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Local adaptation of phenology revealed in outcrosses between spawning segments of a salmonid population

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Abstract

Local adaptation has been demonstrated in spatially or temporally distant animal populations but seldom in proximate populations. To address the scale of local adaptation in Pacific salmon (*Oncorhynchus* spp.), two generations of hybrids between temporally separated spawning segments were made in a population of pink salmon (*O. gorbuscha*) and compared with controls to evaluate the genetic architecture underlying adult migration time and to test for declines in marine survival that resulted from outbreeding depression. Bayesian mixed-effects models revealed that adult migration times in hybrid lines were intermediate to those of controls and that additive sources of genetic variation were significant, thereby indicating that local adaptation has acted on additive genetic variation in shaping this trait. Similarly, a line cross analysis revealed that an additive model best described the genetic architecture of adult migration time. In contrast, marine survival was generally similar between control and hybrid lines, which suggested that the effect of outbreeding upon marine survival was minimal at such a fine scale of genetic divergence. The implications of these results are that (1) local adaptation can facilitate genetic divergence of life history traits between proximate subpopulations; (2) artificial relaxation of natural barriers to gene flow can cause maladaptive shifts in life history traits; and (3) wild populations may harbor fine-scale adaptive variation that supports productivity and sustainability.

Introduction

Genetic variation in breeding time has been demonstrated in populations spanning a wide range of taxa, and individuals within these populations often have life history traits that are locally adapted to environmental conditions characteristic of their breeding time (e.g. birds: Møller 2001; Sheldon *et al.* 2003; fish: Smoker *et al.* 1998; Hendry *et al.* 1999; Quinn *et al.* 2000; plants: Weis and Kossler 2004; Hall and Willis 2006). Consistent differences in breeding time constrains gene flow between population segments, which facilitates genetic divergence through local adaptation, provided the effects of divergent selection exceed those of genetic drift and gene flow and there is sufficient genetic variability in the traits under selection (Kawecki and Ebert 2004). By optimizing fitness in individuals that breed at different times, local adaptation staggers the use of resources over time, which may increase the carrying capacity of resource-limited populations (Gharrett *et al.* 2013). Additionally, local adaptation promotes genetic divergence of phenology, which can enable populations to sustain productivity during climate changes (Greene *et al.* 2010; Schindler *et al.* 2010). Hence, local adaptation is likely an important component of the productivity and sustainability of populations that exhibit genetic variation in breeding time.

Because of their characteristic ability to home to spawn in their natal habitat with high temporal precision and the wealth of life history variation that they exhibit over their extensive range, anadromous Pacific salmon have been the focus of many studies on local adaptation (Fraser *et al.* 2011). The high fidelity with which salmon typically home enables adaptation to local niches within heterogeneous ecological landscapes, while constraining gene flow between diverging salmon populations. This pattern of population divergence due to local adaptation is probably the primary means by which many genetically distinct populations have come to exist in each of the anadromous Pacific salmon species (Carvalho 1993). Local adaptation is well documented in salmonid populations separated by large geographic distances (Taylor 1991), but there has been a dearth of research on fine-scale adaptive differences that may arise between proximate salmon populations. Stock transfers and hatchery propagation, which have been increasingly used for Pacific salmon, are typically conducted at a fine spatiotemporal scale, and there is concern that these artificial propagation practices are enabling introgression of non-adapted genetic material into wild populations, thereby disrupting local adaptation and reducing

diversity that is a crucial buffer against inexorable environmental changes (Waples 1991; Eldridge and Naish 2007; Naish *et al.* 2008). Furthermore, interbreeding between locally adapted populations can reduce fitness in hybrids, a phenomenon known as outbreeding depression (Lynch 1991; Gharrett *et al.* 1999; Edmands 1999; Gilk *et al.* 2004).

Outbreeding depression manifests through two different mechanisms that can occur independently or jointly. Outbreeding between populations from different local environments can depress fitness by disrupting fitness-related genotype-by-environment interactions (Edmands 2007). This mechanism, which is termed ecological outbreeding depression, may manifest as early as the first generation because the additive effects of genes contributed from each parent can result in an immediate and disruptive shift in the phenotype (Lynch 1991). Alternatively, outbreeding between genetically isolated populations can disrupt complexes of genes at epistatic loci, leading to a decline in fitness. Epistatic gene complexes can arise through joint selection for multiple loci during local adaptation and random drift (Lynch 1991). Epistatic outbreeding depression typically does not manifest until the second generation or later (Emlen 1991) because epistatic gene complexes are maintained in the gamete contributed by each parent.

Pink salmon that home to Auke Creek, Alaska provide an excellent model system for examining local adaptation that results from varying scales of genetic isolation. The strict two-year anadromous life cycle of pink salmon (Anas 1959; Turner and Bilton 1968) has resulted in genetically distinct odd- and even-year brood lines in Auke Creek that are completely genetically isolated. Within each brood line, adults return to Auke Creek in two distinct spawning segments; 'early-migrating' adults return between mid- and late August, and 'late-migrating' adults return between early and mid-September (Taylor 1980). The early- and late-migrating segments are partially genetically isolated and exhibit genetically-based differences in phenology (Hebert et al. 1998) despite high levels of gene flow that have resulted in minimal differentiation in selectively neutral alleles (McGregor et al. 1998). The structuring of the spawning migration appears to be maintained by the high heritability of migration time within each of these segments ($h^2 = 0.4$; Smoker et al. 1998). The odd- and even-year brood lines provide a model of large-scale genetic isolation, whereas the early- and late-spawning segments provide a model of fine-scale genetic isolation.

Local adaptation that results from large-scale genetic isolation has been addressed by evaluating hybrids between the even- and odd-brood lines at Auke Creek. In a study of two generations of hybrids between the brood lines, reduced marine survival relative to controls was observed in second-generation hybrids only, which indicated that outbreeding depression had resulted from disruption of epistatic gene complexes (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, Kodiak, Alaska (Gilk *et al.* 2004). These studies demonstrated that large temporal and spatial barriers have enabled populations of salmon to diverge, most likely through some combination of local adaptation and genetic drift, and that removal of such barriers can have detrimental effects on overall fitness. However, the effects of disrupting fine-scale barriers to gene flow, such as the barrier separating the seasonal spawning segments, have not yet been investigated in this population.

