

Genetic quality and offspring performance in Chinook salmon: implications for supportive breeding

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Abstract Each year salmon and other fishes are caught and used for supportive breeding programs that attempt to augment natural populations that are threatened with extinction. These programs typically mate individuals randomly and as such they overlook the importance of genetic quality to offspring fitness and ultimately to ensuring population health. Here, we use Chinook salmon (*Oncorhynchus tshawytscha*) and a fully crossed quantitative genetic breeding design to partition genetic variance in offspring performance (growth and survival) to additive and non-additive genetic effects as well as maternal effects. We show that these three effects contribute about equally to the variation in survival, but only non-additive genetic and maternal effects contribute to variation in growth. Some of the genetic effects could be assigned to variation at the class IIB locus of the major histocompatibility complex, but the maternal effects were not associated with egg size and we found no relationship between dam phenotypic measures and offspring survival or growth. We also found no relationship between sire sexually selected characters and offspring survival or growth, which is inconsistent with a “good genes”

hypothesis. Finally, we show that incorporation of genetic quality into supportive breeding programs can increase offspring growth or survival by between 3% and 19% during the endogenous feeding stage alone, and projections to adulthood suggest that survivorship could be over four fold higher.

Keywords Supportive breeding · Chinook salmon · Genetic quality · Major histocompatibility complex · Mate choice · Good genes · Compatible genes

Introduction

Conservation and management programs routinely use supportive breeding, the practice of augmenting vulnerable wild populations with individuals that were bred in captivity, in an attempt to circumvent population decline. Many of these programs mate individuals randomly, or attempt to mate dissimilar individuals to maximize genetic diversity and thus minimize inbreeding depression (Wang et al. 2001; Keller and Waller 2002). However, there is an increasing recognition that random mating or genetic diversity does not capture the complexity of genetic quality (Grahn et al. 1998; Wedekind 2002; Wedekind and Müller 2004; Neff and Pitcher 2005). Here, we begin to address this issue in Chinook salmon (*Oncorhynchus tshawytscha*) by using a quantitative genetic breeding design to examine genetic quality.

Genetic quality consists of two components comprising the independent effects of parental genotypes (additive genetic effects) and the interaction between parental genotypes (non-additive genetic effects) (reviewed in Neff and Pitcher 2005). Quantitative

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genetic breeding designs can be used to partition variance in fitness (or fitness related traits) between additive and non-additive genetic effects. For example, one powerful design is the North Carolina Design II whereby a set of sires and dams are crossed in all pairwise combinations (Lynch and Walsh 1998, p. 598). A two-way ANOVA can then be used to partition the variance in fitness among additive genetic effects (good genes), non-additive genetic effects (compatible genes), and maternal effects. To date, only a few studies exist that use such quantitative genetic breeding designs to examine the architecture of genetic quality, but these studies have shown both additive and non-additive genetic effects on fitness (e.g. Wedekind et al. 2001; Wedekind and Müller 2004; Rudolfson et al. 2005). Furthermore, some studies have been able to attribute additive and non-additive genetic effects to specific loci such as those of the major histocompatibility complex (MHC) (Pitcher and Neff 2006; reviewed in Bernatchez and Landry 2003).

The MHC genes code for proteins that present pathogens to the immune system. In many populations, MHC loci are highly polymorphic and most individuals are heterozygous (Bernatchez and Landry 2003). Heterozygous individuals may have increased survivorship because they are able to present a broader array of antigens and thereby resist a broader array of pathogens (Doherty and Zinkernagel 1975; Penn et al. 2002; Kurtz et al. 2004). Alternatively, some studies have found that individuals with specific MHC alleles have increased survivorship (Arkush et al. 2002; Lohm et al. 2002; Grimholt et al. 2003). Thus, the genes of the MHC can show both additive and non-additive genetic effects on fitness. Complementary to those findings, other studies have shown that females prefer to mate with males that differ from them at the MHC or that have the specific MHC alleles associated with improved offspring fitness (reviewed in Milinski 2003; Ziegler et al. 2005). Because of this potential benefit of improved immune response, incorporation of MHC genetic architecture into the design of supportive breeding programs has been advocated (Hughes 1991; Grahn et al. 1998). However, few studies have examined the feasibility and consequences of this suggestion (for an exception, see Schreiber et al. 1993).

Additive genetic effects have also been studied in the context of sexual selection, where the “good genes” hypothesis proposes that males with the most elaborate secondary sexual traits have additive genetic variance for increased fitness (reviewed in Andersson 1994; Møller and Alatalo 1999). For example, Petrie (1994) found that peahens (*Pavo cristatus*) mated to males with more elaborate tails produced offspring

that had higher survivorship and growth than females mated to males with less elaborate tails (for other examples, see Welch et al. 1998; Barber et al. 2001). Thus, secondary sexual traits can provide a measurement of additive genetic variance, and because sexually selected traits are easily measured, these traits could be used in supportive breeding programs to identify individuals of high genetic quality (Grahn et al. 1998; Wedekind and Müller 2004).

