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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.

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 microsatellite 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 phenotypically 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).