To address whether local adaptation can arise from fine-scale genetic isolation, out crosses between the early- and late-spawning segments at Auke Creek were evaluated in this study. Specifically, comparisons were made between controls and two generations of hybrids between these spawning segments to look for evidence of outbreeding effects on two traits that contribute to lifetime fitness: adult migration time and marine survival. Because adult migration time is closely associated with spawning time, the assumed driver of local adaptation, we might expect this trait to show a considerable degree of local adaptation. The association between marine survival and spawning time is less clear, but we might expect epistatic outbreeding depression to manifest as a decline in marine survival in hybrids, as has been demonstrated in previous hybridization studies at Auke Creek. This study evaluates the importance of fine-scale local adaptation by addressing the following questions (1) Do hybrids exhibit migration times intermediate to early- and late-migrating controls that would provide evidence of local adaptation of migration time? (2) To what extent does additive genetic variation contribute to migration time and are other sources of genetic variation (e.g. dominance) important? and (3) Does outbreeding depression result in reduced marine survival of second-generation hybrids that would indicate that local adaptation has produced distinct epistatic gene complexes in the spawning segments?

Materials and Methods

Field methods

Auke Creek, a short (323 m) outlet steam of Auke Lake that drains into Auke Bay, is a spawning ground and migratory corridor of pink salmon. Located at the mouth of Auke Creek and at the head of tidewater is Auke Creek Station, a permanent counting weir and experimental salmon hatchery, which are operated by the U.S. National Marine Fisheries Service. Early- and late-migrating pink salmon were collected at the weir and artificially spawned at the hatchery in the summers of 2005 and 2006 to create first-generation (F_1) hybrid and control lines, which were propagated into the second generation (F_2) by artificially spawning returning F_1 progeny that were collected at the weir in 2007 and 2008. This experiment was carried out in accordance with policy and regulations of the State of Alaska and in accordance with ethical standards for the care and use of vertebrate animals approved by the University of Alaska Fairbanks Institutional Animal Care and Use committee.

The first-generation breeding design was structured to enable the development rates of hybrids to be compared with those of early and late controls (Echave et al. 2017). To minimize environmental effects, each control line was incubated in the same thermal regime as its hybrid counterpart; since early and late controls were spawned on separate days, this required semen to be cryopreserved to create separate early and late hybrid lines. Although cryopreservation may result in mutations of genes related to migration time, we note that in zebrafish (*Danio rerio*) only small mutation rates between 10^{-4} and 10^{-5} have been observed from cryopreservation (Riesco and Robles 2014), too low to substantially influence genes that contribute to migration time. The run source (i.e. early vs. late) of each experimental line was defined by the run source of the female parent. For instance, an early-female by late-male cross was considered an early hybrid. The first-generation breeding design was accomplished by using gametes collected from the earliest migrating males and females (22 August 2005; 3, 4, and 5 August 2006) to produce early controls in each brood year; semen collected from the early-migrating males in 2005 was cryopreserved to create late hybrids in the odd-brood line with eggs collected from the latest migrating females on 7 September 2005. We did not produce a late hybrid line in the even-brood line because cryopreservation of semen from early-migrating males in 2006 failed. We used cryopreserved

semen collected from the latest migrating males two brood years prior (11 September 2001; 6 and 9 September 2002) and eggs collected from early- and late-migrating females on the aforementioned dates to produce early hybrids and late controls in each brood year. Because of our failure to produce late hybrids in 2006, we did not release a corresponding late control line in the even-brood line.

The first-generation breeding design resulted in the production of eighty full-sib families (Table 1). Second-generation experimental lines were created from returning first-generation adults. The requirement of using only experimental fish as brood stock led to an imbalanced breeding design with smaller numbers of families in the second generation of the experiment (Table 1).

Identical methods were used to rear first- and second-generation progeny. Control and hybrid embryos were incubated in separate stacks of vertical incubator trays at Auke Creek Station in ambient temperature water that was pumped from the creek. Developing embryos were incubated until they were ~5% yolk by weight, whereupon the fish were anesthetized and subsequently marked with an experiment-identifying adipose fin excision and contrasting 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 outmigration, and all marked adults were collected at Auke Creek Station as they migrated into the stream during summer of the following year and examined for the absence of an adipose or pelvic fin to determine if they belonged to one of our experimental lines. Tissue samples were obtained from each marked adult by clipping the axillary process at the base of the remaining pelvic fin. During the summers of 2007 and 2008, marked adults were tagged with numbered Floy^{TM} (Floy Tag Inc., Seattle, WA) tags and held captive until full maturity. A randomly selected sample of those fish was used as broodstock for the second-generation crosses. Identical methods were used to sample marked second-generation fish that returned in the summers of 2009 and 2010, with the exception that none were retained as broodstock.

Laboratory methods

Tissue samples from experimental broodstock and their returned progeny were stored in numbered vials of preservative solution (Seutin *et al.* 1991) and stored at approximately -20 ˚C in a conventional freezer. We isolated total genomic DNA with DNeasy Blood and Tissue kits

(QIAGEN, Inc., Valencia, CA). Five microsatellite loci (*Ogo1a* [Olsen *et al.* 1998]; *Oki10* [Smith *et al.* 1998]; *One102* [Olsen *et al.* 2000]; *One109* [Olsen *et al.* 2000]; and *OtsG311* [Williamson *et al.* 2002]) were chosen to unequivocally assign parental pairs to progeny. Amplification and separation of target fragments of microsatellite loci were accomplished with methods that are detailed in the supporting information.

Statistical methods

Parentage - Microsatellite genotype information was used to assign parental pairs to returning adults with PROBMAX (Version 1.2; Danzmann 1997). Parentage assignment was confirmed when the genotype of an individual was consistent with those of a prospective parental pair for all five loci (10/10 alleles). In instances where a near perfect match (9/10 alleles) was observed between an individual and a pair of parents, an additional pair of microsatellite loci (*Ots103* [Small *et al.* 1998] and *Ots208* [Greig *et al.* 2003]) was used to confirm the assignment of those parents. Parentage information was used to determine the experimental line to which each returning adult fish belonged and to assign fish to their respective full-sib families.

Adult migration time - Components of variation of adult migration time of first- and second-generation experimental lines were quantified with linear mixed-effects models under a Bayesian framework. Additive genetic components of variation arising from covariance among siblings were estimated as random effects, while components of variation arising from experimental treatment (e.g. type of cross) were estimated as fixed effects. Samples from the posterior distribution of each effect were drawn with the MCMC algorithm, which was conducted in R (R Core Team 2015) with the package 'MCMCglmm' (Hadfield 2010). This analysis was performed separately for the early and late experiments in each generation in order to avoid confounding the effects of run source with those of cross.

