Short communication

Non-additive genetic effects contribute to larval spinal deformity in two populations of Chinook salmon (*Oncorhynchus tshawytscha*)

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ABSTRACT

Losses due to larval spinal deformities are widespread in hatchery production. However, the aetiology of this disease remains unclear in most fishes, despite overwhelming evidence for a genetic role in other vertebrate taxa. We examined the contribution of additive and non-additive genetic effects and maternal effects to the incidence of spinal deformity in 50,800 larval Chinook salmon (*Oncorhynchus tshawytscha*) derived from a full factorial quantitative genetic breeding experiment conducted on two populations from British Columbia, Canada. The overall incidence of spinal deformity was low at only 0.69% and 0.05% of offspring in the Big Qualicum and Quinsam populations, respectively. However, spinal deformities affected 34% and 13% of families within the two respective populations, and up to 21% of offspring were affected within susceptible families. Non-additive genetic effects, but not additive or maternal effects, were significantly associated with spinal deformity in larvae. In the Big Qualicum population, non-additive genetic effects explained 100% of the total phenotypic variance in spinal deformity, whereas 80% of the phenotypic variance was explained by non-additive genetic effects in the Quinsam population. Relatedness between parents and offspring sex was not associated with spinal deformity. These results contrast to other studies of salmonids that have shown the effects of additive genetic variance on spinal deformity in later life-history stages and relatedness between parents on larval spinal deformity. Our results instead indicate that the interaction between parental genomes outside of inbreeding plays an important role in the occurrence of spinal deformity in Chinook salmon larvae.

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1. Introduction

Deformity of the spine is a commonly observed disease across vertebrate taxa. In fishes, spinal deformities can take the form of lordosis (swayback), scoliosis (curvature from side to side), and kyphosis (hunchback). These deformities are generally referred to as LSK. The occurrence of spinal deformity in fishes has been reported for many species including the economically important Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*; McKay and Gjerde, 1986; Andrades et al., 1996; Divanach et al., 1997). These species are commonly reared in an aquaculture setting and the occurrence of spinal deformity has been cause for significant economic and animal welfare concern (Sullivan et al., 2007a). Spinal deformity may lower levels of production due to decreased survival (Andrades et al., 1996) and deformed individuals are often unacceptable to consumers (Gjerde et al., 2005). Moreover, spinal deformities are relatively widespread in aquaculture and hatchery settings relative to what has been observed in the wild, so there is significant interest in addressing the potential

causes of these deformities (Boglione et al., 2001). Nevertheless, the aetiology of spinal deformity remains poorly understood.

It has been suggested that spinal deformities result from both environmental and genetic factors (Valentine, 1975). However, studies examining the development of spinal deformities in fish species have largely focused on assessing the role of only the environmental factors. For example, a study on goldfish (*Carrassius auratus*) has shown that water temperature is associated with spinal abnormalities (Wiegand et al., 1989). Kyphosis of the spine is associated with infection by a *Myxozoan* parasite in Japanese mackerel (*Scomber japonicus*; Yokoyama et al., 2005). Insufficient dietary components (see Cahu et al., 2003 for review) or exposure to pollutants such as organophosphates or organochlorines (Mount and Stephen, 1967; Couch et al., 1977) has also been implicated in the development of spinal abnormalities, as have high water current settings during development in hatcheries (Chatain, 1994; Divanach et al., 1997).

Relatively few studies in nonmodel fishes have examined the potential role of genetics in spinal deformities despite overwhelming evidence for a genetic basis in humans and in model systems (Pourquié and Kusumi, 2001; Gorman and Breden, 2007; Heary and Madhaven, 2008). Research in humans has suggested that spinal deformities are X-chromosome linked, as females exhibit a two-fold higher incidence of the disease than do males (Justice et al., 2003). Yet, other research

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suggests that spinal deformity is autosomal dominant and influenced by multiple genes (Wise et al., 2000; Chan et al., 2002; Salehi et al., 2002; see Heary and Madhaven, 2008 for a review). In fishes, early studies conducted on rainbow trout have shown that inbreeding (a non-additive genetic effect) contributes to spinal deformity in juveniles (Aulstad and Kittelsen, 1971; Kincaid, 1976). More recently, Gjerde et al. (2005) examined the prevalence of spinal deformities in 14–17 month old Atlantic salmon from full and half-sib families and found a significant additive genetic effect contributing to the development of spinal deformity. McKay and Gjerde (1986) also found that spinal deformity in 2-year old Atlantic salmon was heritable. However, other studies in Atlantic salmon and tilapia (*Oreochromis aureus*) have suggested that spinal deformity is not heritable (Tave et al., 1982; Sullivan et al., 2007a). These data suggest a complex aetiology of the disease with many contributing factors.

