A TEST OF LOCAL ADAPTATION IN SEASONALLY SEPARATE SUBPOPULATIONS OF PINK SALMON (*ONCORHYNCHUS GORBUSCHA*)

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A

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Abstract

Differences in fitness related traits were observed between first generation (F_1) hybrid and control lines of temporally distinct subpopulations of pink salmon (*Oncorhynchus gorbuscha*). The lines were cultured in a common freshwater environment, released to sea together, and collected at their natal stream as adults. Early- and late-run pink salmon, which are partially genetically isolated by the time at which they return to Auke Creek in Southeast Alaska to spawn, were crossed to create F_1 and F_2 hybrid groups in the even- and odd-year brood lines. Marine survival of controls exceeded that of F_1 hybrids of the even-year brood line, whereas no difference in marine survival between those experimental groups was detected in the odd-year brood line. First generation hybrids expressed intermediate time of return relative to controls in both brood lines. Second generation hybrids exhibited similar embryonic development rates to controls in both brood lines. These results demonstrate that removal of a genetic barrier as fine as that which occurs within a brood line and location can disrupt local adaptation in a population of pink salmon, which may cause outbreeding depression in hybrids and may potentially reduce the overall biodiversity and productivity of the population.

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Introduction

Pacific salmon are characterized by a propensity to home to their natal stream, a trait that has enabled many genetically distinct populations of salmonids to arise through adaptation to local environments (Carvalho 1993). Local adaptation, a process in which natural selection increases the frequency of traits that confer a survival or reproductive advantage in a local environment, is well documented in salmonid populations separated by large geographic distances (Taylor 1991). Hatchery salmon propagation, which has been widely used to rehabilitate wild stocks and enhance fisheries, can create opportunities for non-native genetic material to intogress into wild populations. There is concern that introgression of genetic material from hatchery fish into wild populations will cause the wide variety of locally adapted stocks to be replaced by a smaller number of relatively homogenous ones, thereby reducing diversity that is a crucial buffer against inexorable environmental changes (Waples 1991). Interbreeding between genetically divergent stocks can also reduce fitness in hybrids, a phenomenon known as outbreeding depression (Gharrett and Smoker 1991; Edmands 1999; Gilk et al. 2004).

Outbreeding depression manifests through two different mechanisms that can occur independently or jointly. Interbreeding between populations from different local environments can produce hybrids that are maladapted to either of the parental environments. Accordingly, hybrids could suffer a loss of fitness through disruption of interactions between genes and environment (Edmands 2007). This mechanism for outbreeding depression (termed "extrinsic" or "ecological") acts on first and subsequent

generations (Lynch 1991) and may involve underdominance, genotype-by-environment interactions, or epistatic interactions (Edmands 1999). Alternatively, outbreeding depression can act through the disruption of co-adapted gene complexes. Complexes of alleles at epistatic loci can arise through joint selection for multiple loci during local adaptation and random drift (Lynch 1991). Populations may evolve different epistatic gene complexes under similar selection pressures because random drift participates in determining the genetic material that is available for co-adaptation (Lenski and Travisano 1994) and because favorable allele combinations are maintained by natural selection. Hybridization can disrupt favorable interactions between alleles at different loci, leading to a loss of fitness. This mechanism for outbreeding depression (termed "intrinsic" or "epistatic") typically does not manifest itself until the second generation or later (Emlen 1991) because epistatic gene complexes are maintained in the gamete contributed by each parent. In fact, first generation hybrids may exhibit heterosis as a result of masking of deleterious recessive alleles in heterozygotes (Lynch 1991). However, independent segregation and recombination in subsequent generations can separate gene complexes. Some epistatic gene complexes may be comprised of tightly linked loci that could take many generations to disrupt (Edmands 1999).

Prior research on pink salmon has examined outbreeding depression by removing large temporal and spatial barriers between populations. One such barrier, a consequence of the two-year life cycle to which pink salmon rigidly adhere (Davidson 1934; Bilton and Ricker 1965; Turner and Bilton 1968), has enabled two genetically isolated brood lines to arise; one brood line returns to spawn in even years and the other returns in odd

years. In separate studies of two generations of hybrids between the even- and odd-year brood lines, reduced survival was observed in F_2 hybrids only, which indicated that outbreeding depression had manifested primarily through the intrinsic mechanism (Gharrett and Smoker 1991; Gharrett et al. 1999). Similarly, more pronounced outbreeding depressive effects were observed in the second generation of hybridization between pink salmon from Auke Creek and spatially distant (~1000 km) Pillar Creek (Gilk et al. 2004). These studies demonstrated that large spatial and temporal barriers have enabled populations of pink salmon to diverge, most likely through local adaptation, and that removal of such barriers can have detrimental effects on overall fitness. More recent research has focused on the effects of hybridization on embryonic development. A study on embryonic development in hybrids between the even- and odd-year brood lines detected no difference in timing to completion of epiboly (an adaptive trait) between controls and F_2 hybrids, but did reveal significant variation among families of female parents (Wang et al. 2006). A pronounced dam effect, coupled with a non-significant sire effect, indicated that maternal effects or additive effects can occur before expression of the paternal genome during development. In addition, embryonic development time (measured by the number of accumulated temperature days elapsed when 50% of embryos were hatched in an incubator compartment) was examined in first- and second-generation hybrids between Pillar and Auke Creek pink salmon. Comparisons between F_2 crosses and backcrosses suggested that a model which incorporated both extrinsic and intrinsic outbreeding depression effects best explained differences in development time that were observed in that study (Wang et al. 2007).

Previous research on Auke Creek pink salmon evaluated the consequences of removing a gene flow barrier between populations that are completely temporally or spatially isolated. However, there is a dearth of studies that have examined the effects of disrupting fine-scale temporal or spatial structure. This study addressed that gap in our understanding by evaluating the effects of hybridizing seasonally distinct spawning segments that return to Auke Creek in the same year. Within the even- and odd-year brood lines, time of return for spawning pink salmon follows a bimodal distribution; "early" spawners generally enter Auke Creek in peak volumes between mid and late August, while "late" spawners generally peak between early and mid September. The median return times for the two groups are offset by about 20 days, although the length of separation varies inter-annually with rainfall patterns and stream temperatures (Taylor 2008). The early- and late-spawning segments are partially isolated genetically and probably adapted to different local environments (Gharrett et al. 2001; Echave 2010). The partitioning of the pink salmon run is likely the result of adaptation to limited spawning substrate (Smoker et al. 1998).

The short separation between the two spawning segments provides a unique opportunity to investigate local adaptation operating at a fine scale of temporal divergence. Evidence of local adaptation can be inferred by changes (relative to controls) in hybrids between early- and late-run pink salmon in three fitness-related attributes: marine survival, embryonic development rate, and time of return. Marine survival, the proportion of released fry that return as adults to spawn, is a direct indicator of fitness. Observations of indirect measures of fitness, such as time of return and embryonic

development rate, can reveal deleterious shifts in life history attributes. Time of return (McGregor et al. 1998; Smoker et al. 1998) and embryonic development rate (Goddard 1995; Hebert et al. 1998; Echave 2010) have a genetic basis in Auke Creek pink salmon; consequently, hybrids may express either different means or increased variance of these traits relative to the parental types. The rationale is that, although the early- and late-spawning segments share the same stream, seasonal changes in Auke Creek during the spawning season produce different environmental regimes for the two groups; accordingly, the two spawning segments have likely developed distinct life history strategies (i.e. time of return, development rate) as a consequence of adaptation to different local environments. Hybridization has the potential to perturb the genetic architecture of these life history traits, thereby disrupting local adaptation and causing outbreeding depression. In this sense local adaptation and outbreeding depression are closely associated; local adaptation creates genetic divergence between populations, which potentiates outbreeding depression in hybrids.

Our objective was to test whether local adaptation has structured the early- and late-spawning segmentation of the population by evaluating the effects of hybridizing these seasonally distinct subpopulations of pink salmon. The primary questions addressed were: (1) Does extrinsic outbreeding depression result in reduced marine survival of F_1 hybrids? (2) Does extrinsic outbreeding depression cause phenotypic changes in time of return of F_1 hybrids? and (3) Does outbreeding depression (extrinsic or intrinsic) cause phenotypic changes in embryonic development rate of F_2 hybrids? Differences in survival or fitness-related traits between hybrids and controls are indicative of outbreeding

depression and suggest that early- and late-run pink salmon are genetically distinct groups that have diverged through local adaptation. We repeated this experiment in both the even- and odd-year brood lines.

Methods

Field Methods

Auke Creek, Alaska is a short (350 m) and steep (20 m) outlet of Auke Lake that drains into Auke Bay and serves as a spawning ground and migratory corridor for pink salmon. Located at the mouth of the creek and at the head of tidewater is a permanent weir and salmon hatchery, which is operated by the U.S. National Marine Fisheries Service. Early- and late-run pink salmon were collected at the weir and artificially spawned at the hatchery in the summers of 2005 and 2006 (Echave 2010) to create F_1 hybrid and control lines (Table 1), which were propagated into the F_2 generation (Table 2) by artificially spawning returning F_1 progeny in 2007 and 2008. We used cryopreserved semen collected four years prior (2001, 2002) from late-run fish to produce late-male by early-female hybrids and late-male by late-female controls in each brood year. We used semen collected from early-run fish to produce early-male by early-female controls in each brood year; semen from those males was also cryopreserved to produce early-male by late-female hybrids in brood year 2005. We were unsuccessful in cryopreserving semen from early-run males in 2006 and produced no early-male by late-female hybrids that year. Consequently, we did not release late-run controls in brood year 2006.

The F_1 mating scheme, a blocked incomplete-factorial design, was intended for analysis of F_1 embryonic development (Echave 2010). We produced 20 blocks of F_1 families for both spawning segments in 2005, and another 20 blocks for the early-

spawning segment in 2006. Each block consisted of two early- and two late-run females crossed with each of two early- and two late-run males, and each full-sib cross was equally divided between two randomly selected compartments within FAL™ (Marisource, Milton, WA) vertical incubation trays. Control and hybrid embryos were incubated at the Auke Creek hatchery in ambient temperature water that was pumped from the creek and treated twice a week with dilute formalin (1:6000 in static water) for 1 hour to inhibit growth of fungus and bacteria. Daily observations of incubation temperature and hatching status enabled us to estimate average development time in each compartment by the accumulation of temperature days (ATUs) on the date of mid-hatch (50% or more eggs hatched).