In this study, we use Chinook salmon as a model system to partition genetic variance in offspring performance (survival and growth) among additive and non-additive genetic effects as well as maternal effects. Many Chinook salmon populations are threatened or endangered (e.g. Fisher 1994; Yoshiyama et al. 1998; Nemeth and Kiefer 1999) and supportive breeding is now widely used in an attempt to enhance wild populations (Hedrick et al. 2000). Here, we cross 11 females with 11 males in a quantitative genetic breeding design and rear the resulting 121 sib groups (in replicate) through the endogenous feeding period. We attempt to attribute variation in offspring growth and survival to parental phenotype and genetic effects including MHC genotype at the class IIB locus. Genetic variation at this locus is associated with survival in Chinook salmon through, for example, resistance to infectious haematopoietic necrosis virus (Arkush et al. 2002). Finally, we model the value of incorporating genetic quality into supportive breeding programs for Chinook salmon through increases in offspring survivorship and growth.

Methods

Chinook salmon biology

Chinook salmon are a Pacific salmon that typically breed in freshwater streams on the west coast of North America. They now have been introduced to other parts of North America, including the Great Lakes (Crawford 2001). The mating system of Chinook salmon is like many of the other semelparous Pacific salmon (Groot and Margolis 1991; de Gaudemar 1998). Females spawn multiple times in a series of nests and defend those nests from superimposition by nests of other females. Male Chinook salmon mate with several females during the spawning season, but provide no parental care and therefore no material benefits to their offspring (Healey 1991; Berejikian and Tezak 2005). Nevertheless, in one study, females exhibited mate choice for larger males by delaying spawning in the presence of smaller males (Berejikian et al. 2000).

Parental collection

The Chinook salmon used in this study were collected using standard electroshock methods from a winter run in the Credit River, which flows into Lake Ontario. Chinook salmon have been stocked in Lake Ontario for about 36 years (Crawford 2001). On 6 October 2000, 11 males and 11 females were selected to encompass a range of body sizes. For this study, we elected to focus on migratory males only and therefore did not use any male smaller than 45 cm in total body length (i.e. resident males). Eggs or milt were collected from each individual by applying gentle pressure to the fish's abdomen. The eggs or milt were then placed in a plastic bag. Care was taken to ensure that the gametes were not exposed to any water to prevent egg hardening or activation of the milt. A subsample of 10 eggs were preserved in Stockard's solution and these eggs were later used to estimate mean egg diameter for each female using digital calipers (nearest 0.1 mm). The bags were stored at approximately 10°C (the temperature of the river water) and transported back to Parkview hatchery. A sample of tissue from the tail was also taken from each adult and preserved in 95% ethanol for genetic analyses.

Each male and female was weighed to the nearest 0.1 kg and mid-eye-to-hypural-flexure length (MEH length), was measured to the nearest cm using a tape measure. We used these two measurements to calculate Fulton's condition factor ($=\text{mass}/\text{length}^3$). Fulton's condition factor has been correlated with non-polar lipid density in Atlantic salmon (*Salmo salar*) parr (Sutton et al. 2000) other fishes including sexually mature bluegill (*Lepomis macrochirus*) (Neff and Cargnelli 2004). For males, we estimated age using the mean number of rings on two scales (Fukuwaka and Kaeriyama 1997). The two scale counts were highly correlated ($r = 0.74$, $P = 0.01$, $n = 11$). Male growth rate was calculated from MEH length/mean number of rings. We also took three additional measurements that have been implicated in male dominance (terminology from Kinnison et al. 2003): hump depth (distance from the lateral line to the apex of the hump at the front insertion of the dorsal fin), snout length (distance from the middle of the eye to the tip of the upper jaw), and upper jaw length (distance from mouth opening to the tip of the upper jaw). We later used principle components analysis (PCA) to construct a single index from these three measurements. We then took the residuals from a linear regression of the PCA (first axis) onto MEH length to control for allometry.

Breeding design

We performed all 121 possible crosses of 11 males and 11 females in replicate ($n = 242$ families total). Eggs from each female were divided into 11 pairs of containers with 150 eggs each. Sperm concentration was determined for each male prior to the milt addition using a haemocytometer (methods in Pitcher et al. 2003), and the volume of milt was adjusted to ensure that equal numbers of sperm were introduced into each container. Egg samples were used in the same order as the females were collected and were timed to ensure that fertilization occurred about 90 min after collection. This fertilization protocol has been shown to result in fertilization rates in excess of 99% for salmon (Vronskiy 1972; Healey 1991). Next, each full-sib family was randomly allocated to a cell in a Heath incubation tray. Each tray had plexi-glass partitions that formed 16 equal sized cells. The Heath trays were then exposed to natural, untreated river water that ranged from 6°C to 13°C during the experiment.