Determining the potential role of genetics in the development of spinal deformities is further complicated by the varied and potentially differing causes of deformities throughout development. Indeed, spinal deformities may arise during embryonic (congenital) development or in later life-history stages (Andrades et al., 1996). In humans, it is thought that congenital and adolescent spinal deformity exhibit differing pathologies (Heary and Madhaven, 2008). In fishes, to our knowledge, studies have only examined the contribution of genetics to the occurrence of spinal deformity in older fishes; yet congenital deformities may also represent significant losses to aquaculture (Andrades et al., 1996; Sullivan et al., 2007b; see Gorman and Breden, 2007 for review). Studies from model vertebrate species suggest an important role for genetics in the development of congenital spinal deformities. For example, mouse, *Xenopus*, and zebrafish model systems indicate that genetic factors are involved in regulating the development of spinal patterning during embryonic development and may influence the expression of spinal deformity (see Pourquié and Kusumi, 2001 for review). Moreover, familial and twin studies in humans have shown a clear genetic contribution to the occurrence of congenital spinal deformity (Heary and Madhaven, 2008). Thus, it is likely that genetics also play a role in congenital spinal deformities in fishes.

In this study, we used a full factorial quantitative genetic breeding approach to examine the genetic contribution to spinal deformity in larval Chinook salmon in two populations from British Columbia, Canada. Our study design allowed us to examine the contribution of additive and non-additive genetic effects as well as maternal effects to spinal deformity. Larvae were tracked to the end of the endogenous feeding stage, so dietary factors did not influence the development of spinal deformity. Moreover, larvae within each population were raised in a common environment, so as to control for potentially confounding environmental factors such as differences in water currents or temperature within the rearing environment. We also examined the potential contribution of inbreeding to larval spinal deformity in each of our populations. This was achieved by examining the relatedness between the breeding males and females using hypervariable micro-satellite genetic markers. The potential for sex-biased expression of spinal deformity in the larvae was also assessed by genetically sexing a subset of individuals with spinal deformity and a subset of phenotyp-ically normal individuals.

2. Materials and methods

2.1. Collection of Chinook salmon

We conducted crosses between eight male and eight female Chinook salmon in each of two populations located in the Big Qualicum and Quinsam rivers in British Columbia, Canada. Wild Chinook salmon were collected from the Big Qualicum River using diversion channels located at the Fisheries and Oceans Canada Big Qualicum salmon hatchery. At the Quinsam River, wild adults were collected by seine netting from natural holding ponds during the migration to the spawning grounds.

Crosses were conducted on October 8, 2007 at the Big Qualicum hatchery (located near Qualicum Beach, B.C.), and on October 15, 2007 at the Quinsam hatchery (located near Campbell River, B.C.). Males and females were euthanized prior to gamete collection using carbon dioxide. Milt was collected from males by applying pressure to the male's abdomen and eggs were collected by dissecting the abdomen of the female and removing the eggs. Eggs and milt were stored in dry buckets and Whirl-paks bags (Nasco Plastics, Newmarket, Canada), respectively, until the crosses were conducted.

