.044	0.053	0.047	0.069	0.011	0.048
265	0	0	0	0.019	0	0.012
267	0.019	0.021	0.020	0.019	0	0.012
269	0	0	0	0.006	0.023	0.012
271	0.006	0	0.004	0.019	0.011	0.016
275	0.013	0.011	0.012	0.031	0.023	0.028
277	0	0	0	0.013	0	0.008

Table E.2. Continued.

	1996			1997		
	Auke	Pillar	Total	Auke	Pillar	Total
279	0.019	0.021	0.020	0	0	0
281	0	0	0	0.006	0	0.004
285	0	0	0	0	0.011	0.004
287	0	0	0	0	0.011	0.004
291	0	0.021	0.008	0.006	0	0.004
293	0	0	0	0.006	0	0.004
297	0	0	0	0	0.011	0.004
301	0	0	0	0.006	0	0.004
303	0	0	0	0	0.011	0.004
305	0	0	0	0.006	0	0.004
307	0.013	0	0.008	0	0	0
309	0	0	0	0.013	0	0.008
313	0	0	0	0.006	0	0.004
321	0	0	0	0	0.011	0.004
323	0.006	0	0.004	0	0	0
325	0	0	0	0	0.011	0.004

Table E.3. Observed allele frequencies for the *Oki*11 microsatellite locus in Auke and Pillar Creek pink salmon used for breeding experiments in 1996 and 1997.

	1996			1997		
	Auke	Pillar	Total	Auke	Pillar	Total
No. of fish	76	40	116	62	40	102
*Oki*11 alleles						
76	0	0	0	0.016	0	0.010
82	0	0	0	0.089	0.125	0.103
84	0.026	0.050	0.034	0.016	0	0.010
86	0.414	0.388	0.405	0.476	0.363	0.431
90	0.079	0.125	0.095	0.065	0.063	0.064
94	0.026	0	0.017	0	0.025	0.010
96	0.013	0.025	0.017	0.008	0	0.005
98	0.020	0.025	0.022	0	0	0
102	0	0.013	0.004	0.024	0.025	0.025
104	0.007	0	0.004	0.032	0.038	0.034
106	0.046	0	0.030	0.008	0	0.005
108	0.007	0	0.004	0.016	0.013	0.015
110	0.072	0.013	0.052	0	0.013	0.005
112	0.020	0.038	0.026	0.008	0	0.005
114	0.026	0.013	0.022	0	0.013	0.005
116	0.020	0	0.013	0	0.013	0.005
118	0.007	0.038	0.017	0	0	0
120	0	0	0	0.024	0.025	0.025
122	0.013	0.050	0.026	0	0.013	0.005
124	0.007	0	0.004	0.016	0	0.010
128	0	0	0	0.016	0.038	0.025
130	0	0	0	0.008	0	0.005
132	0	0	0	0	0.013	0.005
134	0	0	0	0.008	0.013	0.010
136	0	0	0	0	0.013	0.005
138	0.007	0	0.004	0.008	0	0.005
140	0.007	0	0.004	0.016	0	0.010
144	0.007	0	0.004	0	0.013	0.005
146	0	0.013	0.004	0	0	0
148	0	0	0	0	0.025	0.010
150	0	0	0	0.016	0.025	0.020
151	0	0.013	0.004	0	0	0
152	0.007	0.013	0.009	0.008	0.013	0.010
156	0.013	0	0.009	0	0	0
158	0.013	0	0.009	0.016	0.013	0.015

Table E.3. Continued.