The linear mixed-effects model that describes all pertinent fixed and random effects on adult migration time within a brood year (2005, 2006, 2007, or 2008) and run (early or late) was:

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

where y_{ijkl} is the Julian date of weir passage (i.e. migration time) of an individual. The overall mean migration time is μ, C_i is the fixed effect of the i^{th} cross (hybrid or control), D_{ij} is the random effect of the j^{th} dam within the i^{th} cross, 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 l^{th} replicate of the j^{th} dam and k^{th} sire within the i^{th} cross.

Non-informative, yet proper priors were used for the fixed and random effects. A normal, zero-mean prior with a large variance was selected for C_i:

$$C_i \sim N(\mu = 0,\ \sigma^2 = 1\ x\ 10^{10})$$

The inverse-gamma is the classical prior distribution for variance components, but this prior can cause inefficient sampling of the posterior distribution of small variances. To address this, a method known as parameter expansion (Gelman *et al.* 2008; Browne *et al.* 2009) was used to give more flexibility to the MCMC algorithm by partitioning each random effect R_t into two independent components:

$$R_t = \alpha\eta_t$$

$$\alpha \sim N(0, 1000)$$

$$\eta_t \sim N\left(0,\ \sigma_\eta^2\right)$$

$$\sigma_\eta^2 \sim InvGam(0.5, 0.5)$$

A useful feature of the MCMCglmm function is that it enables computations of the posterior distribution of functions of variance components. For instance, the proportion of variation that is attributable to covariance among maternal (P_D) and paternal (P_S) siblings can be calculated as functions of the posterior values of the variance components:

$$P_D = \frac{D_{ij}}{D_{ij} + S_{ik} + \varepsilon_{ijkl}}$$

$$P_S = \frac{S_{ik}}{D_{ij} + S_{ik} + \varepsilon_{ijkl}}$$

This gives the posterior distributions of meaningful parameters that describe how additive genetic components contribute to adult migration time.

In each model, the statistical significance of the fixed effect of cross was evaluated simply by determining whether the 95% Bayesian credible interval of its posterior distribution included zero. Statistical significance of variance components was interpreted with the deviance information criterion (DIC). Since smaller DIC values are indicative of a better model fit, the difference in the DIC between a full model and one with the variance component of interest removed (ΔDIC) was calculated and included in the model summary. In general, a ΔDIC value

252 of five is considered substantial, and a ΔDIC value of greater than ten is adequate to rule out the
253 model with the higher DIC (Spiegelhalter *et al.* 2002).

254 *Line cross analysis* - Because returning F_1 fish carried a mark that denoted the type of
255 cross but not the run source, some F_1 hybrids (i.e. early control x late control) were produced in
256 the second generation of the odd-broodline experiment (Supporting information; Table S2).
257 Consequently, F_1 and F_2 hybrid lines were reared with early and late control lines that year. The
258 existence of F_1 hybrids presented the opportunity to use a line cross analysis to evaluate the
259 genetic architecture underlying adult migration time. Adopting the nomenclature of Lynch and
260 Walsh (1998), the early and late control lines are referred to as the parental lines (P_1 and P_2,
261 respectively) in this analysis. The types of genetic parameters that can be estimated in a line
262 cross analysis are determined by the number of lines, and the four lines present in the second
263 generation of the odd-broodline experiment (P_1, P_2, F_1, and F_2) provide sufficient degrees of
264 freedom to test for additive and dominance effects. The joint scaling procedure described in
265 Lynch and Walsh (1998) was used to perform the line cross analysis. This procedure involves
266 first testing the fit of a null model with additive effects only. If the additive model is rejected by
267 a chi-square test, a higher order null model with additive and dominance effects can be tested. A
268 statistical power analysis was performed for the null model with a bootstrap simulation. New
269 data were simulated by drawing a new mean for each line from a normal distribution with mean
270 and variance equal to the weighted mean of the line ($\bar{Z}_J$) and its standard error, respectively; the
271 null model was then re-fit with the new data. The statistical power was estimated as the
272 percentage of times in which the null model was rejected over 10,000 iterations. The statistical
273 power analysis was conducted over different scenarios in which the number of full-sib families
274 present in each line ranged from 1 to 100.

275 *Marine survival* - A Bayesian hierarchical analysis was used to quantify marine survival
276 in the first and second generation. The Bayesian approach produces posterior distributions of
277 parameters of interest, which enables straightforward comparisons of parameters between
278 experiments. Samples from the posterior distribution of each parameter were drawn with the
279 Markov chain Monte Carlo (MCMC) algorithm, which was performed by using the package
280 'R2WinBUGS' (Sturtz *et al.* 2005) to call WinBUGS (Lunn *et al.* 2000) from R. The likelihood

of observing y_{ij} returned adults from the i^{th} family and within the j^{th} experimental line followed a binomial distribution:

$$y_{ij} \sim Bin(p_{ij}, n_{ij})$$

where p_{ij} is the marine survival proportion of n_{ij} released fry from a family.

The hierarchical framework specifies that the marine survival proportions of individual families are drawn from a common distribution that is specific to a given experimental line. This assumption is based on the idea that, within an experimental line, offspring from different families should exhibit marine proportions that are similar because of experimental treatment, but different because of environmental and genetic effects that contribute to marine survival. Specifically, the logit (i.e. log-odds ratio) of the survival proportion of each family (l_{ij}) followed a normal distribution:

$$l_{ij} = \log\left(\frac{p_{ij}}{1 - p_{ij}}\right)$$

$$l_{ij} \sim N(\mu_j, \tau_j)$$

where the hyperparameters that govern the distribution of the logits are the mean (μ_j) and the inverse (τ_j) of the variance, which WinBUGS accommodates as the variance parameter in normal distributions.

Non-informative, yet proper priors were used for the hyperparameters, μ_j and τ_j. A normal zero-mean prior with a large variance was used for μ_j, and a gamma prior with parameters that yield a large variance was used for τ_j:

$$\mu_j \sim N(\mu = 0,\ \sigma^2 = 1\ x\ 10^6)$$

$$\tau_j \sim Gam(\alpha = 0.001, \beta = 0.001)$$

The overall mean survival proportion of the j^{th} experimental line (p_j) and its standard deviation on the logit scale (σ_j), were calculated as functions of μ_j and τ_j:

$$p_j = \frac{\exp(\mu_j)}{1 + \exp(\mu_j)}$$

$$\sigma_j = \sqrt{1/\tau_j}$$

Because the parameter σ_j provided a measurement of variation in survival among families, comparisons of its posterior distributions among experimental lines enabled us to evaluate whether outbreeding affected some families more than others.