Each day for 80 days post fertilization (about the end of the endogenous feeding stage), the trays were checked and the number of dead offspring within each full-sib family (cell) were counted and removed. Although we did not assay the specific causes of mortality, common sources of offspring mortality in Ontario salmon hatcheries include water molds (*Saprolegnia* spp.), furunculosis (*Aeromonas salmonicida*), infectious pancreatic necrosis virus, and Flavobacterium diseases such as bacterial gill disease and cold-water disease (Bruneau et al. 1999). On days 73 and 80 post-fertilization, we also measured the total body length of five haphazardly chosen offspring from each cell using digital calipers (nearest 0.1 mm). Mean apparent growth rate of the offspring in each cell was then calculated as the difference in mean body length divided by 7 days. Overall survivorship was determined for each cell by dividing the number of surviving offspring by 150. Thus, for each family, we had a total of 10 measurements of offspring body size (at day 80) and 2 measurements of apparent growth rate and survivorship. At the end of the experiment, 5 offspring from each cell (i.e. 10 from each full-sib family) were preserved in 95% ethanol.

Genetic analysis

Elsewhere we describe the methods we used to obtain MHC class IIB genotypes for our adults and offspring (Pitcher and Neff 2006). Briefly, DNA was extracted from either adult or offspring tissue using a simple proteinase K digestion (Neff et al. 2000). Using primers

published in Docker and Heath (2002), we used PCR to amplify 294 bases of the class IIB region of the major histocompatibility complex. These 294 bases encompass the variable part of the class II protein binding region, which is responsible for binding foreign peptides in white blood cells and presenting them to T cells. The PCR products from duplicate reactions were digested using either restriction enzymes *RsaI* or *DdeI* and run on agarose gels. This combination of enzymes allows the identification of the three primary alleles in our population. Each of these alleles shows high homology to previously published sequences in Chinook salmon comprising *Onts-1*, *Onts-1b*, and *Onts-wr3* (Miller and Withler 1996; Arkush et al. 2002; see Pitcher and Neff 2006). Using the computer algorithm developed in Pitcher and Neff (2006), we assigned additive and non-additive genetic effects on offspring survivorship and body length to MHC alleles and genotypes (see Online material and <http://www.publish.uwo.ca/~bneff/links.htm> for Genetic effects software).

Statistical analyses

We began by using a three-way ANOVA with stack number (4 levels), tray position (4 levels) and cell location (16 levels) to examine potential rearing location effects on offspring performance. Next, our fully crossed quantitative genetic breeding design allowed us to use the North Carolina Design II approach to partition variance in offspring performance to additive genetic effects, non-additive genetic effects, and maternal effects (Lynch and Walsh 1998, p. 598). A two-way ANOVA was used with female identity (*Dam*), male identity (*Sire*) and the interaction (*Dam* × *Sire*) all entered as random factors. Because males in our experiment provided only genes (sperm) to the offspring, the *Sire* effect provided an estimate of additive genetic effects. Specifically, assuming that epistatic genetic variance is of negligible importance, the additive genetic effects were calculated from four times the *Sire* component of variance, the non-additive genetic effects were calculated from four times the *Dam* × *Sire* component of variance, and the maternal effects were calculated from the difference between the *Dam* and *Sire* components of variance (Lynch and Walsh 1998, p. 601).

To examine adult phenotypic correlates (mass, MEH length, Fulton's condition factor, for males and females, the age, growth rate, and dominance traits residuals for males, and mean egg diameter for females) of mean offspring performance, we used linear regression with more than one value of *Y* per value

of *X* (Sokal and Rohlf 1995, p. 476). This analysis allowed us to incorporate the half-sib family data. For example, when analyzing the female phenotypic data, we had 11 *X*-values (one for each female), but 121 *Y*-values (11 half-sib families per female).

Mate choice

We constructed three models of mate choice to examine the potential benefits of genetic quality to supportive breeding programs: (1) *random mating*; (2) *MHC mating*; and (3) *optimal mating*. The first model repeatedly selected a random female (from the 11) and “mated” her to a randomly selected male. The survivorship or mean body length of their offspring was determined based on our empirical data (from the 121 replicated families) for that pair. The routine was repeated for a total of 10,000 times and the expected (average) survivorship or body length was determined. The second model repeatedly selected a random female and allowed her to choose from 2 to 11 randomly selected males. We assumed that the female would mate with the optimal male based only on the males' MHC genotype (i.e. the mate that would maximize either mean survivorship or body length of her offspring based on the MHC values as determined by the genetic algorithm; see Pitcher and Neff 2006; Online material). The routine was repeated for a total of 10,000 times for each of the possible number of mates (i.e. 2–11) and the expected survivorship or body length was determined. The third model was analogous to the second model except we assumed that the female would select the optimal mate based on the observed survivorship or body length data (i.e. the mate that maximized these offspring traits).