	1996			1997		
	Auke	Pillar	Total	Auke	Pillar	Total
160	0.013	0	0.009	0	0	0
162	0.020	0.013	0.017	0	0	0
164	0.013	0.013	0.013	0.008	0	0.005
166	0.007	0.050	0.022	0	0.013	0.005
167	0.007	0	0.004	0	0	0
168	0	0.013	0.004	0	0	0
170	0.007	0	0.004	0	0	0
172	0	0.013	0.004	0	0	0
174	0.007	0	0.004	0	0	0
176	0.007	0.013	0.009	0	0	0
179	0.007	0	0.004	0	0	0
180	0	0	0	0.008	0.013	0.010
181	0	0.013	0.004	0	0	0
182	0.007	0	0.004	0	0	0
184	0	0.013	0.004	0.008	0.013	0.010
186	0	0	0	0	0.013	0.005
188	0	0	0	0	0.013	0.005
189	0.007	0	0.004	0	0	0
190	0.007	0.013	0.009	0	0	0
191	0.013	0	0.009	0	0	0
193	0.007	0	0.004	0.008	0	0.005
194	0	0	0	0.008	0	0.005
195	0	0.013	0.004	0	0	0
197	0	0	0	0.008	0	0.005
200	0.007	0	0.004	0.008	0.013	0.010
203	0.007	0	0.004	0	0	0
204	0	0	0	0.008	0	0.005
206	0	0	0	0	0.025	0.010
207	0	0.013	0.004	0	0	0
208	0	0	0	0.008	0	0.005
209	0	0	0	0	0.013	0.005
211	0.007	0	0.004	0	0	0
215	0	0.013	0.004	0	0	0
217	0	0	0	0.008	0	0.005
229	0	0	0	0.008	0	0.005
239	0	0	0	0.008	0	0.005
252	0	0	0	0.008	0	0.005

Table E.4. Observed allele frequencies for the *Ots*103 microsatellite locus in Auke and Pillar Creek pink salmon used for breeding experiments in 1996 and 1997.

	1996			1997		
	Auke	Pillar	Total	Auke	Pillar	Total
No. of fish	79	47	126	80	44	124
*Ots*103 alleles						
208	0.006	0	0.004	0	0	0
260	0.013	0	0.008	0	0	0
268	0.006	0	0.004	0	0	0
272	0.006	0	0.004	0.006	0	0.004
280	0.006	0	0.004	0	0	0
284	0.006	0	0.004	0	0	0
288	0.006	0.011	0.008	0.013	0	0.008
292	0.006	0.011	0.008	0.006	0	0.004
294	0.013	0	0.008	0	0	0
296	0	0	0	0.019	0	0.012
300	0.038	0.011	0.028	0.013	0	0.008
304	0.019	0.032	0.024	0.019	0.034	0.024
308	0	0.032	0.012	0.050	0.034	0.044
312	0.038	0.053	0.044	0.050	0.011	0.036
314	0	0.011	0.004	0	0	0
316	0.025	0	0.016	0.006	0.034	0.016
318	0	0	0	0	0.023	0.008
320	0.032	0.011	0.024	0.019	0.011	0.016
324	0.051	0.053	0.052	0.031	0.023	0.028
326	0	0	0	0.006	0	0.004
328	0.025	0.053	0.036	0.031	0.011	0.024
332	0.057	0.043	0.052	0.031	0.023	0.028
336	0.038	0.064	0.048	0.031	0.011	0.024
338	0	0	0	0.006	0	0.004
340	0.038	0.043	0.040	0.025	0.045	0.032
344	0.063	0.021	0.048	0.056	0.034	0.048
346	0	0	0	0.006	0	0.004
348	0.051	0.021	0.040	0.025	0.068	0.040
352	0.032	0.021	0.028	0.075	0.080	0.077
356	0.025	0.032	0.028	0.056	0.080	0.065
358	0.006	0	0.004	0	0	0
360	0.063	0.032	0.052	0.050	0.011	0.036
364	0.057	0.053	0.056	0.050	0.080	0.060
368	0.032	0.064	0.044	0.031	0.057	0.040
372	0.044	0.011	0.032	0.031	0.045	0.036

Table E.4. Continued.

	1996			1997		
	Auke	Pillar	Total	Auke	Pillar	Total
376	0.025	0.053	0.036	0.013	0.011	0.012
380	0.038	0.032	0.036	0.044	0.023	0.036
384	0.013	0	0.008	0.050	0.057	0.052
386	0	0	0	0	0.011	0.004
388	0.025	0.032	0.028	0.038	0	0.024
390	0	0.011	0.004	0	0	0
392	0.013	0.032	0.020	0.025	0.045	0.032
396	0.013	0.064	0.032	0.019	0	0.012
398	0	0	0	0	0.011	0.004
400	0	0.021	0.008	0.025	0.023	0.024
404	0.013	0.011	0.012	0	0.045	0.016
408	0.019	0.011	0.016	0.006	0	0.004
412	0.006	0.011	0.008	0.006	0.011	0.008
416	0	0	0	0.006	0	0.004
420	0.006	0.011	0.008	0	0.011	0.004
424	0.006	0.021	0.012	0	0	0
428	0	0	0	0.013	0	0.008
432	0	0	0	0	0.034	0.012
436	0	0	0	0.006	0	0.004
444	0.006	0	0.004	0	0	0
448	0	0	0	0.006	0	0.004
450	0.006	0	0.004	0	0	0
452	0	0.011	0.004	0	0	0
497	0.006	0	0.004	0	0	0

Table E.5. Observed allele frequencies for the *Ots*1 microsatellite locus in Auke and Pillar Creek pink salmon used for breeding experiments in 1996 and 1997.