2.2. Breeding crosses

Within each population, all males and females were crossed, resulting in 64 unique family groups. Each cross was replicated 4 times ($N = 256$ family groups) and each replicate consisted of 100 eggs. Eggs were stored under cool conditions until fertilization could take place (<2 h from collection) and were fertilized in the order in which they were collected. Families were stored in individual egg tubes (Dynamic Aqua-supply Ltd, Surrey, Canada) and were randomly allocated to a location within a Heath incubation tray system located at each hatchery. The tray location of each tube was noted as location within the stack system has previously been shown to influence the survival of offspring (Pitcher and Neff, 2007). Family groups were monitored bi-weekly throughout the larval stage (between ~500 and 1000 accumulated thermal units (ATUs in °C)) for external evidence of spinal deformity. Monitoring began on December 10 2007 in the Big Qualicum population and on December 8 2007 in the Quinsam population. All families within a population were examined for spinal deformity on the same day. Between populations, we examined families for deformed larvae within 2 days of each other. In our analysis we used the total number of offspring with spinal deformities within each family group at the end of the larvae stage (~1000 ATUs). All forms of spinal deformity (including humpback, swayback, curvature from side to side, or a combination of these) were analyzed as a single grouping, as we were unable to distinguish the affected vertebrae without radiographic equipment.

2.3. Relatedness

Chinook salmon parents were genotyped at seven microsatellite loci (Table 1). Loci were amplified using polymerase chain reaction (PCR) following the protocol outlined in Heath et al. (2006). PCR products were analyzed using the Li-Cor 4300 DNA analyzer (Li-Cor Biosciences, Lincoln, Nebraska). For each population we calculated the number of alleles and the expected (H_E) and observed (H_O) heterozygosity at each locus using Genepop v. 3.4 (Raymond and Rousset, 1995). Pairwise relatedness estimates between the dams and sires used in the crosses were calculated using the method of Ritland (1996) in GeneAlEx v. 6.2 (Peakall and Smouse, 2006).

2.4. Offspring sex determination

We amplified intron-E of the growth hormone (GH) gene to identify the sex of larvae. The GH gene is duplicated in most salmonids, but males possess a third pseudogene (GH-Ψ) presumptively located on the Y-chromosome, thereby allowing for easy sex determination (Du et al., 1993; Zhang et al., 2001). Intron-E of the GH-I, GH-II and GH-Ψ loci were amplified using PCR following the methods outlined in Zhang et al. (2001). PCR products were visualized on agarose gels, and individuals were identified as males when a fragment corresponding to the GH-Ψ pseudogene (~273 bp) was present on the gel in addition to fragments corresponding to the GH-I (~782 bp) and GH-II (~400 bp) loci (Du et al., 1993).

Table 1

Microsatellite loci used to assess relatedness between Chinook salmon (*Oncorhynchus tshawytscha*) parents. Forward and reverse primer sequences, annealing temperatures (T_m) used in PCR reactions, observed (H_O) and expected heterozygosity (H_E) and number of alleles (A) found in the parents in each population are indicated.

Locus	Primer sequence[a]	T_m (°C)	Big Qualicum			Quinsam		
			H_O	H_E	A	H_O	H_E	A
Ots107	F-ACAGACCAGACCTCAACA? R-ATAGAGACCTGAATCGGTA	58	16	15.0	14	16	14.6	11
Ots104	F-GCACTGTATCCACCAGTA R-GTAGGAGTTTCATTTGAATC	64–49[b]	15	14.9	16	13	13.6	12
OtsG311	F-GCGGTGCTCAAAGTGATCTCAGTCA R-TCCATCCCTCCCCCATCCATTGT	64–49[b]	15	14.0	18	16	15.2	16
OtsG249	F-TCTCAGAGGGTAAAATCTCAGTAAG R-GTACAACCCCTCTCACCTACCC	64–49[b]	16	15.1	15	15	15.3	18
Omy325	F-TGTGAGACGTGCAGATTTTGC R-CGGAGTCCGTATCCTTCCC	56	13	13.2	8	10	11.4	6
OtsG83B	F-TAGCCCTGCACTAAAATACAGTTC R-CATTAATCTAGGCTTGTCAGCAGT	60	15	15.1	15	16	14.9	13
OtsG68	F-CATGTACGTGGCGAAGCCCTC R-CATGTCGGCTGCTCAATGTA	55	16	15.3	16	13	14.1	14

[a] Primer sequences published in: Nelson and Beacham, 1999; Williamson et al., 2002; O'Connell et al. 1997.

[b] Touchdown PCR: annealing temperature set at 64 °C for initial cycle, then dropped by 1 °C per subsequent cycle until a final annealing temperature of 49 °C was reached and continued through the remaining cycles.