Results

There were a total of 36,300 eggs across the 242 families. At the termination of our experiment, survivorship among these families averaged $71 \pm 19\%$ (range = 13–99%; Fig. 1a), offspring body length averaged $28.0 \text{ mm} \pm 1 \text{ mm}$ (range = 25–31 mm; Fig. 1b), and apparent growth rate averaged $0.9 \pm 0.2 \text{ mm/d}$ (range = 0.3–1.5 mm/day). A three factor ANOVA revealed no effect of stack number, tray position and cell location on survivorship ($P > 0.48$ for the three main effects). However, there was an effect of these location parameters on offspring length (stack: $F_{3,1188} = 5.74$, $P < 0.001$; tray: $F_{3,1188} = 2.31$, $P < 0.075$; cell: $F_{15,1188} = 6.62$, $P < 0.001$). We therefore used the residuals from this latter ANOVA in the subsequent North Carolina Design II

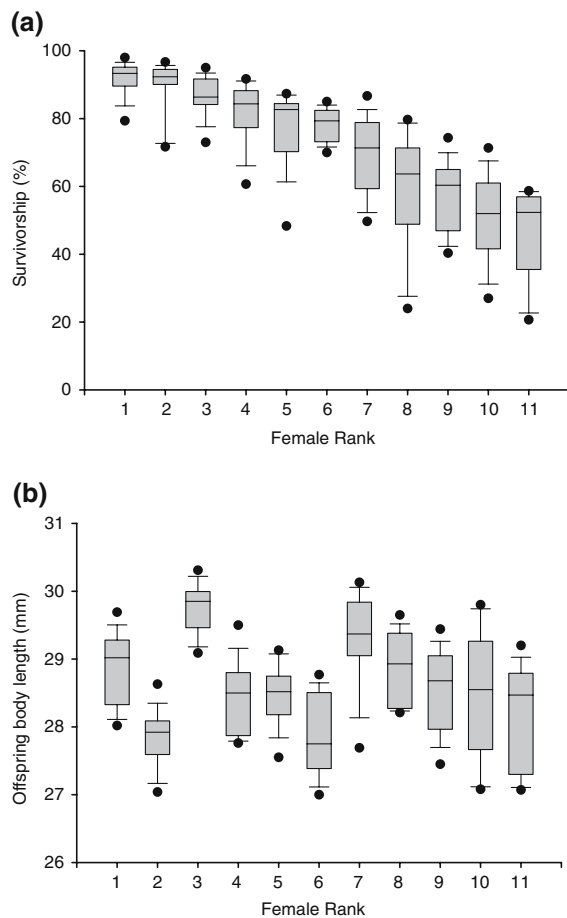


Fig. 1 Box plots summarizing the variation in offspring survivorship (a) and offspring body length (b) among the 121 families grouped by female identity in Chinook salmon. For the purpose of displaying these data we assigned female identity based on the rank of the median survivorship of her families. We used the same order for the offspring body length data. Box plots show the 10, 25, 50, 75 and 90 percentiles and the dots are data lying outside this range

analysis and all other analyses using offspring body length.

Across family replicates, there was a correlation in survivorship ($r = 0.92$, $df = 120$, $P < 0.001$), offspring body length ($r = 0.55$, $df = 120$, $P < 0.001$), and apparent growth rate ($r = 0.43$, $df = 120$, $P < 0.001$). There was a high correlation between mean offspring body length and apparent growth rate ($r = 0.90$, $df = 241$, $P < 0.001$). Thus, for brevity, we present analyses involving body length and omit those involving apparent growth rate. There was a weak, non-significant correlation between mean family survivorship and offspring length ($r = 0.11$, $df = 241$, $P = 0.086$).