Developing fry were incubated until they were ~5% yolk by weight, and then each fish was anesthetized by immersion in an aqueous MS-222 (Tricaine Methanesulfonate) solution (100 mg/liter) for approximately 3 minutes. Anesthetized fish were immediately marked with an experiment-identifying adipose fin excision and opposing pelvic fin excisions to distinguish controls from hybrids. Controls and hybrids were concurrently released into Auke Creek in April, at the peak of natural pink salmon emigration. Returning adult pink salmon were collected at Auke Creek weir and examined for the absence of an adipose or pelvic fin to determine if they belonged to one of our experimental lines. Marked fish were tagged with numbered Floy™ (Floy Tag Inc., Seattle, WA) tags, and a randomly selected sample of those fish was held in pens to be used as broodstock. Fish that were not chosen as broodstock were euthanized by

cranial concussion followed by exsanguination. Tissue samples were obtained from each marked fish by clipping the axillary process at the base of the remaining pelvic fin.

Table 1 - First generation (F_1) crosses in each of two brood lines observed in these experiments. Parental run types are abbreviated Early (E) and Late (L).

		2005 Sire		2006 Sire	
Dam		E	L	E	L
2005	E	EE	EL		
	L	LE	LL		
2006	E			EE	EL

Table 2 - Second generation (F_2) crosses in each of two brood lines observed in these experiments. Parental run types are abbreviated Early (E) and Late (L).

		2007 Sire				2008 Sire	
Dam		EE	EL	LE	LL	EE	EL
2007	EE	EEEE					
	EL		ELEL	ELLE			
	LE		LEEL	LELE			
	LL				LLLL		
2008	EE					EEEE	
	EL						ELEL

Laboratory Methods

Tissue samples from F_1 parents were stored in numbered vials of preservative solution (Seutin et al. 1991) and refrigerated at -22 °C. We isolated total genomic DNA with DNeasy Blood and Tissue kits (QIAGEN, Inc., Valencia, CA). Five microsatellite loci (*Ots1* [Banks et al. 1999]; *Ots208* [Greig et al. 2003]; *Ogo1a* [Olsen et al. 1998]; *Oki10* [Smith et al. 1998]; and *One109* [Olsen et al. 2000]) were chosen to unequivocally assign parental pairs to progeny (Table A1). Polymerase chain reaction (PCR) was used to amplify microsatellite loci. The PCR reaction mixtures (Table A2) were 10 μL volumes: 1 x PCR buffer (50 mM KCl, 10 mM Tris-HCl at pH 9.0); 1.5-3 mM $MgCl_2$; 0.125-0.2 mM each deoxynucleotide triphospate (dNTP); 0.3-0.5 μM each forward and reverse primer (Integrated DNA Technologies, Inc., Coralville, IA); 0.01-0.05 μM labeled primer (Eurofins MWG Operon, Huntsville, AL); approximately 1 unit of generic *Taq* polymerase; and 50-100 ng DNA. The general amplification profile (Table A1) was 1 cycle at 95°C for 3 min; 30-40 cycles at 95°C for 30 s, 49-59°C for 30 s, and 72°C for 45 s; and 1 cycle at 72°C for 5 min. After amplification, PCR 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. Target fragments were separated by loading approximately 1 μL of PCR product into polyacrylamide denaturing gels containing 6% of a PAGE-PLUS™ 40% concentrate (AMRESCO Inc., Solon, OH), 8 M Urea, and 5X TBE (445 mM Tris-Borate and 10 mM EDTA, pH 8.0), in a reaction catalyzed by ammonium persulfate and TEMED (N,N,N',N'-tetramethylethylenediamine). Electrophoresis was

performed in LI-COR automated sequencers (4300^{TM} DNA Analysis System, LI-COR, Inc., Lincoln, NE) in 1X TBE buffer, with running conditions 1,500 V, 40 W, 40 mA, and 45°C plate temperature. Allele sizes were scored by using Saga (Ver. 3.2.1, LI-COR) software to compare allele band patterns with LI-COR IRD700^{TM} or IRD800^{TM} standard ladders (Lincoln, NE).

Statistical Methods

We used microsatellite genotype information to assign parental pairs to returning adults with PROBMAX (Version 1.2; Danzmann 1997), which uses exclusion analysis based on known parental mating combinations. The type of cross for each returning adult fish was determined from parentage information.

Marine survival, the proportion of released experimental fry that returned to Auke Creek as adults, was our primary indicator of outbreeding depression. Analysis of log-linear models of marine survival was performed in SYSTAT (Version 11; SYSTAT Software Inc.), which enabled us to estimate interactions between survival and two explanatory variables: type of cross and spawning date. We used R software (Version 2.12.2; R Foundation 2011) to examine power curves for tests of homogeneity of survival.

We documented the date of collection at the Auke Creek weir of all returning F_1 experimental pink salmon and examined temporal patterns of return by analysis of those dates. Analysis of variance of time of return was performed with restricted maximum likelihood (REML) for mixed models (PROC MIXED; SAS Version 9.2, SAS Institute

Inc., Cary, NC), which is more robust than standard analysis of variance for datasets that include both fixed and random effects, unbalanced data, and departures from normality (Littell et al. 1996). Additionally, REML provides better estimation properties than maximum likelihood (ML) because it accounts for the loss of degrees of freedom in estimating the mean, and it yields unbiased estimates of variance parameters (Smyth and Verbyla 1996). In REML analyses, variances are estimated directly and likelihood is maximized by removing fixed effects from the model and testing them separately. The significance of each random effect was estimated with a log-likelihood ratio test between a full model and a model without the effect of interest (Littell et al. 1996). The analysis was performed separately for the early- and late-spawning segments in order to avoid confounding the effects of run and type of cross. The return data were comprised of incomplete and non-equivalent blocks, which precluded reliable estimation of a block effect or of sire by dam interaction. Consequently, those terms were not included in our linear model. The linear model that describes all pertinent random and fixed effects on time of return within a brood year (2005 or 2006) and run (early or late) was:

$$y_{ijkl} = \mu + C_i + D_j + S_{ik} + \varepsilon_{ijkl}$$

where y_{ijkl} is the dependent variable (Julian days elapsed on return date). The overall population mean is μ, C_i is the fixed effect of the i^{th} cross (hybrid or control), D_j is the random effect of the j^{th} dam, S_{ik} is the random effect of the k^{th} sire within the i^{th} cross, and ε_{ijkl} is the residual random error associated with the j^{th} dam and k^{th} sire within the i^{th} cross.

We defined development time in F_2 progeny as ATUs at mid-hatch. Analysis of variance of development time was performed with a mixed model procedure that was similar to that used for time of return, and the analysis was performed separately for the early and late runs. Sparse returns of F_1 progeny resulted in an unbalanced F_2 mating design with incomplete and non-equivalent blocks. Consequently, our F_2 development data did not provide a basis for reliably estimating a block effect or a sire by dam interaction, and those terms were excluded from the model. The F_2 mating design was carried out over the course of several spawning dates in each brood year. To account for discrepancies in incubation temperature regimes, spawning date was incorporated as a fixed effect. The linear model that describes all pertinent random and fixed effects on development time within a brood year (2007 or 2008) and run (early or late) was:

$$y_{ijklm} = \mu + T_i + C_j + D_{ijk} + S_{ijl} + \varepsilon_{ijklm}$$

where y_{ijklm} is the dependent variable (ATUs at mid-hatch). The overall population mean is μ, T_i is the fixed effect of the i^{th} spawning date, C_j is the fixed effect of the j^{th} cross (hybrid or control), D_{ijk} is the random effect of the k^{th} dam within the i^{th} spawning date and j^{th} cross, S_{ijl} is the random effect of the l^{th} sire within the i^{th} spawning date and j^{th} cross, and ε_{ijklm} is the residual random error associated with the k^{th} dam and l^{th} sire within the i^{th} spawning date and j^{th} cross.

Results

Marine survival

We captured 176 marked pink salmon at Auke Creek hatchery in 2007 and genotyped each fish at five microsatellite loci. Based on parentage analysis, 169 F_1 progeny (96%) were conclusively linked to parental pairs from 2005, and 7 fish did not appear to belong to our experiment. Type of cross was determined for each experimental fish, and all line designations (control, hybrid) were concordant with documented pelvic fin clips. The total recovery of 169 returned adults from 44,728 released fry corresponded to a total marine survival of 0.38% (Table 3), less than 1/10 of the survival (4.47%) observed in wild pink salmon from this brood year. Marine survival was 0.12% lower in hybrids than in controls and 0.20% lower in late- than in early-run fish. Log-linear analysis of marine survival (Table 4) revealed interaction between survival and run time ($P = 0.001$), survival and type of cross ($P = 0.042$), and a three-way interaction between survival, run time, and type of cross ($P = 0.046$).

In 2008, we recovered 122 marked fish at Auke Creek hatchery. Parentage analysis conclusively linked 112 F_1 progeny (92%) to parental pairs from 2006 and excluded 10 fish from our experiment. All line designations were concordant with documented pelvic fin clips. The total recovery of 112 returned adults from 35,159 released fry corresponded to a marine survival of 0.32%, less than 1/10 of the survival (3.83%) observed in wild pink salmon from this brood year. Marine survival was 0.05%

lower in hybrids than in controls, but log-linear analysis (Table 4) did not reveal a significant interaction between survival and type of cross ($P = 0.465$).

Time of return

Data from 169 returned F_1 adults of broodyear 2005 (Table A3) were used in the REML analysis of 2007 time of return (Table 5). Early-run controls returned 3.8 days before early-run hybrids on average, and late-run controls returned 3.7 days after late-run hybrids (Figure 1). Type of cross significantly influenced time of return for both the early ($P = 0.013$) and late experiment ($P = 0.002$). Neither dam nor sire effects significantly affected time of return.