Results

Mixed-effects model of adult migration time - Data from 169 returned adults from broodyear 2005 and 112 adults from broodyear 2006 were used in the analysis of migration time of adults from the first generation of this experiment (Table 2). In broodyear 2005, the mean posterior estimate of adult migration date was 3.6 days later in early hybrids relative to early controls, and 3.8 days earlier in late hybrids relative to late controls (Figure 1). Further, 95% Bayesian credible intervals (BCIs) indicated that the effect of cross was significant in both the early (0.8 – 6.5) and late (-6.2 – -1.5) experiments of this brood year. Similar results were observed in broodyear 2006, in which the mean posterior estimate of adult migration date was 10 days later in early hybrids relative to early controls (Figure 1), and the effect of cross was significant (6.6 – 13.6). In both experiments from broodyear 2005, BCIs of the random effects of dam and sire were broad and included zero, and only the inclusion of the dam term from the late experiment produced a moderate change in DIC (-6.7). In the early experiment from broodyear 2006, only the inclusion of the sire term produced a large change in DIC (-19.2), and the BCI of P_S (6.4 – 56.9%) suggested that the sire term explained a significant amount of the variation in migration time.

Data from 606 returned adults from broodyear 2007 and 521 adults from broodyear 2008 were used in the analysis of migration time of second-generation adults (Table 3). In broodyear 2007, the mean posterior estimate of adult migration date was 4.4 days later in early hybrids relative to early controls and 4.9 days earlier in late hybrids relative to late controls (Figure 2), and BCIs indicated that the effect of cross was significant in both the early (1.6 – 7.5) and late (-9.7 – -0.3) experiments. Similar results were observed in broodyear 2008, in which the mean posterior estimate of adult migration date was 7.4 days later in early hybrids relative to early controls (Figure 2), and the effect of cross was significant (4.8 – 10.1). In broodyear 2007, large

changes in DIC accompanied the inclusion of the dam term in the early experiment (-11.1) and the sire term in the late experiment (-14.4), and the BCI of P_D in the early experiment (2.0 – 28.9%) and P_S in the late experiment (2.8 – 32.8%) suggested that these terms explained a significant amount of the variation in migration time. In the early experiment of broodyear 2008, only the inclusion of the sire term produced a large change in DIC (-9.3); the BCI of P_S, however, had a lower limit close to zero (0.0 – 29.9%).

Line cross analysis - A line cross analysis was conducted to evaluate migration time of adults from the second-generation odd-year broodline experiment. The number of families that had at least one returning adult ranged from 6 in the P_2 line to 37 in the F_2 line. The weighted mean Julian dates of migration in the F_1 and F_2 lines were similar and approximately intermediate to those of the P_1 and P_2 lines (Supporting information; Table S3). The estimated additive composite effect from the additive null model was significant ($\widehat{\alpha_1^c}$ = -3.460; SE = 0.537). The chi-square test of the additive null model was not significant (P = 0.352) and, hence, a higher order model that incorporated directional dominance was not tested.

Hierarchical model of marine survival - Marine survival proportions from 194 families from broodyear 2005 and 112 families from broodyear 2006 were used in the Bayesian hierarchical analysis of first-generation marine survival (Table 4). In broodyear 2005, 95% BCIs indicated that the marine survival percentage (p_j x 100) of early controls (0.45 – 0.77%) exceeded that of early hybrids (0.09 – 0.36%), but that late controls (0.08 – 0.32%) and late hybrids (0.12 – 0.60%) had similar survival percentages. In broodyear 2006, early controls (0.19 – 0.38%) and early hybrids (0.18 – 0.47%) had similar marine survival percentages. Except for the early control line from broodyear 2005, the posterior distributions of p_j overlapped substantially among all first-generation experimental lines. In broodyear 2005, the logit-scale standard deviation of marine survival was similar between early controls (0.03 – 0.63) and early hybrids (0.04 – 1.64) and between late controls (0.05 – 1.60) and late hybrids (0.03 – 1.75). Similarly, in broodyear 2006, the logit-scale standard deviation was similar between early controls (0.11 – 1.08) and early hybrids (0.03 – 0.93).

Marine survival proportions from 65 families from broodyear 2007 and 69 families from broodyear 2008 were used in the Bayesian hierarchical analysis of second-generation marine survival (Table 5). In broodyear 2007, BCIs indicated that the mean marine survival percentages

were similar between early controls (1.13 – 1.64%) and early hybrids (1.06 – 1.77%) and between late controls (1.01 – 2.18%) and late hybrids (1.13 – 1.89%). Similarly, in broodyear 2008, the marine survival percentages were similar between early controls (1.08 – 1.51%) and early hybrids (0.75 – 1.53%). Collectively, the posterior distributions of p_j overlapped substantially among all second-generation experimental lines. In broodyear 2007, the logit-scale standard deviation of family-specific marine survival was similar between early controls (0.07 – 0.49) and early hybrids (0.03 – 0.61) and between late controls (0.03 – 0.76) and late hybrids (0.25 – 0.75). In broodyear 2008, early controls (0.25 – 0.61) and early hybrids (0.13 – 0.97) had similar logit-scale standard deviations.

Discussion

Adult migration time - The highlight of this study was a clear demonstration of intermediate phenotypic expression of adult migration time in hybrids relative to controls over two generations of outbreeding, which indicates that local adaptation has acted to shape this trait, even in the presence of substantial gene flow. Furthermore, the pattern of trait expression was consistent with genes influencing migration time in an additive manner. This is reinforced by the fact that, when large numbers of observations were available, significant dam and sire components of variation in this trait were detected. The line cross analysis provided further support of this observation by demonstrating that significant additive effects contribute to adult migration time and suggesting that a model with only additive effects was sufficient to explain the genetic architecture underlying this trait. However, the power of a line cross analysis is, in part, a function of the number of families that are included in the experimental design. This experiment was not designed for a line cross analysis, and some of the experimental lines had few representative families (e.g. P_2 and F_1). Consequently, the standard errors of the weighted-mean migration times were high in those lines (Supporting information; Table S3). Indeed, a statistical power analysis revealed that the power of the chi-square test of the null additive model was only modest (0.41). This means that, even if directional dominance effects contribute to this trait, the experimental design only yielded a moderate chance of detecting them. The use of a balanced design with the same average number of families per line would have increased the

power to 0.5. In order to attain a statistical power of 0.8, the average number of families per line would have had to have been nearly doubled.