Overall, *Dam*, *Sire* and *Dam* × *Sire* effects explained 92% of the phenotypic variation in survivorship (Table 1). Although all three effects were statistically significant, most of the explained variation was attrib-

uted to *Dam* effects, which include both maternal additive genetic effects and environmental effects such as egg nutrients. From the *Sire* variance component we estimated the additive genetic effects to be 2.24×10^{-2} ($= 4 \times 0.56 \times 10^{-2}$), which represents about 56% ($= 2.24 \times 10^{-2} / 3.99 \times 10^{-2}$) of the phenotypic variance in survivorship. From the *Dam* × *Sire* variance component we estimated the non-additive genetic effects to be 2.16×10^{-2} ($= 4 \times 0.54 \times 10^{-2}$), which represents about 54% of the phenotypic variance in survivorship. Finally, from the difference between the *Dam* and *Sire* variance components we estimated the maternal effects to be 2.04×10^{-2} ($= 2.6 \times 10^{-2} - 0.56 \times 10^{-2}$), which represents about 51% of the phenotypic variance in survivorship. Thus, additive genetic variance, non-additive genetic variance and maternal effects variance respectively account for 56%, 54% and 51% of the total variance in survivorship. When there is epistatic genetic variance, the estimated genetic effects will be overestimated (Lynch and Walsh 1998, p. 601). This may explain why the percentages add up to more than 100%.

For offspring length, *Dam*, *Sire* and *Dam* × *Sire* effects explained about 29% of the total phenotypic variance (Table 1). The *Sire* variance component was not significant, indicating that there were no additive genetic effects on offspring length during the course of the experiment. The non-additive genetic effects and maternal effects accounted for 73% and 11%, respectively, of the phenotypic variance in offspring length.

For females, the regression analysis revealed no relationship between either offspring survivorship or body length and MEH length (survivorship: $r^2 = 0.01$, $F = 0.32$, $df = 1,9$, $P = 0.59$; offspring body length: $r^2 = 0.02$, $F = 0.91$, $df = 1,9$, $P = 0.37$), mass (survivorship: $r^2 = 0.01$, $F = 0.78$, $df = 1,9$, $P = 0.40$; offspring body length: $r^2 = 0.01$, $F = 0.97$, $df = 1,9$, $P = 0.35$), or Fulton’s condition factor (survivorship: $r^2 = 0.11$, $F = 0.10$, $df = 1,9$, $P = 0.76$; offspring body length: $r^2 = 0.05$, $F = 0.002$, $df = 1,9$, $P = 0.97$) (Fig. 2; Online material). There also was no relationship between mean egg diameter and mean offspring survivorship ($r^2 = 0.23$, $F = 1.61$, $df = 1,9$, $P = 0.24$) or offspring body length ($r^2 = 0.55$, $F = 1.15$, $df = 1,9$, $P = 0.31$; Fig. 2).

For males, the regression analysis revealed no relationship between either offspring survivorship and body length and MEH length (survivorship: $r^2 = 0.01$, $F = 0.69$, $df = 1,9$, $P = 0.73$; offspring body length: $r^2 = 0.01$, $F = 0.03$, $df = 1,9$, $P = 0.87$), dominance traits residuals (survivorship: $r^2 = 0.01$, $F = 0.97$, $df = 1,9$, $P = 0.35$; offspring body length: $r^2 = 0.01$, $F = 1.88$, $df = 1,9$, $P = 0.20$), mass (survivorship: $r^2 = 0.01$, $F = 0.43$, $df = 1,9$, $P = 0.53$; offspring body

Table 1 Summary of the two-way ANOVA results for survivorship and offspring length in Chinook salmon

| Source of variation | DF | SS | MS | F | P | $\sigma^2 \times 10^{-2}$ | % Total variance |
|-------------------------|-----------|-------|------|------|---------|---------------------------|------------------|
| Survivorship | | | | | | | |
| Dam | 10, 100 | 5.78 | 0.58 | 42.0 | < 0.001 | 2.6 ± 1.1 | 65.2 |
| Sire | 10, 100 | 1.37 | 0.14 | 9.9 | < 0.001 | 0.56 ± 0.26 | 14.0 |
| Dam × Sire | 100, 121 | 1.38 | 0.01 | 4.7 | < 0.001 | 0.54 ± 0.07 | 13.5 |
| Residual | 121 | | | | | 0.29 ± 0.04 | 7.3 |
| Offspring length | | | | | | | |
| Dam | 10, 100 | 224.7 | 22.5 | 5.8 | < 0.001 | 16.9 ± 2.9 | 11.0 |
| Sire | 10, 100 | 43.0 | 4.3 | 1.1 | 0.36 | 0.39 ± 1.7 | 0.2 |
| Dam × Sire | 100, 1089 | 387.8 | 3.9 | 3.6 | < 0.001 | 28.0 ± 5.5 | 18.3 |
| Residual | 1089 | | | | | 108.0 ± 4.6 | 70.5 |

The results include source of variation, degrees of freedom (DF, where appropriate including the numerator and denominator values separated by a comma), sum of squares (SS), mean square (MS), *F* statistic, *P*-value, variance component (σ^2) and the percent of total variance