The REML analysis of 2008 time of return (Table 5) was conducted with return data from 112 returned F_1 adults of broodyear 2006 (Table A3). Early-run controls returned 9.4 days before early-run hybrids on average. Type of cross ($P < 0.001$) and sire ($P = 0.002$) significantly influenced time of return. There was no significant dam effect.

Embryonic development

Mid-hatch data from 64 F_2 families of broodyear 2007 (Table A4) were used in the REML analysis of embryonic development time (Table 6). Early-run controls accumulated approximately 1.2 more ATUs at mid-hatch than early-run hybrids, and late-run controls accumulated 2.3 fewer ATUs than late-run hybrids. Spawning date moderately influenced ATUs at mid-hatch in both experiments ($P < 0.11$). Type of cross did not significantly affect ATUs at mid-hatch in either the early ($P = 0.444$) or late ($P = 0.135$) experiment. Time (ATUs) to mid-hatch had a significant dam effect in both the

early ($P < 10^{-6}$) and late experiment ($P = 0.004$), whereas a moderate sire effect ($P = 0.090$) was observed in the late experiment only.

Mid-hatch data from 67 F_2 families (Table A4) were used for the REML analysis of development time in brood year 2008 (Table 6). Early-run controls accumulated approximately 12 more ATUs at mid-hatch than early-run hybrids. We observed significant effects of spawning date ($P = 0.008$), dam ($P < 10^{-6}$), and sire ($P < 10^{-5}$) on ATU's at mid-hatch. Type of cross did not significantly affect ATU's at mid-hatch.

Table 3 – Numbers released and return proportions by brood year, cross, and run in two brood years of pink salmon outbred over one generation. Returning odd- and even-year progeny were collected at Auke Creek weir in 2007 and 2008 respectively.

		Broodyear 2005		Broodyear 2006	
Cross	Run	Released	Returned proportion	Released	Returned proportion
Control	Early	12,517	0.0060	25,294	0.0033
	Late	12,084	0.0026	0	
Hybrid	Early	13,047	0.0033	9,865	0.0028
	Late	7,080	0.0028	0	

Table 4 – Log-linear analysis of first generation marine survival in two brood years of outbred pink salmon.

	Broodyear 2005			Broodyear 2006		
Interaction term	X^2	Df	P	X^2	df	P
Survival * Cross	4.146	1	0.042	0.530	1	0.465
Survival * Run	11.538	1	0.001			
Survival * Cross * Run	3.972	1	0.046			

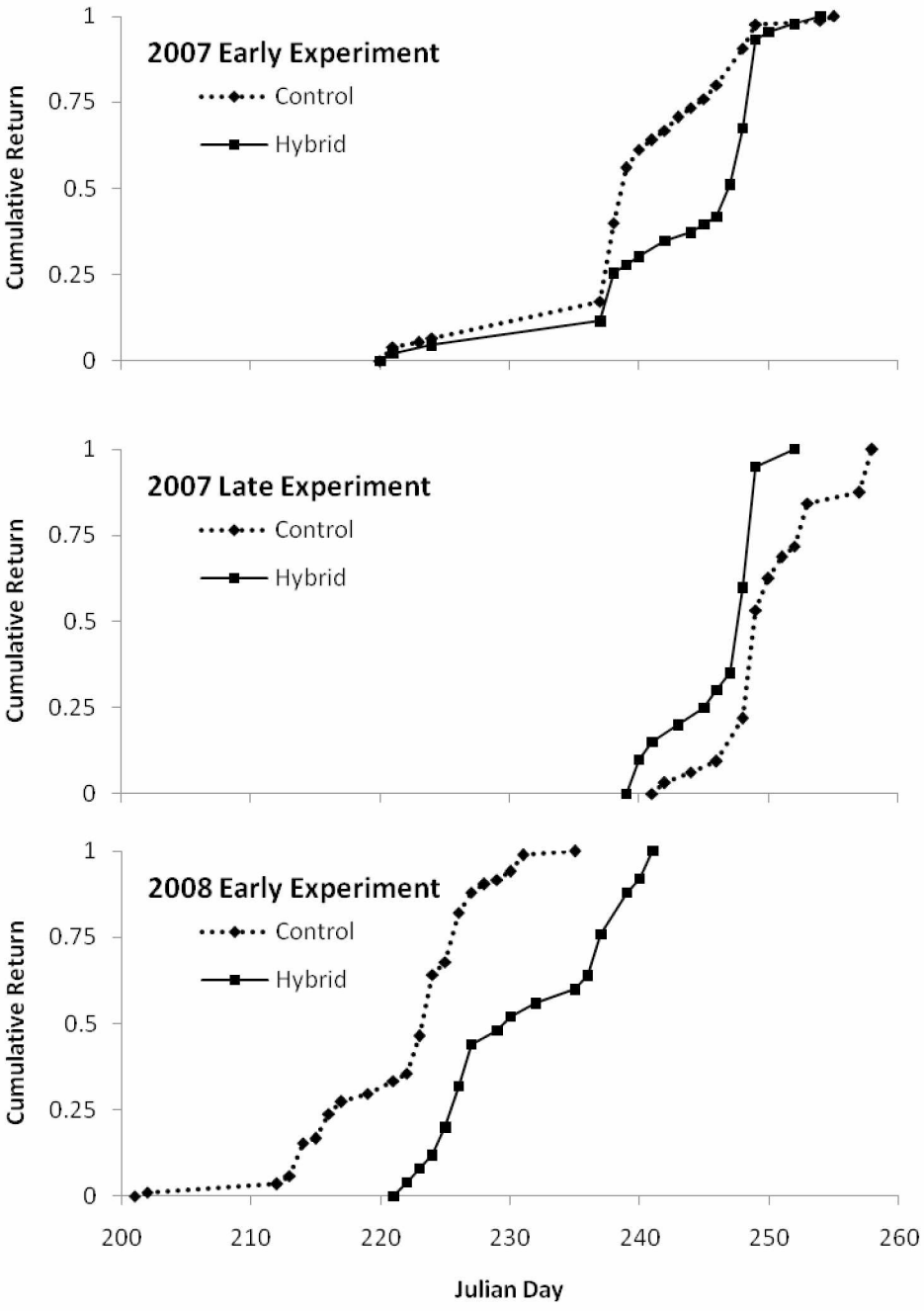

Figure 1 - Cumulative return proportions of F_1 progeny from each experimental line that returned to Auke Creek. Experimental fish from broodyears 2005 and 2006 returned to Auke Creek in 2007 and 2008 respectively. Date of return was measured by Julian day. Julian day 230 is near August 1st.

Table 5 – Significance values of factors that affect time of return of first generation outbred pink salmon in the early and late experiments of broodyear 2005 and in the early experiment of broodyear 2006. Factors include cross (C), dam (D), and sire (S). Analyses were conducted with restricted maximum likelihood (REML) for mixed models (PROC MIXED) in SAS (version 9.2).

	Brood (Run)		
Source of variation	2005 (Early)	2005 (Late)	2006 (Early)
Cross (C)[a]	0.013	0.002	<0.001
Dam (D)	0.376	0.061	0.376
Sire (S)	0.201	0.376	0.002

[a]Fixed effects

Table 6 – Significance values of factors that affect embryonic development time of second generation outbred pink salmon in the early and late experiments examined in 2007 and in the early experiment examined in 2008. Factors include spawning date (T), cross (C), dam (D), and sire (S). Analyses were conducted with restricted maximum likelihood (REML) for mixed models (PROC MIXED) in SAS (version 9.2).

	Brood (Run)		
Source of variation	2007 (Early)	2007 (Late)	2008 (Early)
Spawning date (T)[a]	0.108	0.061	0.008
Cross (C)[a]	0.444	0.135	0.568
Dam (D)	$<10^{-6}$	0.004	$<10^{-6}$
Sire (S)	0.327	0.090	$<10^{-5}$

[a]Fixed effects

Discussion

Local adaptation is an important driver of genetic divergence between populations, which in turn enables outbreeding depression to manifest in hybrids. Hence, we might expect that outbreeding depression can serve as an indicator of local adaptation. We looked for evidence of local adaptation in early- and late-run pink salmon by examining hybrids between the two groups for outbreeding depressive effects. To accomplish this, we evaluated marine survival as a direct indicator of outbreeding depression, and we looked for phenotypic shifts of two fitness related traits: embryonic development time and time of return. Since these traits are likely optimized for different environmental regimes encountered by early- and late-spawning pink salmon, perturbations of them could decrease fitness in hybrids.

Marine survival of even-broodyear F_1 hybrids between early- and late-run pink salmon was similar to controls. This result is similar to those of previous studies, in which marine survival of controls did not differ from F_1 hybrids between even- and odd-year pink salmon (Gharrett and Smoker 1991; Gharrett et al. 1999) and F_1 hybrids between spatially isolated populations of even-broodyear pink salmon (Gilk et al. 2004). The power of this test, however, was low (0.11). An F_1 hybrid marine survival of 0.21%, approximately 3/4 of that observed, would be required for this test to attain a power of 0.5.

In contrast to the even-year brood line, marine survival of F_1 hybrids was significantly lower than controls in the odd-year brood line. This result is similar to the reduced survival that was observed in odd-broodyear F_1 hybrids between spatially

isolated pink salmon (Gilk et al. 2004) and suggests that extrinsic outbreeding depression may influence marine survival.

We observed reduced survival of experimental fish relative to wild fish, which was consistent with hatchery and marking effects observed in prior experiments at Auke Creek (Lane et al. 1990). Various aspects of the culturing process might adversely impact developing fry and consequently reduce survival in the marine environment. In addition, the comparatively benign hatchery environment may favor survival to emigration of many embryos that would have perished in the harsher natural environment. Upon introduction to the natural environment during emigration, many of these fry likely succumb to mortality. Hence, delayed mortality may reduce marine survival. To account for this, controls and hybrids were reared under identical incubation conditions and released at the same time, which gave each fish an equal opportunity to prepare for the marine environment. Therefore, we would expect that any discrepancies in return rates between controls and hybrids are the result of differential performance in the marine environment.