2.5. Statistical analysis

Within each population, we initially examined the potential that tray location (Big Qualicum: 14 trays; Quinsam: 14 trays) in addition to Sire and Dam identities influenced the occurrence of spinal deformity using a three-way ANOVA. Tray, Dam, and Sire were treated as random factors. However, this analysis revealed that tray did not significantly influence spinal deformity rate in either population; thus, tray effects were excluded from subsequent analyses. A two-way ANOVA was then used to partition variance in rate of offspring spinal deformity to maternal effects, non-additive genetic effects, and additive genetic effects (Lynch and Walsh, 1998). Sire and Dam identities and the interaction between Dam and Sire (Dam×Sire) were treated as random factors within the model. Following Lynch and Walsh (1998), the contribution of additive genetic effects to spinal deformity rate was estimated as 4× the Sire contribution to variance. Non-additive genetic effects were estimated as 4× the Dam×Sire contribution to variance (Lynch and Walsh, 1998). Maternal effects were calculated as the difference between the variances associated with Dams and Sires (Lynch and Walsh, 1998). Due to the unbalanced design of the Big Qualicum dataset, we also used REML analysis to partition variation in spinal deformity across family groups to Dam, Sire and tray effects. However, we only report the results of the ANOVA, as both methods of analysis provided analogous results.

Independent-sample *t*-tests were used to examine whether rates of spinal deformity within families or relatedness between parents were significantly different between populations. Spearman's rank correlations were used to assess the statistical significance of the relationship between relatedness and rate of spinal deformity within families. Fisher's exact tests were used to examine whether offspring sex ratios differed in larvae with spinal deformity versus in phenotypically normal larvae. All statistical analyses were run in SPSS v.17. Means are reported ±1 SE. All significance values reported are two-tailed and we used a significance value of $\alpha = 0.05$ throughout.

3. Results

3.1. Quantitative genetics

We monitored rates of spinal deformity in a total of 50,800 larvae derived from 8×8 crosses conducted in the Quinsam and Big Qualicum populations, respectively. Five replicates were excluded from the analysis in the Big Qualicum population because families generated from crosses between female 8 and male 5 ($N = 1$ replicate), female 8 and male 7 ($N = 1$ replicate), and female 8 and male 1 ($N = 3$ replicates) had 0% survivorship to the larval stage. Overall, spinal deformity affected 34% of families in the Big Qualicum population and 13% of families in the Quinsam population. Rates of spinal deformity within family groups averaged $0.69 \pm 0.17\%$ (range = 0–21%) in the Big Qualicum population and $0.05 \pm 0.01\%$ (range = 0–2%) in the Quinsam population (Fig. 1, Supplementary materials). Overall, Big Qualicum families exhibited a significantly higher overall rate of spinal deformity than did the Quinsam families (independent-samples $t_{126} = 3.81$, $P < 0.001$).

In the Big Qualicum population, Dam, Sire, and Dam×Sire effects explained 37.1% of the variation in spinal deformity (Table 2). However, only Dam×Sire effects were significantly associated with variation in spinal deformity rate among families, representing 34.8% of the total variance in spinal deformity (Table 2). We estimated the non-additive genetic effects from the Dam×Sire component of variance as 0.5×10^{-3} ($= 4 \times 12.5 \times 10^{-5}$) which represents approximately 100% ($= 0.5 \times 10^{-3}/0.5 \times 10^{-3}$) of the total phenotypic variance in spinal deformity rate. The Sire component of variance was not significant indicating that there was no evidence of an additive genetic effect on larval spinal deformity. Similarly, the Dam component of variance was not significant, indicating that maternal effects, which include additive genetic effects and maternal environmental effects such as egg quality, were not significant contributors to spinal deformity in the larvae.