NB: Variance components are expressed ±1 SE

length: $r^2 = 0.01$, $F = 0.01$, $df = 1,9$, $P = 0.93$), Fulton's condition factor (survivorship: $r^2 = 0.21$, $F = 0.09$, $df = 1,9$, $P = 0.77$; offspring body length: $r^2 = 0.37$, $F = 0.65$, $df = 1,9$, $P = 0.44$), age (survivorship: $r^2 = 0.01$, $F = 0.69$, $df = 1,9$, $P = 0.43$; offspring body length: $r^2 = 0.01$, $F = 0.34$, $df = 1,9$, $P = 0.57$) or growth rate (survivorship: $r^2 = 0.31$, $F = 0.69$, $df = 1,9$, $P = 0.43$; offspring body length: $r^2 = 0.02$, $F = 0.001$, $df = 1,9$, $P = 0.98$) (Fig. 2; Online material).

Our mate choice model revealed that random mating would provide an expected offspring survivorship during the endogenous feeding period of 70.7% (Fig. 3a). If females instead selected mates based on MHC genotype for offspring survivorship, then the expected survivorship would range from 72.5% (when females are given only two randomly selected males to pick from) to 74.9% (when females are given 11 males to select from). If females selected mates optimally based on overall survivorship, then the expected survivorship would range from 77.4% to 84.0% (for 2–11 males). Thus, MHC and optimal mating can increase survivorship by 6% (= 74.9/70.7) and 19% (= 84/70.7), respectively. If females instead selected mates for increased offspring body length, random mating would provide an expected length by the end of the endogenous feeding period of 28.6 mm (Fig. 3b). If females instead selected mates based on MHC genotype for offspring body length, then the expected length would range from 28.8 mm to 29.0 mm (for 2–11 males). If females selected mates optimally based on offspring body length, then the expected length would range from 28.9 mm to 29.5 mm (for 2–11 males). Thus, MHC and optimal mating can increase offspring body length by 1% (= 29.0/28.6) and 3% (= 29.5/28.6), respectively.

Discussion

We used a fully crossed quantitative genetic breeding design to assess the importance of genetic quality on offspring performance during the endogenous feeding stage in Chinook salmon. Our results add to a growing number of studies on fishes that demonstrate genetic components to larval growth or survivorship. Consistent with our study, Wedekind and colleagues (2001) found in whitefish (*Coregonus* sp.) that additive and non-additive genetic variance contributed about equally to the phenotypic variance in survivorship (also see Wedekind and Müller 2004). In Atlantic cod (*Gadus morhua*), Rudolfsen and colleagues (2005) found significant non-additive genetic effects on survivorship, but failed to find additive genetic effects. Although we failed to find a significant additive genetic effect on body size, several other studies in salmonidae have found such effects, including Rainbow trout (*Oncorhynchus mykiss*) (Gjerde and Schaeffer 1989), Atlantic salmon (Rye and Refstie 1995), Coho salmon (*Oncorhynchus kisutch*) (Hershberger et al. 1990), Arctic charr (*Salvelinus alpinus*) (Nilsson 1992), and several other populations of Chinook salmon from British Columbia (Winkelman and Peterson 1994). Perhaps not surprisingly, the genetic architecture of growth and survivorship is complex and likely varies among species, populations, environments, and stages of ontogeny.

The MHC class IIB locus appears to play a role in survivorship during early development of Chinook salmon. We found a significant association between MHC class IIB genotype and survivorship during the endogenous feeding stage in our population. Although there are no data yet available for Chinook salmon, the