We tested for disruption of local adaptation that could cause outbreeding depression in hybrid F_1 pink salmon by estimating genetic influences on time of return. Despite having low statistical power, we observed differences of time of return between controls and hybrids in each run within both brood lines. The return distribution pattern for each type of cross was consistent with our initial expectations based on local adaptation of time of return: hybrids returned to Auke Creek later than controls in the early experiment of both brood lines, and hybrids returned earlier than controls in the late

experiment of the odd-year brood line. Our results demonstrate intermediate phenotypic expression of time of return in hybrid pink salmon relative to controls and indicate that there is a strong genetic basis for this trait. While a phenotypic shift in time of return does not directly demonstrate outbreeding depression, it does suggest that hybridization can reduce spawning success. The reason for this is that timing of egg deposition is critical to maximizing offspring survival during embryonic development. This is particularly relevant for embryos of early-spawning pink salmon, which are vulnerable to disturbance by subsequent spawning activity. Embryos that complete epiboly, a developmental process in which the germ ring closes over the yolk plug (Ballard 1973), gain heightened resistance to mechanical agitation. Accordingly, offspring that complete epiboly are more likely to survive redd superimposition by late-spawning adults (Smoker et al. 1998). The influence of late-run genetic material could delay egg deposition in hybrid pink salmon; consequently, hybrid embryos might be more prone to mortality from superimposition by late-spawning adults.

Our results confirm that there is a genetic basis for time of return, which explains why gene flow is restricted between the early- and late-spawning segments. Previous research has yielded insight into the notion that the early and late subpopulations are partially isolated genetically. Lane et al. (1990) conducted a study in which a genetic marker was bred into the late-run subpopulation of the odd-year brood line in 1979 and 1981. Between 1983 and 1989, the allele frequencies of the marker were monitored in the early- and late-spawning segments (Gharrett et al. 2001). Introgression of the marker into the early-run subpopulation was minimal, which suggested that gene flow was restricted

between the two spawning segments and that fine-scale temporal structure existed within brood lines. Temporal structure enhances the productivity of each brood line by maximizing the number of offspring that can be produced from limited spawning substrate (Smoker et al. 1998). Hence, it is likely that erosion of the temporal barrier that separates the early- and late-spawning segments would cause decreased productivity of Auke Creek pink salmon.

Embryonic development rate is an important life history trait, which has a genetic basis in Auke Creek pink salmon (Goddard 1995; Hebert et al. 1998). Embryonic development exhibits phenotypic plasticity in salmonids, and warmer incubation temperatures typically accelerate development (Murray and McPhail 1988). Early-run fish arriving at Auke Creek in late August experience warmer stream conditions than late-run fish arriving in September. However, the offset in fry emergence between the two groups suggests that early- and late-run pink salmon develop at similar rates despite incubating in different temperature regimes (Joyce 1986). It is likely that, through adaptation to different local regimes, a compensatory mechanism has evolved that enables both groups to emerge concurrently with favorable estuarine conditions each spring. Accordingly, we might expect to observe extrinsic effects on development rate in F_1 hybrids.

The analysis of embryonic development time (measured by ATU's at mid-hatch) of F_1 hybrids between early- and late-run pink salmon yielded contrasting results between the even- and odd-year brood lines (Echave 2010). In the odd-year brood line, hybrids developed slower than early controls. This result was surprising because Hebert (1998)

observed that late-run pink salmon from Auke Creek developed faster than early-run fish, irrespective of incubation temperature regime; hence, we would expect a hybrid embryo to develop faster than an early-control embryo because of the influence of late-run genetic material. In the even-year brood line, early hybrids developed faster than early controls, which was consistent with a phenotypic expression intermediate to the parental types as predicted by Hebert's results. In addition, late hybrids from the odd-year brood line, which developed more slowly than late controls, expressed an intermediate phenotype. Echave (2010) proposed that the unexpected results of the early component of odd-year experiment may have been influenced by unusual creek temperatures during the summer of 2005, which were among the warmest on record. One important consequence of warm creek temperatures in 2005 was that weir passage of the earliest arriving pink salmon was delayed; therefore, early-run broodstock were not collected until 22 August, after more than 50% of the pink salmon run had already passed through the weir. Hence, it is possible that early-run broodstock from 2005 were unusually mixed with late-run individuals, whose genetic contribution could have hastened embryonic development of the early controls. Although the results were mixed, there was evidence of intermediate expression of development time in F_1 hybrids, which suggests that differences in embryonic development rate between early- and late-run pink salmon have arisen through adaptation to local environmental regimes. Additionally, development time was strongly influenced by sire and dam effects in each of the F_1 experiments, which indicates that additive genetic effects are significant contributors to variation of embryonic development rate.

We compared embryonic development time between controls and hybrid F_2 pink salmon to look for evidence of local adaptation. Whereas shifts in development time can be attributed to extrinsic effects in F_1 hybrids, both extrinsic and intrinsic effects could contribute to shifts in development time in F_2 hybrids. Neither the even- nor the odd-year analyses indicated that embryonic development time differed between controls and hybrids. However, the F_2 mating design complicated the interpretation of the development analysis. Spawning of the F_2 crosses occurred on three different dates in each brood year. Developing embryos were incubated in ambient temperature water that was pumped from Auke Creek; consequently, incubation temperature regimes differed for fish that were spawned on different dates. The analyses of development time showed that spawning date was moderately influential for the odd-year experiment and strongly influential for the even-year experiment. However, our experimental design does not enable us to accurately partition the variation in development time between spawning date and type of cross. The analysis revealed strong dam effects on development time for each of the F_2 experiments and moderate to strong sire effects for two out of three F_2 experiments, which reinforces the idea that additive genetic effects contribute to variation of development rate.

Conclusion

A long-term series of water temperature data and biological observations collected at Auke Creek indicated that a general warming trend ($0.03^{\circ}C\ yr^{-1}$) is affecting the pink salmon population. Earlier migration times for spawning adults and elevated

incubation temperatures are causing fry to emigrate earlier (Taylor 2008; Kovach et al. 2012). The abundance of phytoplankton, which is limited by incident light and typically peaks in early April, is tightly linked to the survival of juvenile pink salmon (Ziemann et al. 1990). Prior to the primary plankton bloom, environmental conditions in Auke Bay are characterized by cooler waters and limited prey availability (Coyle and Paul 1990), both of which can constrain growth and reduce survival of early emigrating fry. Early emigrating juveniles may benefit from reduced size-selective predation by growing in the estuarine environment prior to the rapid increase of near-shore predators that typically occurs in May. However, an adaptive advantage of reduced size-selective predation is likely constrained by longer exposure to predators, poorer growth in early spring, and reduced survival to adulthood (Mortensen 2000). Consequently, a continued trend toward earlier migration could result in reduced average fitness of emigrating juveniles and an overall decline in the productivity of Auke Creek pink salmon. Local adaptation, by altering life history traits such as embryonic development rate and time of return, can adjust emigration times to optimal environmental conditions. Spawning segregation increases genetic diversity, which entails higher adaptability to changing local regimes. The findings of our study suggest that hybridization has the capacity to erode the temporal barrier separating the early- and late-runs, thereby hindering the ability of pink salmon to sustain productivity in a changing climate.

The Pacific Decadal Oscillation (PDO), a pattern of marine climate variability that shifts polarity on an inter-decadal time scale, is associated with Alaskan salmon production cycles. In particular, pink salmon are influenced by marine climate variability

during the early ocean phase of their life cycles. Environmental changes related to the PDO likely influence productivity in near-shore environments through a "bottom-up" model, in which phytoplankton and zooplankton abundances fluctuate with marine conditions, and the ramifications of these lower trophic level fluctuations spread upward to top-level predators such as salmon (Hare and Francis 1995). Stream-flow variations are also linked to the PDO and influence the survival of emigrating juvenile salmon. It is probable that fluctuations in near-shore plankton abundance and stream-flow variations jointly influence the productivity of pink salmon. Research indicates that marked changes in salmon production regimes in the North Pacific have accompanied shifts in the polarity of the PDO (Mantua et al. 1997). Alaskan salmon stocks are currently abundant, but productivity may decline with the next climatic shift (Hare and Francis 1995), which will increase reliance on hatchery supplementation of wild stocks. Hence, the coming decades will likely bring an increase in the introgression of non-native genetic material into local wild populations. Understanding the influence of hybridization on local adaptation should be of paramount importance to the future management of Alaska's salmon resources.

This study demonstrated that seasonally separate subpopulations can adapt to unique local environments, thereby creating sufficient genetic divergence to potentiate outbreeding depression in hybrids. The implications emphasize the importance of fine-scale population structure to overall fitness, which make it unique to the Auke Creek hybridization experiments. Fine-scale structure is often impossible to resolve without genetic analyses, yet failure to maintain it could be detrimental to the biodiversity and productivity of wild salmon populations. Our observations suggest that prudent

management of wild salmon stocks should be conducted not only with regard for genetic structure that arises from isolation of populations by great distance or time, but also fine-scale genetic structure that can occur within streams and brood lines.

Cited Literature

Ballard, W.W. 1973. Normal embryonic stages for salmonid fishes, based on *Salmo gairdneri* Richardson and *Salvelinus fontinalis* (Mitchill). Journal of Experimental Zoology 184:7–26.

Banks, M.A., M.S. Blouin, B.B. Baldwin, V.K. Rashbrook, H.A. Fitzgerald, S.M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tshawytscha*). Journal of Heredity 90:281-288.

Bilton, H.T., and W.E. Ricker. 1965. Supplementary checks on the scales of pink salmon (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*). Journal of the Fisheries Research Board of Canada 22:1477-1489.

Carvalho, G.R. 1993. Evolutionary aspects of fish distribution: Genetic variability and adaptation. Journal of Fish Biology 43 (Supplement sA): 53-73.