In the Quinsam population, we similarly found that Dam×Sire effects were the only significant source of variation in spinal deformity representing 18.3% ($= 9.7 \times 10^{-7}/4.8 \times 10^{-6}$) of the total variance in spinal deformity (Table 2). Among Quinsam family groups, we estimated that the non-additive genetic effects were 3.9×10^{-6} ($= 4 \times 9.7 \times 10^{-7}$), which explained 80% ($= 3.9 \times 10^{-6}/4.8 \times 10^{-6}$) of the phenotypic variation in spinal deformity. Also, we did not find a significant Sire or

Table 2

Summary of two-way ANOVA results for rate of larval spinal deformity in two populations of Chinook salmon (*Oncorhynchus tshawytscha*).

Population	Source of variation	DF	SS	MS	F	P	σ^2	% Total variance
Big Qualicum	Dam	7	0.013	0.002	1.6	0.166	1.1×10^{-5}	3.1
	Sire	7	0.007	0.001	0.9	0.551	-0.3×10^{-6}	−0.8
	Dam×sire	49	0.060	0.001	5.4	<0.001	12.5×10^{-5}	34.8
	Residual						36.1×10^{-5}	63.0
Quinsam	Dam	7	0.5	0.07	0.9	0.542	-3.6×10^{-8}	−0.7
	Sire	7	0.4	0.05	0.7	0.716	-9.2×10^{-8}	−1.7
	Dam×sire	49	4.1	0.08	1.9	0.001	9.7×10^{-7}	18.3
	Residual						4.0×10^{-6}	84.1

Sources of variation, degrees of freedom (DF), sum of squares (SS), mean square (MS), *F* statistic, *P*-value, variance component (σ^2) and the percent of total variance explained by each source are indicated.

Dam effect within the Quinsam population, indicating that additive and maternal effects are not significant contributors to variance in spinal deformity.

3.2. Relatedness and offspring sex

Relatedness between Quinsam Dams and Sires averaged -0.009 ± 0.004 (range: -0.058 to 0.081). Relatedness between Big Qualicum Dams and Sires averaged -0.013 ± 0.004 (range: -0.060 to 0.086). The observed relatedness between Dams and Sires did not differ significantly between the Big Qualicum or Quinsam populations (independent-samples $t_{126} = 0.75$, $P = 0.46$). We did not find that relatedness between Dams and Sires was correlated with the rate of spinal deformity observed within families in either the Big Qualicum population (Spearman's rank correlation: $r = -0.09$, $P = 0.48$, $n = 64$) or the Quinsam population (Spearman's rank correlation: $r = 0.12$, $P = 0.34$, $n = 64$). We assessed the sex ratio of offspring in 48 larvae with spinal deformities and 43 phenotypically normal larvae randomly selected from both populations. We did not find that sex ratios differed between individuals with spinal deformities (Males: $n = 27$; Females: $n = 21$) or in phenotypically normal individuals (Males: $n = 24$; Females: $n = 19$; Fisher's exact test: $P = 1.00$).

4. Discussion

We used a full factorial breeding design to assess the role of genetics in spinal deformity in Chinook salmon larvae in two populations in British Columbia, Canada. Our results are the first to indicate a non-additive genetic effect on larval spinal deformity in fishes. Strong evidence was found for non-additive genetic effects in both the Big Qualicum and Quinsam populations, despite large differences in the overall rate of spinal deformity between the two populations. These results suggest that the interactions between the genomes of parents play a key role in the development of spinal deformity in larval Chinook salmon. In contrast, McKay and Gjerde (1986) and Gjerde et al. (2005) found evidence for additive genetic effects on spinal deformity expressed in adult Atlantic salmon. They each found that the offspring of particular males exhibited higher levels of spinal deformity, presumably because they inherited alleles predisposing them to the disease from the father. However, the experimental design employed in both studies did not allow the researchers to determine whether there were significant non-additive or environmental effects on the observed variance in spinal deformity; although, inbreeding may have been a factor in the Gjerde et al. (2005) study as a significant correlation between inbreeding and deformity in full-sib families was found. Sullivan et al. (2007a) were unable to replicate the results of either of those studies, as they found no evidence of additive genetic effects on spinal deformity in adult Atlantic salmon. Thus, it remains unclear whether additive genetic effects play a role in spinal deformity in older Atlantic salmon. Furthermore, in contrast to what has been documented thus far for adult spinal deformity in fishes, our results indicate that non-additive genetic effects, but not additive genetic effects, play a role in spinal deformity in larval Chinook salmon. Of course, as has been suggested for humans, it is possible that differing pathologies are associated with the onset of spinal deformity in juvenile and later life-history stages (see Heary and Madhaven, 2008).