Because optimal adult migration time is involved in maximizing reproductive success in this population (Gharrett *et al.* 2013), the observed shift in this trait provided the basis for ecological outbreeding depression. A significant source of mortality in developing pink salmon embryos is mechanical agitation resulting from redd disturbance by subsequent spawners (Fukushima *et al.* 1998). The bimodal migration distribution of Auke Creek pink salmon enables early-run embryos to develop to a mechanically-resistant developmental stage known as epiboly (Ballard 1973) before the arrival of late-run spawners about two weeks later, thereby reducing mortality from redd disturbances (Smoker *et al*. 1998). The adaptive significance of embryonic development rate is supported by common-garden experiments that have revealed that early- and late-run embryos exhibit genetically-based differences in development patterns (Hebert *et al.* 1998) and that early-run embryos complete epiboly faster than late-run embryos (Joyce 1986). Further, early-run embryos require approximately two weeks to complete epiboly, which is consistent with the two weeks that have historically separated the peaks of the early- and late-spawning segments; the implication is that embryonic development trajectory is adapted to the time of egg deposition in the early-migrating subpopulation. Hence, our demonstration of an intermediate migration date (Figures 1, 2) and, by extension, intermediate egg deposition time in hybrids suggests that outbreeding between these spawning segments can disrupt local adaptation by rendering hybrid embryos more prone to mortality from redd superimposition by late-spawning adults. This mechanism has likely contributed to the maintenance of genetically distinct spawning segments in this population.

Marine survival - Similar marine survival rates were observed among five of the six experimental lines from the first generation, with only the early controls from the odd-year brood line exhibiting divergent survival rates (Tables 4, 5). The mechanisms contributing to the comparatively high marine survival rate in this experimental line are unknown and, although considerable care was taken to minimize systematic differences in survival arising from experimental treatment, the possibility that experimental biases underlie this observation cannot be eliminated. The lack of similarly high marine survival in the two other control lines, relative to their hybrid counterparts, suggests that we have no reasonable basis for concluding that one

418 generation of outbreeding between the early and late subpopulations influenced the likelihood of
419 surviving the marine stage. This is not a surprising result, given that previous studies conducted
420 on local adaptation in highly segregated pink salmon populations revealed that depression of
421 marine survival generally did not occur in the first generation of hybridization (Gharrett *et al.*
422 1999; Gilk *et al.* 2004). Furthermore, although early- and late-run juveniles transitioning to the
423 nearshore marine environment generally encounter seasonal differences in growth conditions and
424 predator abundance (Mortensen *et al.* 2000), differences in traits that confer adaptation to the
425 marine environment have not yet been characterized in these subpopulations. Hence, there is no
426 hypothetical basis to support the potential for ecological outbreeding depression of marine
427 survival, and this is the mechanism that would be most likely to cause depression of this trait in
428 first generation hybrids.

429 Similar marine survival rates were observed among all six second-generation experiment
430 lines, and there was therefore no evidence of effects of outbreeding on marine survival in F_2
431 hybrids. These results contrast those of experiments on populations of pink salmon separated by
432 large temporal or spatial barriers to gene flow, which revealed significantly lower marine
433 survival in F_2 hybrids than in controls (Gharrett *et al.* 1999; Gilk *et al.* 2004). Those experiments
434 detected reduced survival almost exclusively in the second generation, which suggested that
435 outbreeding had disrupted local adaptation primarily by the segregation of co-adapted gene
436 complexes (i.e. epistatic outbreeding depression). This hypothesis was particularly well
437 supported in the experiment that examined hybridization between the even- and odd-year brood
438 lines; because individuals from the two brood lines spawn in the same habitat and are likely to
439 encounter, on average, similar environmental conditions, there was little reason to expect
440 hybridization to disrupt genotype-by-environment interactions. However, without regular
441 exchange of migrants, natural selection and genetic drift may drive the formation of distinct co-
442 adapted gene complexes within those isolated populations. Conversely, there is opportunity for
443 interbreeding between the early- and late-migrating segments at Auke Creek, depending on
444 environmental characteristics (e.g. stream flow and temperature) that determine the date of creek
445 entry of adults. Regular exchange of migrants probably constrains the development of distinct
446 co-adapted gene complexes (Edmands 1999), which could explain why experiments that

examined local adaptation at large scales of genetic isolation yielded results that differed from those of this experiment, which examined local adaptation at a fine scale.

Family-specific marine survival - Although two generations of outbreeding did not produce evidence of differences in family survival between controls and hybrids, outbreeding between spawning segments may still have an impact on population viability if outbreeding effects manifest differently among hybrid families. That is, if marine survival of some families is impacted by outbreeding more so than others, an increase in variance of family size and a corresponding decline in the effective population size, N_e, could occur. To address this, posterior inference of family-specific marine survival proportions was made within the framework of Bayesian hierarchical models. The Bayesian hierarchical models of first- and second-generation marine survival indicated that, while some brood years appeared to display more variability in family survival than others, there was no apparent association between variability in family survival and type of cross (i.e. control or hybrid). This observation was supported by the posterior distributions of a measurement of among-family variance in marine survival (σ_j), which had considerable overlap among experimental lines. Hence, the results of this analysis did not provide any evidence of increased variance of marine survival in hybrids between the early- and late-migrating segments, and there is no basis for concluding that outbreeding resulted in a decrease in N_e.

Conclusions - Our results complement those of a study on adaptation of embryonic development time in first-generation hybrids between early- and late-run pink salmon (Echave et al. 2017), which was conducted as part of the same experiment. That study demonstrated that embryonic development time differed between controls and hybrids in a significant, compensatory way. Furthermore, although dam and sire components of variation of development time were significant, their interactions were not, which indicated that adaptation of this trait has primarily exploited additive genetic variation. Early-run fish appear to compensate for higher water temperatures during incubation by slowing their development rate relative to late-run fish after reaching epiboly (Joyce 1986; Hebert *et al*. 1998); this compensatory mechanism results in delayed emergence of early-run fry and a shortened gap in migration time between early- and late-run fry. Delayed emergence may provide an adaptive advantage for early-run fry by synchronizing their transition to the nearshore environment with more favorable growth

conditions, and a shortened temporal gap between early- and late-migrating fry may provide beneficial effects of predator saturation.