Coyle K.O., and A.J. Paul. 1990. Interannual variations in zooplankton population and biomass during the spring bloom in an Alaskan subarctic embayment. In: Association of Primary Production and Recruitment in Subarctic Ecosystems – Interannual Variability and Fisheries Recruitment (D.A Ziemann., and K.W. Fulton-Bennett), pp. 179–228. The Oceanic Institute, Honolulu, HI.

Danzmann, R.G. 1997. PROBMAX: a computer program for assigning unknown parentage in pedigree analysis from known genotypic pools of parents and progeny. Journal of Heredity 88:333.

Davidson, A. 1934. The homing instinct and age at maturity of pink salmon (*Oncorhynchus gorbuscha*). Bulletin of the United States Bureau of Fisheries 48:27-39.

Echave, J.D. 2010. First generation effects of outcrossing between geographically isolated and seasonally isolated populations of pink salmon (*Oncorhynchus gorbuscha*) on various stages of hatching. M.S. Thesis, University of Alaska Fairbanks, Fairbanks, AK.

Edmands, S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. Evolution 53:1757-1768.

Edmands, S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology 16:463-75.

Emlen, J.M. 1991. Heterosis and oubreeding depression: a multi-locus model and an application to salmon production. Fisheries Research 12:187-212.

Gharrett, A.J., and W.W. Smoker. 1991. Two generations of hybrids between even- and odd-year pink salmon (*Oncorhynchus gorbuscha*): a test for outbreeding depression? Canadian Journal of Fisheries and Aquatic Sciences 48:1744-1749.

Gharrett, A.J., W.W. Smoker, R.R. Reisenbichler, and S.G. Taylor. 1999. Outbreeding depression in hybrids between odd- and even-broodyear pink salmon. Aquaculture 173:117-129.

Gharrett, A.J., S. Lane, A.J. McGregor, and S.G. Taylor. 2001. Use of a genetic marker to examine genetic interactions among subpopulations of pink salmon (*Oncorhynchus gorbuscha*). Genetica 111:259-267.

Gilk, S.E., I.A. Wang, C.L. Hoover, W.W. Smoker, S.G. Taylor, A.K. Gray, and A.J. Gharrett. 2004. Outbreeding depression in hybrids between spatially separated pink salmon, *Oncorhynchus gorbuscha*, populations: marine survival, homing ability, and variability in family size. Environmental Biology of Fishes 69:287-297.

Goddard, P.L. 1995. Quantitative genetic analysis of a fitness related life-history character, development rate, in odd- and even-year populations of pink salmon (*Oncorhynchus gorbuscha*). M.S. Thesis, University of Alaska Fairbanks, Fairbanks, AK.

Greig, C., D.P. Jacobson, and M.A. Banks. 2003. New tetranucleotide microsatellites for fine-scale discrimination among endangered chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology Notes 3:376-379.

Hare, S.R., and R.C. Francis. 1995. Climate change and salmon production in the northeast Pacific Ocean, p. 357-372. In: R.J. Beamish *Climate Change and Northern Fish Populations*. Canadian Special Publication of Fisheries and Aquatic Sciences 121.

Hare, S. R. 1996. Low frequency climate variability and salmon production. Ph.D. Dissertation. Univeristy of Washington, Seattle, WA.

Hebert K.P., P.L. Goddard, W.W. Smoker, and A.J. Gharrett. 1998. Quantitative genetic variation and genotype by environment interaction of embryo development rate in pink salmon (*Oncorhynchus gorbuscha*). Canadian Journal of Fisheries and Aquatic Sciences 55:2048-2057.

Joyce, J.E. 1986. Genetic variation in the embryonic development rate of odd and even year pink salmon. M.S. Thesis, University of Alaska, Fairbanks, Fairbanks, AK.

Kovach, R. P., A. J. Gharrett, and D. A. Tallmon. 2012. Genetic change for earlier migration timing in a pink salmon population. Proceedings of the Royal Society B 279:3870-3878.

Lane S, A.J. McGregor, S.G. Taylor, and A.J. Gharrett. 1990. Genetic marking of an Alaskan pink salmon population, with an evaluation of the mark and the marking process. American Fisheries Society Symposium 7:395-406.

Lenski, R.E., and M. Travisano. 1994. Dynamics of adaptation and diversification: A 10,000-generation experiment with bacterial populations. Proceedings of the National Academy of Sciences 91:6808-6814.

Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Inc. Cary, NC, USA.

Lynch, M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45:622-629.

Mantua, N.J., S.R. Hare, Y. Zhang, J.M. Wallace, and R.C. Francis. 1997. A Pacific interdecadal climate oscillation with impacts on salmon production. Bulletin of the American Meteorological Society 78:1069-1079.

McGregor, A.J., S. Lane, M.A. Thomason, L.A. Zhivotovsky, W.W. Smoker, and A.J. Gharrett. 1998. Migration timing, a life history trait important in the genetic structure of pink salmon. North Pacific Anadromous Fish Commission Bulletin No. 1:262-273.

Mortensen D., A. Wertheimer, S. Taylor, and J. Landingham. 2000. The relation between early marine growth of pink salmon, *Oncorhynchus gorbuscha*, and marine water temperature, secondary production, and survival to adulthood. Fishery Bulletin. 98:319-335.

Murray, C.B., and J.D. McPhail. 1988. Effect of incubation temperature on the development of five species of Pacific salmon (*Oncorhynchus*) embryos and alevins. Canadian Journal of Zoology 66:266-273.

Olsen, J.B., P. Bentzen, and J.E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7:1087-1089.

Olsen, J.B., S.L. Wilson, E.J. Kretschmer, K.C. Jones and J.E. Seeb. 2000. Characterization of 14 tetranucleotide microsatellite loci derived from sockeye salmon. Molecular Ecology 9 (12):2185-2187.

R Development Core Team. 2011. R. version 2.12.2. R Foundation for Statistical Computing,Vienna, Austria.

SAS Institute. 1999-2001. SAS/STAT™ Software. The SAS System for Windows. Release 8.02. Cary, North Carolina.

SYSTAT Software. 2004. SYSTAT 11 statistics. SYSTAT Software, Richmond, California.

Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82-90.

Smith, C.T., B.F. Koop, and R.J. Nelson. 1998. Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. Molecular Ecology 7:1614-1616.

Smyth, G. K. and A. P. Verbyla. 1996. A conditional approach to residual maximum likelihood estimation in generalized linear models. Journal of the Royal Statistical Society B58:565-572.

Smoker, W.W., A.J. Gharrett, and M.S. Stekoll. 1998. Genetic variation of return date in a population of pink salmon: a consequence of fluctuating environment and dispersive selection? Alaska Fishery Research Bulletin 5(1):46-54.

Taylor, E.B. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture 98:185-207.

Taylor, S.G. 2008. Climate warming causes phenological shift in pink salmon, *Oncorhynchus gorbuscha*, behavior at Auke Creek, Alaska. Global Change Biology 14:229-23.

Turner, C.E., and H.T. Bilton. 1968. Another pink salmon (*Oncorhynchus gorbuscha*) in its third year. Journal of the Fisheries Research Board of Canada 25:1993-1996.

Wang, I.A., E.H. Leder, W.W. Smoker, and A.J. Gharrett. 2006. Timing of development during epiboly of second-generation crosses and backcrosses between odd- and even-broodyear pink salmon, *Oncorhynchus gorbuscha*. Environmental Biology of Fishes 75:325-332.

Wang, I.A., S.E. Gilk, W.W. Smoker, and A.J. Gharrett. 2007. Outbreeding effect on embryo development in hybrids of allopatric pink salmon (*Oncorhynchus gorbuscha*) populations, a potential consequence of stock translocation. Aquaculture 272S1:S152-S160.

Waples, R.S. 1991. Genetic interactions between hatchery and wild salmonids: Lessons from the Pacific Northwest. Canadian Journal of Fisheries and Aquatic Sciences 48(Supplement 1):124-133.

Ziemann D.A., L.D. Conquest, K.W. Fulton-Bennett, and P.K. Bienfang. 1990. Interannual variability in the Auke Bay Phytoplankton. In: Association of Primary Production and Recruitment in Subarctic Ecosystems - Interannual Variability and Fisheries Recruitment (D.A. Ziemann., and Fulton-Bennett K.W.), pp. 129–170. The Oceanic Institute, Honolulu, HI.

Appendix

Table A1 - Microsatellite information and PCR conditions for microsatellite loci.
PCR profile: 3 min. 95 C°, x cycles (1 min. 95 C°, 1 min. T_A, 1 min. 72 C°), 5 min. 72 C°

Locus	T_A	Cycles	Accession #
Ogo1a	59	30	AF007827
Ots1	49	35	AF107029
Oki10	55	40	AF055435
One109	55	35	AF574525
Ots208	50	35	G68-AF393187

Table A2 - Specific PCR reagent quantities used for each of five microsatellite loci.
Volumes are given in μL per sample.

	Locus				
Reagent	Ogo1a	Ots1	Oki10	One109	Ots208
Purified H2O	5.14	5.40	5.14	5.50	5.40
10x PCR Buffer	1.00	1.00	1.00	1.00	1.00
25 mM MgCl2	0.60	0.75	0.60	0.60	0.75
dNTP's	0.80	0.50	0.80	0.50	0.50
10 uM F	0.36	0.40	0.36	0.30	0.40
10 uM R	0.40	0.40	0.40	0.40	0.40
1 uM L	0.40	0.25	0.40	0.10	0.25
Taq Polymerase	0.10	0.10	0.10	0.10	0.10

Table A3 - Time of return to Auke Creek of F_1 progeny from brood years 2005 and 2006. Parental sample numbers and type of cross are listed for each fish. Spawning was carried out on several different dates during brood year 2005.