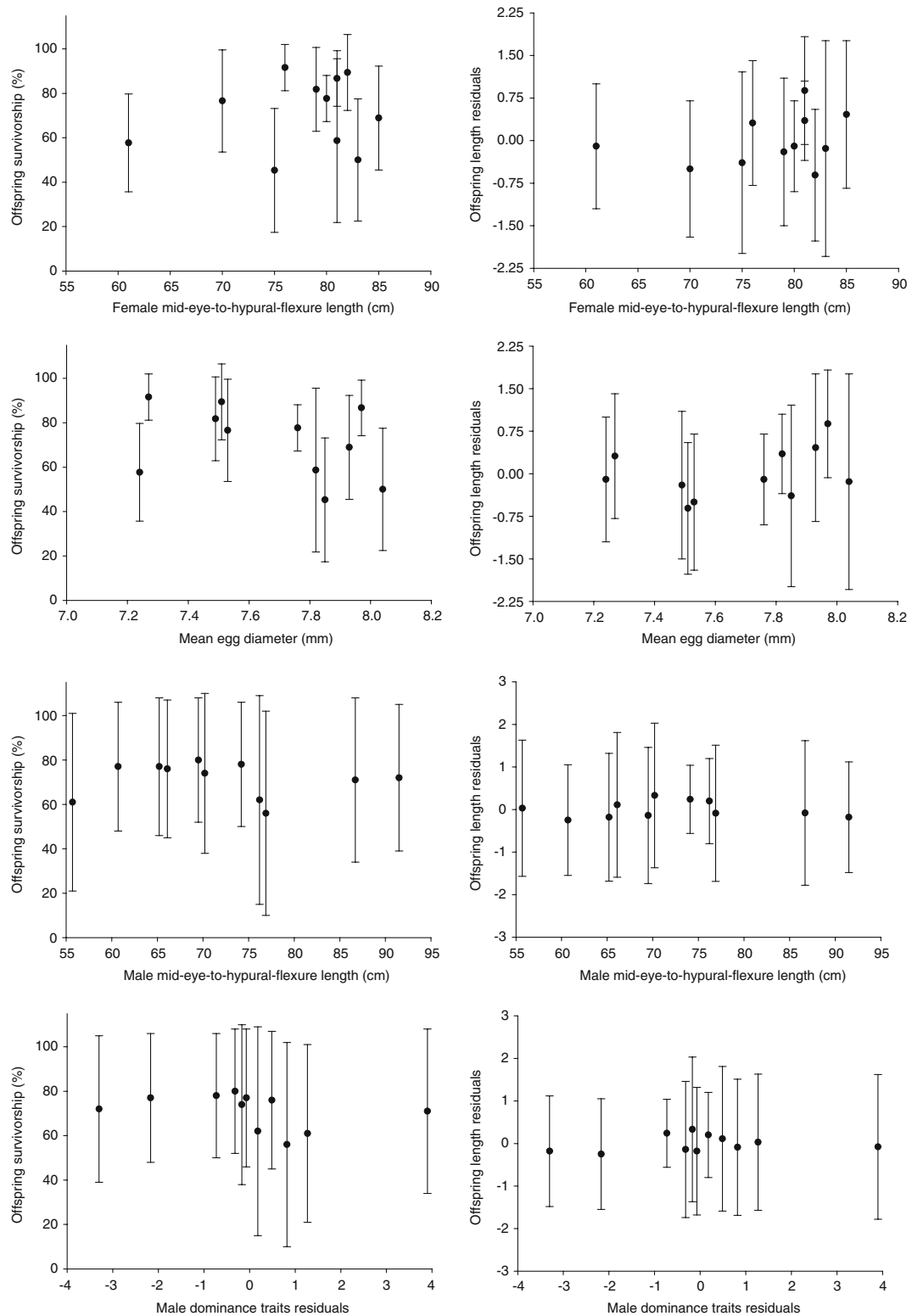


Fig. 2 Relationships between mean offspring survivorship or body length residuals and female MEH length, mean egg diameter, male MEH length and residuals of a dominance trait index in Chinook salmon. The error bars denote the 95% confidence intervals

expression of the immune system during early development has been studied in several fishes (reviewed by Tatner 1996). For example, in Atlantic salmon, the

development of lymphoid organs and the appearance of lymphocytes, both precursors to an MHC immune response, are present as early as 22 days prior to

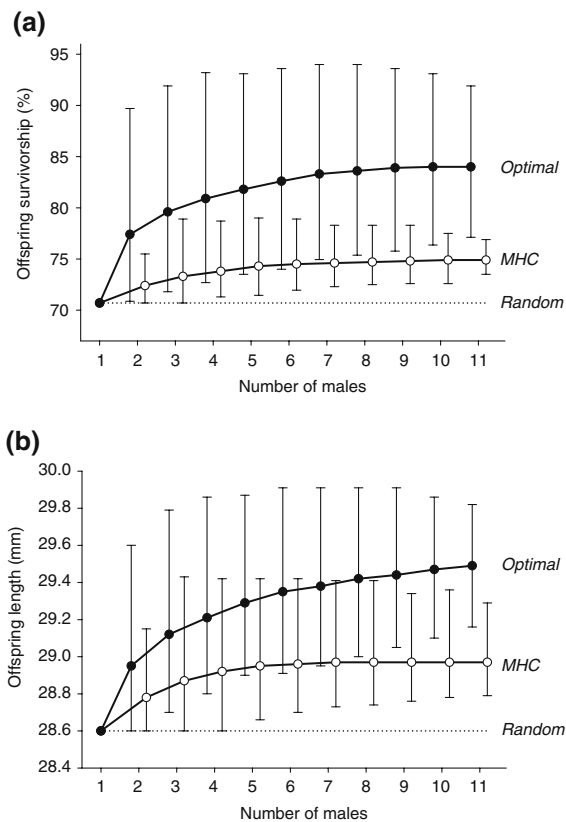


Fig. 3 Model demonstrating the potential increase in (a) offspring survivorship and (b) offspring length from mate choice in Chinook salmon. The *Random* line represents the expected (average) offspring survivorship through to the end of the endogenous feeding period (80 days) for females randomly mated to a single male. The *MHC* line represents the potential benefit to selecting among up to 11 males optimally based on MHC genotype. The *Optimal* line represents the potential benefit to selecting among up to 11 males optimally based on overall survivorship. The error bars denote the 95% confidence intervals. For the purpose of displaying these data we have offset them slightly from their respective *x*-axis values