Inbreeding has previously been implicated as a factor involved in spinal deformity in larval fishes (Aulstad and Kittelsen, 1971; Kincaid, 1976). In our study, relatedness between parents was not significantly correlated with rates of spinal deformity in either the Big Qualicum or Quinsam populations. Also, the relatedness of parents from both populations did not differ, yet the rate of spinal deformity among Big Qualicum families was higher, suggesting that other factors are influencing the observed level of spinal deformity. However, the overall rates of spinal deformity observed among Chinook salmon

larvae in both populations were relatively low, with only 0.69% and 0.05% of fry exhibiting evidence of spinal deformity in the Big Qualicum and Quinsam populations, respectively. This is compared to, for example, 11–43% spinal deformity observed in inbred rainbow trout (Aulstad and Kittelsen, 1971; Kincaid, 1976), and 12% spinal deformity observed in Atlantic salmon (McKay and Gjerde, 1986). It is possible that inbreeding is a factor in populations experiencing high levels of relatedness among brood stock (e.g. in populations derived from a small number of individuals). However, inbreeding is unlikely to be a contributing factor to larval spinal deformity in wild Chinook salmon populations in the Big Qualicum and Quinsam Rivers in British Columbia, as the populations appear to be relatively outbred.

In humans, spinal deformity predominates in adolescent females and studies have found evidence of X-linked inheritance of spinal deformity (Heary and Madhaven, 2008). We did not find evidence for differing sex-ratios in deformed and normal larvae, suggesting that larval spinal deformity is not associated with sex chromosomes in fishes. To our knowledge, our study is the first to suggest that the genetic region(s) involved in spinal deformity in larval fishes is not sex-linked; however, further studies will be necessary to identify likely regions of the genome involved in spinal deformity and to definitively exclude sex chromosome involvement in spinal deformity. Moreover, it is possible that sex-biased occurrence of spinal deformity may result in later life-history stages in fishes, so it will be necessary for future studies of older fishes to examine sex as a possible factor predisposing individuals to spinal deformity.

Differences in the rate of spinal deformity among populations could be related to the differing population genetic structures in the Big Qualicum and Quinsam populations. Both populations are unique stocks exhibiting divergent genetic make-up both at microsatellite loci and at certain functional genetic markers such as the major histocompatibility complex and immunoglobulin heavy-chain (Beacham et al., 2006; Heath et al., 2006). It is conceivable that variation in the gene pool among populations could alter disease susceptibility of individuals if expression of the disease is genetically controlled. Future studies will be necessary to isolate the genetic region(s) responsible for controlling spinal deformity in Chinook salmon in order to begin examining how susceptibility to this disease might vary across populations. A potential focus for further studies could involve the examination of the differing forms of spinal deformity found among individuals and populations. Precise examination of the variation in forms of larval deformities through radiographic study is a necessary first step in identifying the genetic regions that may be involved in the onset of each form of the disease.

In summary, our results suggest that overall susceptibility to larval spinal deformity occurs at a low incidence in both the Big Qualicum and Quinsam River populations, but the potential for large-scale losses (up to 21% of individuals) due to this disease may occur within susceptible families. The interaction between parental genomes and not additive genetic effects, maternal environmental effects or relatedness between parents, appears to drive the susceptibility of offspring to spinal deformity. As such, it is likely that many more individuals carry susceptibility alleles for this disease than can be detected phenotypically. It will be important for future studies to identify the genetic region(s) associated with this disease. Particularly in populations exhibiting high levels of spinal deformity, our results suggest that the brood stock selection process may be able to strategically lower losses due to larval spinal deformity if able to identify unfavourable genetic combinations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.aquaculture.2009.08.018.

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Supplementary Materials Fig 1: Distribution of spinal deformity across family groups in two populations of Chinook salmon (*Oncorhynchus tshawytscha*). Mean percent spinal deformity ± 1 SE is indicated for the Big Qualicum (black) and Quinsam (grey) populations.