2007 F_1 Time of Return					2008 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned	Spawned	Cross	Sire	Dam	Returned
8/27/05	EF x LM	4002	5601	8/26/07	8/12/06	EF x EM	5552	5501	8/3/08
8/27/05	EF x LM	4002	5601	9/5/2007	8/12/06	EF x EM	5552	5501	8/11/08
8/27/05	EF x LM	4002	5601	9/6/2007	8/12/06	EF x EM	5556	5506	8/1/08
8/27/05	EF x LM	4002	5601	8/26/07	8/12/06	EF x EM	5556	5506	8/11/08
9/9/05	LF x LM	4002	5801	9/7/2007	8/12/06	EF x EM	5558	5507	8/3/08
9/9/05	LF x LM	4002	5801	9/14/2007	8/12/06	EF x EM	5558	5507	8/6/08
8/27/05	EF x LM	4003	5603	8/30/2007	8/12/06	EF x EM	5558	5508	7/31/08
8/27/05	EF x LM	4003	5603	9/6/2007	8/12/06	EF x EM	5558	5508	8/1/08
8/27/05	EF x LM	4004	5603	9/5/2007	8/12/06	EF x EM	5558	5508	8/4/08
8/27/05	EF x LM	4004	5604	9/5/2007	8/12/06	EF x EM	5559	5509	8/11/08
9/9/05	LF x LM	4004	5804	9/6/2007	8/12/06	EF x EM	5559	5510	8/11/08
9/9/05	LF x LM	4004	5804	9/7/2007	8/12/06	EF x EM	5559	5510	8/12/08
8/27/05	EF x LM	4006	5606	8/26/07	8/12/06	EF x EM	5559	5510	8/14/08
8/27/05	EF x LM	4006	5606	8/26/07	8/12/06	EF x EM	5559	5510	8/17/08
9/9/05	LF x LM	4006	5806	9/1/2007	8/12/06	EF x EM	5560	5509	8/8/08
8/27/05	EF x LM	4007	5608	9/5/2007	8/12/06	EF x EM	5560	5509	8/9/08
9/9/05	LF x LM	4008	5807	9/15/2007	8/12/06	EF x EM	5560	5510	8/13/08
9/9/05	LF x LM	4008	5808	9/8/2007	8/12/06	EF x EM	5560	5510	8/14/08
9/9/05	LF x LM	4008	5808	9/15/2007	8/12/06	EF x EM	5562	5512	8/15/08
8/27/05	EF x LM	4010	5610	8/28/07	8/12/06	EF x EM	5563	5514	7/20/08
9/9/05	LF x LM	4010	5809	9/7/2007	8/12/06	EF x EM	5564	5513	8/4/08
9/9/05	LF x LM	4010	5810	9/5/2007	8/12/06	EF x EM	5564	5513	8/11/08
9/9/05	LF x LM	4010	5810	9/5/2007	8/12/06	EF x EM	5564	5513	8/11/08
9/9/05	LF x LM	4010	5810	9/5/2007	8/12/06	EF x EM	5564	5513	8/12/08

Table A3 (Continued)

2007 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned
9/9/05	LF x LM	4010	5810	9/6/2007
8/27/05	EF x LM	4011	5611	9/1/2007
8/27/05	EF x LM	4011	5611	9/5/2007
8/27/05	EF x LM	4011	5611	9/6/2007
8/27/05	EF x LM	4011	5612	9/3/2007
8/27/05	EF x LM	4011	5612	9/6/2007
8/27/05	EF x LM	4012	5611	9/4/2007
8/27/05	EF x LM	4012	5611	9/4/2007
8/27/05	EF x LM	4014	5614	8/25/07
8/27/05	EF x LM	4014	5614	8/30/2007
8/27/05	EF x LM	4014	5614	9/7/2007
9/9/05	LF x LM	4014	5813	9/6/2007
9/9/05	LF x LM	4014	5813	9/8/2007
9/9/05	LF x LM	4014	5813	9/10/2007
9/9/05	LF x LM	4014	5813	9/10/2007
9/9/05	LF x LM	4014	5814	8/30/2007
9/9/05	LF x LM	4015	5815	9/9/2007
9/9/05	LF x LM	4016	5815	9/5/2007
9/9/05	LF x LM	4016	5816	9/15/2007
8/27/05	EF x LM	4017	5618	9/6/2007
9/9/05	LF x LM	4019	5820	9/6/2007
8/27/05	EF x LM	4020	5620	8/9/07
8/27/05	EF x LM	4020	5620	8/26/07
8/27/05	EF x LM	4021	5621	9/9/2007
9/9/05	LF x LM	4022	5822	9/10/2007

2008 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned
8/12/06	EF x EM	5564	5514	8/22/08
8/12/06	EF x EM	5566	5516	8/1/08
8/12/06	EF x EM	5567	5517	8/8/08
8/12/06	EF x EM	5567	5518	7/30/08
8/12/06	EF x EM	5568	5517	7/31/08
8/12/06	EF x EM	5568	5517	8/17/08
8/12/06	EF x EM	5568	5517	8/18/08
8/12/06	EF x EM	5568	5518	8/2/08
8/12/06	EF x EM	5568	5518	8/10/08
8/12/06	EF x EM	5568	5518	8/13/08
8/12/06	EF x EM	5571	5521	8/6/08
8/12/06	EF x EM	5571	5521	8/14/08
8/12/06	EF x EM	5571	5521	8/18/08
8/12/06	EF x EM	5571	5522	8/12/08
8/12/06	EF x EM	5571	5522	8/13/08
8/12/06	EF x EM	5571	5522	8/13/08
8/12/06	EF x EM	5571	5522	8/18/08
8/12/06	EF x EM	5572	5521	8/13/08
8/12/06	EF x EM	5572	5522	8/11/08
8/12/06	EF x EM	5572	5522	8/13/08
8/12/06	EF x EM	5574	5523	8/8/08
8/12/06	EF x EM	5574	5524	8/3/08
8/12/06	EF x EM	5574	5524	8/9/08
8/12/06	EF x EM	5574	5524	8/10/08
8/12/06	EF x EM	5574	5524	8/13/08

Table A3 (Continued)

2007 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned
9/9/05	LF x LM	4023	5823	9/6/2007
8/27/05	EF x LM	4024	5623	9/6/2007
8/27/05	EF x LM	4024	5624	9/5/2007
8/27/05	EF x LM	4024	5624	9/6/2007
8/27/05	EF x LM	4027	5628	8/12/07
8/27/05	EF x LM	4027	5628	9/2/2007
9/9/05	LF x LM	4027	5828	9/6/2007
8/27/05	EF x LM	4028	5627	9/5/2007
9/9/05	LF x LM	4028	5828	9/6/2007
8/27/05	EF x LM	4029	5629	9/6/2007
8/27/05	EF x LM	4030	5629	9/6/2007
9/9/05	LF x LM	4030	5829	9/6/2007
9/9/05	LF x LM	4030	5830	9/15/2007
8/27/05	EF x LM	4031	5631	8/26/07
8/27/05	EF x LM	4032	5632	8/27/07
9/9/05	LF x LM	4032	5832	9/6/2007
8/27/05	EF x LM	4036	5635	8/25/07
8/27/05	EF x LM	4036	5635	9/4/2007
8/27/05	EF x LM	4037	5637	9/11/2007
9/9/05	LF x LM	4037	5838	9/3/2007
8/27/05	EF x LM	4038	5637	9/4/2007
8/27/05	EF x LM	4038	5637	9/6/2007
8/27/05	EF x LM	4038	5638	9/6/2007
8/27/05	EF x LM	4039	5640	8/25/07
9/9/05	LF x LM	4039	5839	9/6/2007

2008 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned
8/12/06	EF x EM	5575	5525	8/10/08
8/12/06	EF x EM	5575	5525	8/10/08
8/12/06	EF x EM	5575	5525	8/10/08
8/12/06	EF x EM	5575	5525	8/13/08
8/12/06	EF x EM	5576	5525	8/1/08
8/12/06	EF x EM	5576	5525	8/3/08
8/12/06	EF x EM	5576	5525	8/4/08
8/12/06	EF x EM	5576	5525	8/11/08
8/12/06	EF x EM	5576	5525	8/15/08
8/12/06	EF x EM	5576	5526	8/1/08
8/12/06	EF x EM	5577	5528	8/11/08
8/12/06	EF x EM	5579	5530	8/10/08
8/12/06	EF x EM	5580	5530	8/11/08
8/12/06	EF x EM	5580	5530	8/18/08
8/12/06	EF x EM	5581	5531	8/1/08
8/12/06	EF x EM	5581	5531	8/11/08
8/12/06	EF x EM	5581	5531	8/13/08
8/12/06	EF x EM	5581	5531	8/13/08
8/12/06	EF x EM	5581	5532	8/11/08
8/12/06	EF x EM	5581	5532	8/14/08
8/12/06	EF x EM	5582	5531	8/10/08
8/12/06	EF x EM	5582	5532	8/10/08
8/12/06	EF x EM	5584	5533	8/10/08
8/12/06	EF x EM	5584	5533	8/11/08
8/12/06	EF x EM	5584	5534	8/16/08

Table A3 (Continued)

2007 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned
9/9/05	LF x LM	4040	5840	9/10/2007
9/9/05	LF x EM	5651	5801	8/28/07
9/9/05	LF x EM	5651	5801	9/6/2007
9/9/05	LF x EM	5651	5802	9/5/2007
9/9/05	LF x EM	5651	5802	9/6/2007
8/27/05	EF x EM	5652	5601	8/28/07
9/9/05	LF x EM	5652	5802	9/5/2007
8/27/05	EF x EM	5653	5604	9/6/2007
8/27/05	EF x EM	5654	5603	8/25/07
8/27/05	EF x EM	5654	5604	8/26/07
8/27/05	EF x EM	5655	5606	8/26/07
8/27/05	EF x EM	5655	5606	8/31/2007
9/9/05	LF x EM	5655	5805	8/31/2007
9/9/05	LF x EM	5655	5805	9/6/2007
9/9/05	LF x EM	5655	5805	9/6/2007
8/27/05	EF x EM	5656	5606	8/28/07
8/27/05	EF x EM	5656	5606	8/31/2007
9/9/05	LF x EM	5656	5806	8/29/2007
9/9/05	LF x EM	5656	5806	9/2/2007
9/9/05	LF x EM	5656	5806	9/5/2007
8/27/05	EF x EM	5657	5608	8/26/07
8/27/05	EF x EM	5657	5608	8/30/2007
9/9/05	LF x EM	5658	5807	9/5/2007
8/27/05	EF x EM	5659	5610	8/25/07
8/27/05	EF x EM	5659	5610	8/26/07