hatching (Ellis 1977). Another study in carp (*Cyprinus carpio*) that directly examined expression of the MHC class IIB gene, found that it was expressed one day post hatching (Rodrigues et al. 1998). These other studies indicate that the MHC class IIB is expressed about the time of hatching and possibly considerably earlier in development. Thus, it is conceivable that the MHC dependent survivorship effects detected in our study are a direct result of the MHC immune related function. However, more work is needed to confirm whether or not the survivorship effects detected here are directly linked to differences in MHC expression at the class IIB locus.

The genetic basis of survivorship in Chinook salmon undoubtedly involves many genes in addition to those of the MHC, and our data suggest that these genes may

exhibit epistatic effects. The formulas used to calculate additive and non-additive genetic variance assume that epistasis is negligible (Lynch and Walsh 1998, p. 601). When there is epistasis, however, these formulas can overestimate both additive and non-additive genetic variance. Epistasis may therefore be a factor in our study because the genetic and environmental effects for survivorship exceeded 100% (and the excess could not be explained by the error surrounding the variance in the survivorship estimate; see Table 1). Two other studies of fishes have also found that the variance captured by genetic and environmental effects for survivorship exceeds 100% (Wedekind et al. 2001; Rudolfson et al. 2005). Given that epistasis is likely to be common for complex traits such as survivorship (Kroymann and Mitchell-Olds 2005; reviewed in Carlborg et al. 2004), genetic estimates derived from the North Carolina Design II approach should be considered as maximums. Regardless of the actual magnitude of these genetic effects, the associated statistical significance as derived from the two-way ANOVA is independent of any influence of epistasis.

We were unable to attribute variation in offspring body length or survival to egg size or parental phenotype. In contrast to other work in Chinook salmon, which found a positive relationship between egg mass and survivorship (Heath et al. 1999), we found no relationship between egg diameter and survivorship. It is possible that mass is a better measure of egg quality than diameter. Also, the study by Heath and colleagues (1999) had more than twice the variance in egg size than our study, thus increasing their statistical power. We also failed to find any relationship between sire mass (or length) and offspring performance, which was expected because female Chinook salmon prefer to mate with heavier males presumably because such males provide good genes (Berejikian et al. 2000; Berejikian and Tezak 2005). There also was no relationship between the male dominance traits (i.e. hump depth, snout length, and upper jaw length) and offspring performance. It is possible that benefits from additive genetic effects correlated with these traits show up later in life as has been found in a study of Pink salmon (*Oncorhynchus gorbuscha*; Funk et al. 2005), or were masked by the strong maternal effects.

Our mate choice model revealed several approaches that could be implemented to improve the performance of supportive breeding programs. First, given that many studies have shown that females select males for genetic benefits (Tregenza and Wedell 2000; Neff and Pitcher 2005), incorporating mate choice into supportive breeding programs could significantly increase offspring performance. From our data, we

calculated that a female that selected the optimal male could increase offspring survivorship by as much as 19% or offspring body size by as much as 3% during the endogenous feeding period alone. Mate choice could be incorporated into supportive breeding programs by using natural mating channels (to compare offspring performance from free mating females against randomly mated females (see Partridge 1980)). Second, brood stock could be genotyped at the MHC to identify optimal pairings that produce offspring genotypes associated with high survivorship (also see Hughes 1991). The genotyping may be cost effective for many programs when, for example, restriction enzyme protocols can be utilized as we have in our study (see Docker and Heath 2002).

In conclusion, the genetic architecture of early growth and survivorship in Chinook salmon is complex. We found both additive and non-additive genetic effects that increased survivorship during the endogenous feeding stage by as much as 19%. Provided these genetic effects continue into the exogenous feeding stage at the same level each 80 day period, and assuming the offspring mature between three and five years of age, we can estimate that there would be between 2.6 and 4.3 times more salmon surviving as compared to offspring produced using traditional, random mating protocols (all else being equal). Therefore, incorporating genetic quality into breeding designs could significantly enhance the effectiveness of supportive breeding programs. However, more studies are needed that assess the benefits of free mate choice in captive breeding programs and studies are needed to identify candidate fitness loci or parental phenotypic traits that correlate with fitness, for supportive breeding programs.

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