The results of previous research on Auke Creek pink salmon, when considered alongside those presented here, provide a lucid demonstration of fine-scale local adaptation of migration time and embryonic development time to the seasonally distinct environments encountered by early- and late-migrating fish. The implications are that fine-scale local adaptation contributes to heightened fitness in this population and may also contribute to heightened fitness in other populations that harbor genetic variation in breeding time. Furthermore, since spawning habitat limits the number of progeny that can be produced within the short and narrow confines of Auke Creek, genetically-determined temporal structure likely enhances the carrying capacity of each brood line by staggering the use of this resource over time (Smoker *et al.* 1998; Gharrett *et al.* 2013). Hence, it is likely that erosion of the temporal barrier that separates these spawning segments would cause decreased productivity of this population. Similar temporal structure is likely an important component of the population dynamics of other populations in which breeding habitat is limited. Finally, fine-scale local adaptation may also promote and maintain biodiversity that enhances the ability of populations to sustain productivity as the climate changes. This hypothesis is supported by studies that have demonstrated how adaptive variation of life history traits confers resilience to climatic fluctuations by enhancing the likelihood that some individuals within a population will carry traits that are well-suited to future environmental regimes (Hilborn *et al*. 2003; Greene *et al.* 2010; Schindler *et al.* 2010).

Adaptive variation is often impossible to resolve without genetic analyses, yet failure to maintain it could be detrimental to the productivity, biodiversity, and sustainability of wild populations. Our results suggest that prudent management of wild populations should be conducted not only with regard for genetic and phenotypic variation that arises from isolation of populations by great distance or time, but also fine-scale variation that can occur in the presence of substantial gene flow.

Acknowledgements

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636 **Data accessibility:**

637 Broodstock sources, experimental crosses, adult migration times, parentage assignments, and
638 analyses code are available as Supporting Information on the Molecular Ecology Resources web
639 site.

640 **Tables**

641

642 **Table 1 -** Number of dams, sires, and full-sib families per experimental line in the first and second
643 generations of the hybridization experiment. Lines were created by crossing pink salmon from the early
644 ("E") and late ("L) spawning segments. Asterisks denote sires from which semen was cryopreserved to
645 create crosses. Note that late experimental lines were not produced in 2006 because of failed
646 cryopreservation of semen from early-run males that year.

Year	Line (Dam x Sire)	Line Type	Dams	Sires	Families
2005	E x E	Early Control	40	40	80
	E x L*	Early Hybrid	40	40	80
	L x E*	Late Hybrid	40	40	80
	L x L*	Late Control	40	40	80
2006	E x E	Early Control	40	40	80
	E x L*	Early Hybrid	40	40	80
	L x E	Late Hybrid	--	--	--
	L x L	Late Control	--	--	--
2007	(E x E) x (E x E)	Early Control	14	13	22
	(E x L) x (E x L)	Early Hybrid	7	5	8
	(E x L) x (L x E)	Early Hybrid	7	4	7
	(L x E) x (E x L)	Late Hybrid	9	10	16
	(L x E) x (L x E)	Late Hybrid	4	3	4
	(L x L) x (L x L)	Late Control	6	6	7
2008	(E x E) x (E x E)	Early Control	8	8	41
	(E x L) x (E x L)	Early Hybrid	25	8	26

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655 **Table 2** - Bayesian mixed models of adult migration time of first-generation pink salmon. The effective number of samples from the posterior
656 distribution and 95% Bayesian credible intervals (BCI) are listed for the fixed effect of cross (C_i), the random effects of dam (D_{ij}) and sire (S_{ik}),
657 and the proportion of variation explained by covariance among maternal (P_D) and paternal (P_S) siblings. Delta DIC values, representing the
658 change in DIC accompanying inclusion of a term, are listed for each random effect.

	Broodyear 2005 (early experiment)				Broodyear 2005 (late experiment)				Broodyear 2006 (early experiment)			
Term	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC
C_i	0.796	6.468	9600		-6.175	-1.455	9600		6.662	13.597	9600	
D_{ij}	0.000	13.897	9600	0.184	0.000	12.691	9600	-6.627	0.000	9.198	9260	-1.292
S_{ik}	0.000	16.198	9600	-1.672	0.000	5.712	9600	-0.507	0.000	27.381	9266	-19.177
P_D	0.000	0.253	9600		0.000	0.570	9600		0.000	0.206	9241	
P_S	0.000	0.307	9582		0.000	0.304	9600		0.064	0.569	9323	

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662 **Table 3** - Bayesian mixed models of adult migration time of second-generation pink salmon. The effective number of samples from the posterior
663 distribution and 95% Bayesian credible intervals (BCI) are listed for the fixed effect of cross (C_i), the random effects of dam (D_{ij}) and sire (S_{ik}),
664 and the proportion of variation explained by covariance among maternal (P_D) and paternal (P_S) siblings. Delta DIC values, representing the
665 change in DIC accompanying inclusion of a term, are listed for each random effect.

	Broodyear 2007 (early experiment)				Broodyear 2007 (late experiment)				Broodyear 2008 (early experiment)			
Term	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC
C_i	1.560	7.487	9600		-9.723	-0.274	9600		4.844	10.074	9600	
D_{ij}	0.447	13.416	9600	-11.090	0.000	9.124	9600	0.898	0.000	1.623	8960	1.005
S_{ik}	0.000	5.835	9052	0.614	0.980	25.870	9600	-14.439	0.000	9.718	9227	-9.277
P_D	0.020	0.289	9600		0.000	0.128	9600		0.000	0.062	9286	

P_S	0.000	0.134	9600		0.028	0.328	9600		0.000	0.299	9177	

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668 **Table 4** - Bayesian hierarchical analysis of family-specific marine survival of first-generation pink salmon. Posterior means, 95% Bayesian
669 credible intervals (BCI), and the effective number of posterior samples are listed for two parameters: mean survival proportion (p_j) and the logit-
670 scale standard deviation of marine survival (σ_j) of the j^{th} experimental line.

			p_j				σ_j			
Line	Brood year	No. fam.	Mean	BCI (2.5%)	BCI (97.5%)	Sample	Mean	BCI (2.5%)	BCI (97.5%)	Sample
Early control	2005	66	0.0060	0.0045	0.0077	30000	0.2109	0.0281	0.6329	3200
Early hybrid	2005	42	0.0022	0.0009	0.0036	4700	0.7054	0.0439	1.6430	4300
Late control	2005	60	0.0019	0.0008	0.0032	10000	0.7740	0.0522	1.5960	61000
Late hybrid	2005	26	0.0035	0.0012	0.0060	100000	0.5614	0.0333	1.7530	12000
Early control	2006	69	0.0028	0.0019	0.0038	16000	0.6428	0.1083	1.0830	8700
Early hybrid	2006	43	0.0032	0.0018	0.0047	27000	0.2850	0.0286	0.9312	10000