	1996			1997		
	Auke	Pillar	Total	Auke	Pillar	Total
No. of fish	79	40	119	80	40	120
*Ots*1 alleles						
218	0.006	0.025	0.013	0	0	0
220	0.006	0.038	0.017	0.019	0	0.013
222	0.044	0.025	0.038	0.056	0.038	0.050
224	0.025	0	0.017	0.013	0.013	0.013
226	0.158	0.225	0.181	0.219	0.250	0.229
228	0.095	0.013	0.067	0.013	0.013	0.013
230	0.165	0.175	0.168	0.119	0.175	0.138
232	0.038	0.075	0.050	0.038	0.025	0.033
234	0.196	0.163	0.185	0.306	0.313	0.308
236	0.051	0.025	0.042	0.031	0.038	0.033
238	0.120	0.113	0.118	0.063	0.038	0.054
240	0.019	0.063	0.034	0.031	0.013	0.025
242	0.019	0.025	0.021	0.038	0.025	0.033
244	0.013	0	0.008	0.006	0.013	0.008
246	0.038	0.038	0.038	0	0	0
248	0	0	0	0.031	0.025	0.029
250	0	0	0	0.006	0	0.004
252	0	0	0	0.013	0.013	0.013
254	0	0	0	0	0.013	0.004
256	0.006	0	0.004	0	0	0

Table E.6. Observed allele frequencies for the *Ssa*197 microsatellite locus in Auke and Pillar Creek pink salmon used for breeding experiments in 1996 and 1997.

	1996			1997		
	Auke	Pillar	Total	Auke	Pillar	Total
No. of fish	80	40	120	74	42	116
*Ssa*197 alleles						
116	0	0.013	0.004	0.007	0	0.004
122	0	0	0	0.007	0	0.004
124	0.006	0.038	0.017	0.040	0.012	0.030
128	0.038	0.013	0.029	0.020	0.071	0.039
132	0.025	0.038	0.029	0.040	0.036	0.039
136	0.044	0.100	0.063	0.060	0.036	0.052
140	0.106	0.063	0.092	0.094	0.095	0.094
144	0.144	0.163	0.150	0.101	0.095	0.099
148	0.119	0.125	0.121	0.074	0.107	0.086
150	0	0	0	0	0.024	0.009
152	0.100	0.113	0.104	0.067	0.048	0.060
156	0.088	0.088	0.088	0.074	0.131	0.094
160	0.063	0.038	0.054	0.067	0.083	0.073
162	0	0	0	0	0.012	0.004
164	0.063	0.025	0.050	0.054	0.036	0.047
168	0.044	0.038	0.042	0.040	0.024	0.034
172	0.038	0.013	0.029	0	0	0
176	0.025	0.038	0.029	0.047	0.048	0.047
180	0.044	0	0.029	0.013	0.012	0.013
184	0.006	0.013	0.008	0.040	0	0.026
188	0	0.013	0.004	0.007	0.012	0.009
192	0	0	0	0.007	0	0.004
196	0	0	0	0.007	0	0.004
204	0	0	0	0	0.012	0.004
208	0	0	0	0	0.012	0.004
null	0.050	0.075	0.058	0.134	0.095	0.120

General Conclusion

Hybrids between two spatially separated Pacific salmon populations exhibited reduced

survival due to outbreeding depression, and evidence of both epistatic and non-epistatic

effects was observed. It does not appear that a decreased local homing ability, or

increased variability in family size accounted for the decrease in hybrid survival.

Whether due to non-epistatic or epistatic effects, the decrease in hybrid survival could

jeopardize biodiversity of the wild stocks if repeated introductions are made. Small, one-

time introductions may be of minor concern because natural selection might be expected

to remove maladapted genotypes rapidly from a population; however, repeated

introductions or development of non-local hatchery brood stocks have the potential to

decrease productivity in local wild streams. The possibility of outbreeding depression

suggests that broodstocks should be developed from locally adapted natural populations

and that salmon managers should consider the potentially detrimental effects of

transplanting stocks from one hatchery to another.