2008 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned
8/12/06	EF x EM	5585	5535	8/1/08
8/12/06	EF x EM	5585	5536	8/1/08
8/12/06	EF x EM	5586	5535	8/11/08
8/12/06	EF x EM	5586	5535	8/13/08
8/12/06	EF x EM	5586	5536	7/30/08
8/12/06	EF x EM	5588	5537	8/3/08
8/12/06	EF x EM	5589	5539	8/13/08
8/12/06	EF x EM	5589	5540	8/3/08
8/12/06	EF x EM	5590	5539	8/11/08
8/12/06	EF x EM	5590	5539	8/14/08
8/12/06	EF x LM	5705	5505	8/16/08
8/12/06	EF x LM	5710	5510	8/19/08
8/12/06	EF x LM	5714	5514	8/12/08
8/12/06	EF x LM	5714	5514	8/13/08
8/12/06	EF x LM	5714	5514	8/24/08
8/12/06	EF x LM	5714	5514	8/24/08
8/12/06	EF x LM	5715	5515	8/11/08
8/12/06	EF x LM	5718	5517	8/13/08
8/12/06	EF x LM	5718	5518	8/10/08
8/12/06	EF x LM	5722	5521	8/14/08
8/12/06	EF x LM	5726	5525	8/26/08
8/12/06	EF x LM	5729	5529	8/24/08
8/12/06	EF x LM	5731	5532	8/17/08
8/12/06	EF x LM	5731	5532	8/23/08
8/12/06	EF x LM	5732	5531	8/26/08

Table A3 (Continued)

2007 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned
8/27/05	EF x EM	5659	5610	8/27/07
8/27/05	EF x EM	5659	5610	9/6/2007
9/9/05	LF x EM	5659	5810	9/3/2007
8/27/05	EF x EM	5660	5609	8/9/07
8/27/05	EF x EM	5660	5609	8/26/07
8/27/05	EF x EM	5660	5610	8/26/07
8/27/05	EF x EM	5660	5610	8/27/07
9/9/05	LF x EM	5660	5809	9/4/2007
9/9/05	LF x EM	5660	5809	9/5/2007
9/9/05	LF x EM	5660	5809	9/9/2007
9/9/05	LF x EM	5660	5810	9/6/2007
8/27/05	EF x EM	5662	5611	8/11/07
8/27/05	EF x EM	5662	5611	8/26/07
8/27/05	EF x EM	5663	5613	8/26/07
8/27/05	EF x EM	5663	5614	8/27/07
9/9/05	LF x EM	5663	5813	9/6/2007
8/27/05	EF x EM	5664	5614	8/9/07
8/27/05	EF x EM	5664	5614	8/26/07
9/9/05	LF x EM	5664	5814	8/28/07
8/27/05	EF x EM	5665	5615	9/5/2007
9/9/05	LF x EM	5665	5816	9/6/2007
8/27/05	EF x EM	5666	5615	8/28/07
8/27/05	EF x EM	5666	5616	9/11/2007
8/27/05	EF x EM	5667	5617	8/12/07
8/27/05	EF x EM	5667	5617	8/27/07

2008 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned
8/12/06	EF x LM	5733	5534	8/27/08
8/12/06	EF x LM	5735	5535	8/14/08
8/12/06	EF x LM	5735	5535	8/26/08
8/12/06	EF x LM	5736	5535	8/28/08
8/12/06	EF x LM	5736	5535	8/28/08
8/12/06	EF x LM	5736	5536	8/22/08
8/12/06	EF x LM	5740	5539	8/12/08
8/12/06	EF x LM	5740	5539	8/13/08
8/12/06	EF x LM	5740	5539	8/14/08
8/12/06	EF x LM	5740	5540	8/9/08

2007 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned
8/27/05	EF x EM	5667	5618	8/25/07
8/27/05	EF x EM	5667	5618	8/27/07
8/27/05	EF x EM	5667	5618	8/30/2007
8/27/05	EF x EM	5667	5618	8/31/2007
8/27/05	EF x EM	5668	5617	9/1/2007
8/27/05	EF x EM	5668	5617	9/2/2007
8/27/05	EF x EM	5668	5618	8/26/07
8/27/05	EF x EM	5669	5619	8/9/07
8/27/05	EF x EM	5669	5620	8/26/07
8/27/05	EF x EM	5670	5620	8/25/07
8/27/05	EF x EM	5670	5620	8/25/07
8/27/05	EF x EM	5671	5621	8/27/07

Table A3 (Continued)

2007 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned
8/27/05	EF x EM	5672	5621	9/1/2007
8/27/05	EF x EM	5673	5623	8/26/07
8/27/05	EF x EM	5674	5624	9/6/2007
8/27/05	EF x EM	5676	5625	8/25/07
8/27/05	EF x EM	5676	5625	9/5/2007
8/27/05	EF x EM	5676	5626	8/26/07
8/27/05	EF x EM	5677	5628	8/25/07
8/27/05	EF x EM	5677	5628	9/5/2007
8/27/05	EF x EM	5678	5627	9/2/2007
8/27/05	EF x EM	5678	5628	8/25/07
8/27/05	EF x EM	5678	5628	8/27/07
8/27/05	EF x EM	5678	5628	8/27/07
8/27/05	EF x EM	5680	5629	9/5/2007
8/27/05	EF x EM	5680	5629	9/5/2007
8/27/05	EF x EM	5681	5632	8/26/07
8/27/05	EF x EM	5681	5632	9/3/2007
8/27/05	EF x EM	5681	5632	9/6/2007

2007 F_1 Time of Return				
Spawned	Cross	Sire	Dam	Returned
8/27/05	EF x EM	5682	5631	8/29/2007
8/27/05	EF x EM	5682	5632	8/26/07
8/27/05	EF x EM	5682	5632	9/5/2007
8/27/05	EF x EM	5685	5635	8/28/07
8/27/05	EF x EM	5685	5635	9/6/2007
8/27/05	EF x EM	5685	5636	8/27/07
8/27/05	EF x EM	5685	5636	8/29/2007
8/27/05	EF x EM	5685	5636	9/12/2007
8/27/05	EF x EM	5686	5635	8/27/07
8/27/05	EF x EM	5686	5635	9/5/2007
8/27/05	EF x EM	5686	5636	8/26/07
8/27/05	EF x EM	5686	5636	8/27/07
8/27/05	EF x EM	5688	5637	9/5/2007
8/27/05	EF x EM	5689	5640	8/27/07
8/27/05	EF x EM	5690	5640	8/26/07
8/27/05	EF x EM	5690	5640	9/3/2007

Table A4 - Development rate, measured by accumulated temperature units (ATU's) at mid-hatch, of F_2 progeny from brood years 2007 and 2008. Parental sample numbers and type of cross are listed for each fish. Spawning was carried out on several different dates during each brood year.

2007 F_2 Embryonic Development					2008 F_2 Embryonic Development				
Spawned	Cross	Sire	Dam	ATU	Spawned	Cross	Sire	Dam	ATU
9/4	EF x EM	6102	5901	549.4	8/20	EF x EM	5327	5420	617.7
9/4	EF x EM	6102	5901	559.9	8/20	EF x EM	5327	5426	633.7
9/4	EF x EM	6102	5902	549.4	8/20	EF x EM	5327	5426	633.7
9/4	EF x EM	6102	5902	549.4	8/20	EF x EM	5327	5430	625.7
9/4	EF x EM	6103	5903	552.9	8/20	EF x EM	5327	5430	617.7
9/4	EF x EM	6103	5903	549.4	8/20	EF x EM	5327	5434	621.7
9/4	EF x EM	6103	5904	549.4	8/20	EF x EM	5327	5434	617.7
9/4	EF x EM	6103	5904	552.9	8/20	EF x EM	5327	5437	609.7
9/4	EF x EM	6104	5903	546	8/20	EF x EM	5327	5439	617.7
9/4	EF x EM	6104	5903	546	8/20	EF x EM	5327	5439	617.7
9/4	EF x EM	6104	5904	546	8/20	EF x EM	5327	5441	613.7
9/4	EF x EM	6104	5904	546	8/20	EF x EM	5327	5441	617.7
9/4	EF x EM	6105	5905	556.4	8/20	EF x EM	5329	5420	609.7
9/4	EF x EM	6105	5905	552.9	8/20	EF x EM	5329	5420	613.7
9/4	EF x EM	6105	5906	549.4	8/20	EF x EM	5329	5425	613.7
9/4	EF x EM	6105	5906	552.9	8/20	EF x EM	5329	5425	617.7
9/4	EF x EM	6106	5905	549.4	8/20	EF x EM	5329	5431	617.7
9/4	EF x EM	6106	5905	549.4	8/20	EF x EM	5329	5431	621.7
9/4	EF x EM	6106	5906	552.9	8/20	EF x EM	5329	5432	621.7
9/4	EF x EM	6106	5906	552.9	8/20	EF x EM	5329	5432	617.7
9/4	EF x EM	6107	5907	546	8/20	EF x EM	5329	5443	621.7
9/4	EF x EM	6107	5907	552.9	8/20	EF x EM	5329	5443	621.7
9/4	EF x EM	6107	5908	542.4	8/20	EF x EM	5329	6222	625.7
9/4	EF x EM	6107	5908	542.4	8/20	EF x EM	5329	6222	617.7
9/4	EF x EM	6109	5909	549.4	8/20	EF x EM	5330	5420	613.7
9/4	EF x EM	6109	5909	542.4	8/20	EF x EM	5330	5420	609.7

Table A4 (continued)