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674 **Table 5** - Bayesian hierarchical analysis of family-specific marine survival of second-generation pink salmon. Posterior means, 95% Bayesian
675 credible intervals (BCI), and the effective number of posterior samples are listed for two parameters: mean survival proportion (p_j) and the logit-
676 scale standard deviation of marine survival (σ_j) of the j^{th} experimental line.

			p_j				σ_j			
Line	Brood year	No. fam.	Mean	BCI (2.5%)	BCI (97.5%)	Sample	Mean	BCI (2.5%)	BCI (97.5%)	Sample
Early control	2007	22	0.0138	0.0113	0.0164	10000	0.2732	0.0692	0.4909	9100

Early hybrid	2007	15	0.0142	0.0106	0.0177	3500	0.2198	0.0302	0.6075	7400
Late control	2007	6	0.0153	0.0101	0.0218	10000	0.2099	0.0271	0.7643	10000
Late hybrid	2007	22	0.0150	0.0113	0.0189	1900	0.4666	0.2484	0.7504	10000
Early control	2008	49	0.0130	0.0108	0.0151	10000	0.4132	0.2481	0.6069	2300
Early hybrid	2008	20	0.0114	0.0075	0.0153	10000	0.5012	0.1254	0.9731	7800

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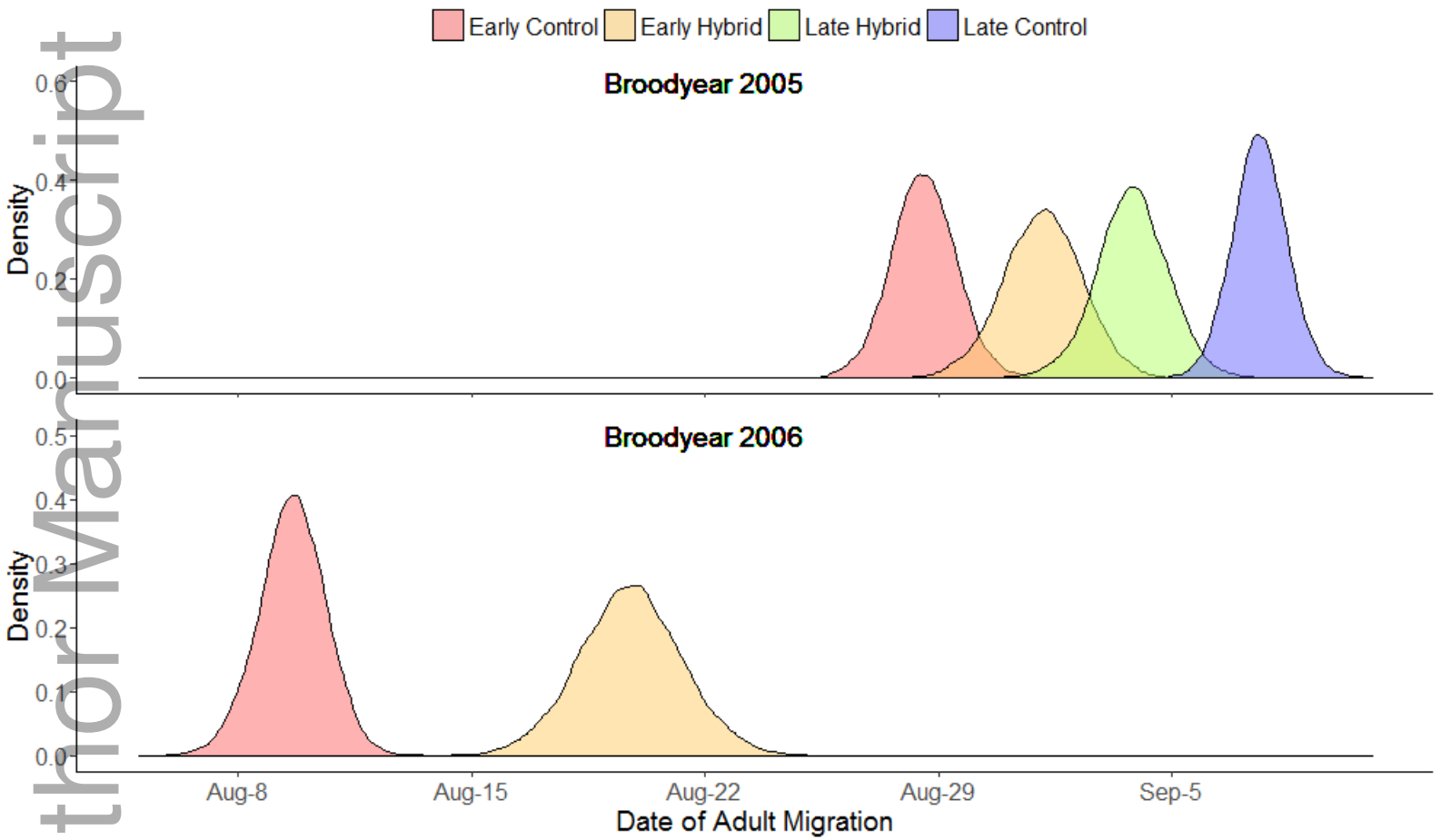
680 **Figures**



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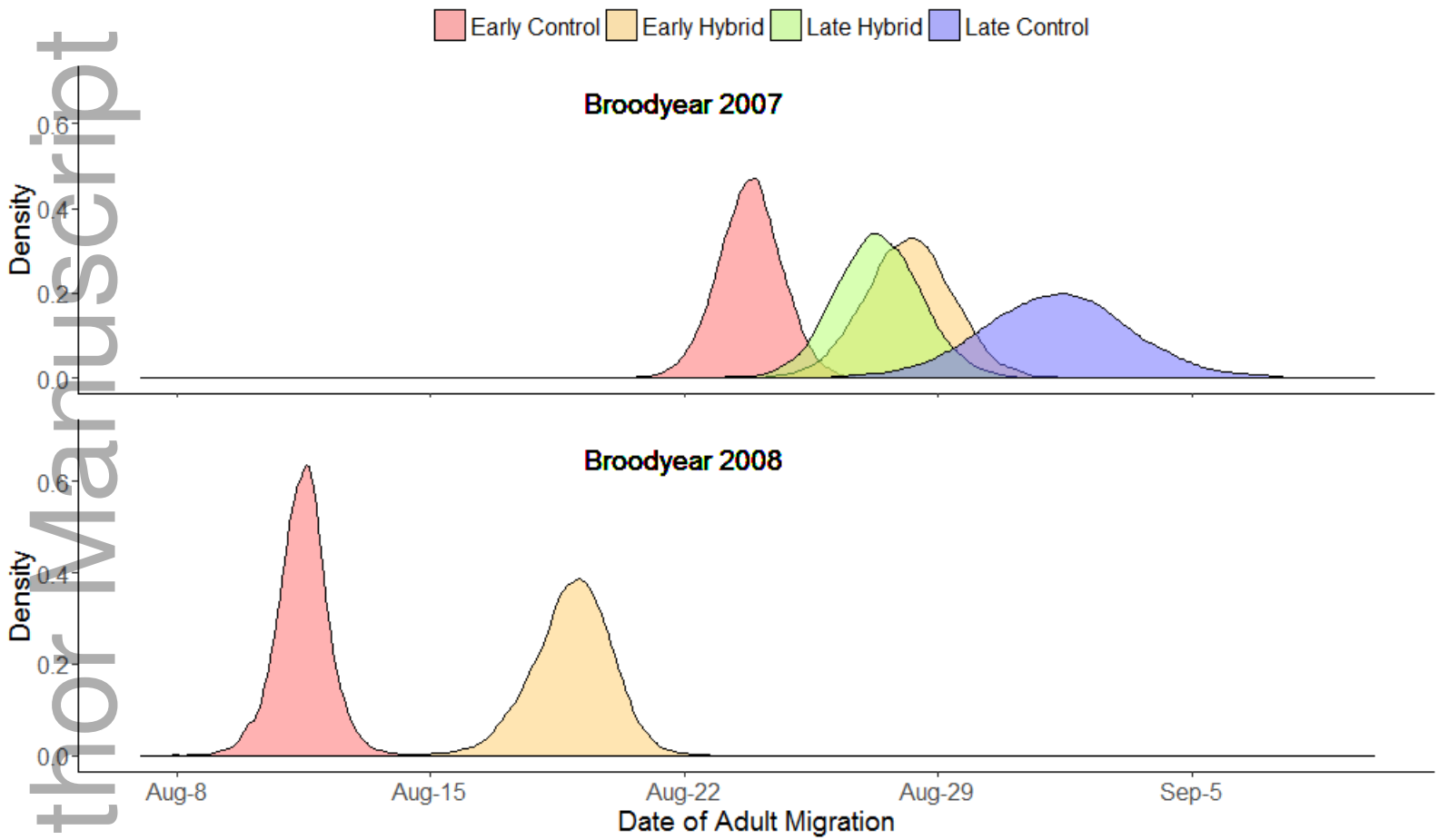
683 **Figure 1 -** Posterior distributions of mean adult migration time in experimental lines of first-generation Auke Creek pink salmon in the odd- and

684 even-year brood lines. Posterior samples (n = 9,600) were obtained under a Bayesian mixed model framework.



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687 **Figure 2 -** Posterior distributions of mean adult migration time in experimental lines of second-generation Auke Creek pink salmon in the odd-
688 and even-year brood lines. Posterior samples (n = 9,600) were obtained under a Bayesian mixed model framework.

Table 1 - Number of dams, sires, and full-sib families per experimental line in the first and second generations of the hybridization experiment. Lines were created by crossing pink salmon from the early ("E") and late ("L) spawning segments. Asterisks denote sires from which semen was cryopreserved to create crosses. Note that late experimental lines were not produced in 2006 because of failed cryopreservation of semen from early-run males that year.

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	(L x E) x (L x E)	Late Hybrid	4	3	4
	(L x L) x (L x L)	Late Control	6	6	7
2008	(E x E) x (E x E)	Early Control	8	8	41

Table 2 - Bayesian mixed models of adult migration time of first-generation pink salmon. The effective number of samples from the posterior distribution and 95% Bayesian credible intervals (BCI) are listed for the fixed effect of cross (C_i), the random effects of dam (D_{ij}) and sire (S_{ik}), and the proportion of variation explained by covariance among maternal (P_D) and paternal (P_S) siblings. Delta DIC values, representing the change in DIC accompanying inclusion of a term, are listed for each random effect.

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Table 3 - Bayesian mixed models of adult migration time of second-generation pink salmon. The effective number of samples from the posterior distribution and 95% Bayesian credible intervals (BCI) are listed for the fixed effect of cross (C_i), the random effects of dam (D_{ij}) and sire (S_{ik}), and the proportion of variation explained by covariance among maternal (P_D) and paternal (P_S) siblings. Delta DIC values, representing the change in DIC accompanying inclusion of a term, are listed for each random effect.

	Broodyear 2007 (early experiment)				Broodyear 2007 (late experiment)				Broodyear 2008 (early experiment)			
Term	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC	BCI (2.5%)	BCI (97.5%)	Sample	ΔDIC
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Table 4 - Bayesian hierarchical analysis of family-specific marine survival of first-generation pink salmon. Posterior means, 95% Bayesian credible intervals (BCI), and the effective number of posterior samples are listed for two parameters: mean survival proportion (p_j) and the logit-scale standard deviation of marine survival (σ_j) of the j^{th} experimental line.

			p_j				σ_j			
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Late hybrid	2005	26	0.0035	0.0012	0.0060	100000	0.5614	0.0333	1.7530	12000
Early control	2006	69	0.0028	0.0019	0.0038	16000	0.6428	0.1083	1.0830	8700
Early hybrid	2006	43	0.0032	0.0018	0.0047	27000	0.2850	0.0286	0.9312	10000

Table 5 - Bayesian hierarchical analysis of family-specific marine survival of second-generation pink salmon. Posterior means, 95% Bayesian credible intervals (BCI), and the effective number of posterior samples are listed for two parameters: mean survival proportion (p_j) and the logit-scale standard deviation of marine survival (σ_j) of the j^{th} experimental line.

			p_j				σ_j			
Line	Brood year	No. fam.	Mean	BCI (2.5%)	BCI (97.5%)	Sample	Mean	BCI (2.5%)	BCI (97.5%)	Sample
Early control	2007	22	0.0138	0.0113	0.0164	10000	0.2732	0.0692	0.4909	9100
Early hybrid	2007	15	0.0142	0.0106	0.0177	3500	0.2198	0.0302	0.6075	7400
Late control	2007	6	0.0153	0.0101	0.0218	10000	0.2099	0.0271	0.7643	10000
Late hybrid	2007	22	0.0150	0.0113	0.0189	1900	0.4666	0.2484	0.7504	10000
Early control	2008	49	0.0130	0.0108	0.0151	10000	0.4132	0.2481	0.6069	2300
Early hybrid	2008	20	0.0114	0.0075	0.0153	10000	0.5012	0.1254	0.9731	7800