2007 F_2 Embryonic Development				
Spawned	Cross	Sire	Dam	ATU
9/4	EF x EM	6109	5910	546
9/4	EF x EM	6109	5910	549.4
9/4	EF x EM	6110	5909	549.4
9/4	EF x EM	6110	5909	549.4
9/4	EF x EM	6110	5910	549.4
9/4	EF x EM	6110	5910	546
9/10	EF x EM	6111	5911	541.3
9/10	EF x EM	6111	5911	544.9
9/10	EF x EM	6113	5914	548.5
9/10	EF x EM	6113	5914	552.1
9/10	EF x EM	6114	5914	548.5
9/10	EF x EM	6114	5914	555.6
9/10	EF x EM	6115	5915	544.9
9/10	EF x EM	6115	5915	544.9
9/10	EF x EM	6116	5915	537.8
9/10	EF x EM	6116	5915	544.9
9/10	EF x EM	6117	5917	537.8
9/10	EF x EM	6117	5917	541.3
9/10	EF x EM	6117	5918	555.6
9/10	EF x EM	6117	5918	552.1
9/10	EF x LM	6205	6005	534.5
9/10	EF x LM	6205	6005	537.8
9/10	EF x LM	6205	6006	548.5
9/10	EF x LM	6205	6006	552.1
9/10	EF x LM	6205	6007	552.1
9/10	EF x LM	6205	6007	544.9
9/10	EF x LM	6207	6008	548.5
9/10	EF x LM	6207	6008	548.5

2008 F_2 Embryonic Development				
Spawned	Cross	Sire	Dam	ATU
8/20	EF x EM	5330	5425	621.7
8/20	EF x EM	5330	5425	617.7
8/20	EF x EM	5330	5431	617.7
8/20	EF x EM	5330	5431	617.7
8/20	EF x EM	5330	5432	621.7
8/20	EF x EM	5330	5432	621.7
8/20	EF x EM	5330	5443	621.7
8/20	EF x EM	5330	6222	625.7
8/20	EF x EM	5330	6222	621.7
8/20	EF x EM	5331	5426	637.7
8/20	EF x EM	5331	5426	641.7
8/20	EF x EM	5331	5430	621.7
8/20	EF x EM	5331	5430	617.7
8/20	EF x EM	5331	5434	625.7
8/20	EF x EM	5331	5434	617.7
8/20	EF x EM	5331	5437	621.7
8/20	EF x EM	5331	5437	621.7
8/20	EF x EM	5331	5439	625.7
8/20	EF x EM	5331	5439	621.7
8/20	EF x EM	5331	5441	617.7
8/20	EF x EM	5331	5441	605.7
8/20	EF x EM	5332	5416	617.7
8/20	EF x EM	5332	5416	613.7
8/20	EF x EM	5332	5418	617.7
8/20	EF x EM	5332	5418	621.7
8/20	EF x EM	5332	5421	613.7
8/20	EF x EM	5332	5421	609.7
8/20	EF x EM	5332	5423	617.7

Table A4 (continued)

2007 F_2 Embryonic Development

Spawned	Cross	Sire	Dam	ATU
9/10	EF x LM	6207	6009	552.1
9/10	EF x LM	6207	6009	548.5
9/10	EF x LM	6207	6010	559.2
9/10	EF x LM	6207	6010	555.6
9/10	EF x LM	6208	6008	555.6
9/10	EF x LM	6208	6008	544.9
9/10	EF x LM	6212	6014	552.1
9/10	EF x LM	6212	6014	548.5
9/10	EF x LM	6212	6015	548.5
9/10	EF x LM	6212	6015	552.1
9/10	EF x LM	6213	6008	534.5
9/10	EF x LM	6213	6008	559.2
9/10	EF x LM	6213	6017	537.8
9/10	EF x LM	6213	6017	537.8
9/10	EF x LM	6214	6008	541.3
9/10	EF x LM	6214	6008	548.5
9/10	LF x EM	6209	6011	548.5
9/10	LF x EM	6209	6011	548.5
9/10	LF x EM	6209	6012	544.9
9/10	LF x EM	6209	6012	548.5
9/10	LF x EM	6211	6016	548.5
9/10	LF x EM	6211	6016	552.1
9/10	LF x LM	6111	5912	548.5
9/10	LF x LM	6111	5912	548.5
9/10	LF x LM	6112	5912	552.1
9/10	LF x LM	6112	5912	552.1
9/10	LF x LM	6119	5919	544.9
9/10	LF x LM	6119	5919	537.8

2008 F_2 Embryonic Development				
Spawned	Cross	Sire	Dam	ATU
8/20	EF x EM	5332	5423	617.7
8/20	EF x EM	5332	5424	625.7
8/20	EF x EM	5332	5424	625.7
8/20	EF x EM	5332	5440	617.7
8/20	EF x EM	5332	5440	613.7
8/20	EF x EM	5333	5416	625.7
8/20	EF x EM	5333	5416	617.7
8/20	EF x EM	5333	5418	621.7
8/20	EF x EM	5333	5418	617.7
8/20	EF x EM	5333	5421	617.7
8/20	EF x EM	5333	5421	613.7
8/20	EF x EM	5333	5423	621.7
8/20	EF x EM	5333	5423	617.7
8/20	EF x EM	5333	5424	617.7
8/20	EF x EM	5333	5424	625.7
8/20	EF x EM	5333	5440	621.7
8/20	EF x EM	5333	5440	617.7
8/20	EF x EM	5334	5435	617.7
8/20	EF x EM	5334	5435	617.7
8/20	EF x EM	5334	5436	609.7
8/20	EF x EM	5334	5436	605.7
8/20	EF x EM	5334	5438	609.7
8/20	EF x EM	5334	5438	605.7
8/20	EF x EM	5334	5442	609.7
8/20	EF x EM	5334	5442	609.7
8/20	EF x EM	5334	5443	609.7
8/20	EF x EM	5334	5443	609.7
8/20	EF x EM	5334	5444	609.7

Table A4 (continued)

2007 F_2 Embryonic Development

Spawned	Cross	Sire	Dam	ATU
9/15	EF x LM	6217	6020	541.2
9/15	EF x LM	6217	6020	537.7
9/15	LF x EM	6216	6021	551.6
9/15	LF x EM	6216	6021	551.6
9/15	LF x LM	6120	5920	520.1
9/15	LF x LM	6120	5920	541.2
9/15	LF x LM	6120	5921	551.6
9/15	LF x LM	6120	5921	534.2
9/15	LF x LM	6122	5924	534.2
9/15	LF x LM	6122	5924	541.2
9/22	LF x LM	6125	5929	527
9/22	LF x LM	6125	5929	527

2008 F_2 Embryonic Development

Spawned	Cross	Sire	Dam	ATU
8/20	EF x EM	5334	5444	609.7
8/20	EF x EM	5334	5445	613.7
8/20	EF x EM	5334	5445	609.7
8/20	EF x EM	6039	5435	617.7
8/20	EF x EM	6039	5435	621.7
8/20	EF x EM	6039	5436	617.7
8/20	EF x EM	6039	5436	609.7
8/20	EF x EM	6039	5438	617.7
8/20	EF x EM	6039	5438	621.7
8/20	EF x EM	6039	5442	617.7
8/20	EF x EM	6039	5442	609.7
8/20	EF x EM	6039	5443	617.7
8/20	EF x EM	6039	5443	609.7

2008 F_2 Embryonic Development				
Spawned	Cross	Sire	Dam	ATU
8/20	EF x EM	6039	5444	617.7
8/20	EF x EM	6039	5444	617.7
8/20	EF x EM	6039	5445	617.7
8/20	EF x EM	6039	5445	617.7
8/20	EF x LM	5103	5204	617.7
8/20	EF x LM	5103	5204	621.7
8/20	EF x LM	5103	5204	621.7
8/20	EF x LM	5103	5205	617.7
8/20	EF x LM	5104	5204	617.7
8/20	EF x LM	5104	5204	617.7
8/20	EF x LM	5104	5205	613.7
8/20	EF x LM	5104	5205	609.7
8/20	EF x LM	5105	5204	617.7
8/20	EF x LM	5105	5204	617.7
8/20	EF x LM	5105	5205	617.7
8/20	EF x LM	5105	5205	617.7
8/20	EF x LM	5107	5204	617.7
8/20	EF x LM	5107	5205	613.7
8/20	EF x LM	5107	5205	609.7
8/20	EF x LM	5107	5205	617.7
8/20	EF x LM	5108	5204	617.7
8/20	EF x LM	5108	5204	613.7
8/20	EF x LM	5108	5205	609.7
8/20	EF x LM	5108	5205	613.7
8/25	EF x LM	5109	5207	597.8
8/25	EF x LM	5109	5207	601.8
8/25	EF x LM	5109	5209	593.8
8/25	EF x LM	5109	5209	597.8

Table A4 (continued)

2008 F_2 Embryonic Development				
Spawned	Cross	Sire	Dam	ATU
8/29	EF x LM	5109	5208	606.4
8/29	EF x LM	5109	5208	602.9
8/29	EF x LM	5109	5210	594.9
8/29	EF x LM	5109	5210	590.9
8/29	EF x LM	5109	5213	590.9
8/29	EF x LM	5109	5213	582.9
8/29	EF x LM	5109	5214	606.4
8/29	EF x LM	5109	5214	602.9
8/29	EF x LM	5111	5208	606.4
8/29	EF x LM	5111	5208	606.4
8/29	EF x LM	5111	5210	602.9
8/29	EF x LM	5111	5210	602.9

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Spawned	Cross	Sire	Dam	ATU
8/29	EF x LM	5111	5213	598.9
8/29	EF x LM	5111	5213	590.9
8/29	EF x LM	5111	5214	602.9
8/29	EF x LM	5111	5214	602.9
8/20	EF x LM	5104	5205	609.7
8/20	EF x LM	5105	5204	617.7
8/20	EF x LM	5105	5204	617.7
8/20	EF x LM	5105	5205	617.7
8/20	EF x LM	5105	5205	617.7
8/20	EF x LM	5107	5204	617.7
8/20	EF x LM	5107	5205	613.7
8/20	EF x LM	5107	5205	609.7