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Rib and vertebral deformities in rainbow trout (*Oncorhynchus mykiss*) explained by a dominant-mutation mechanism

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ABSTRACT

We suggest and investigate a hypothesis of a dominant-mutation mechanism as a possible cause for rib and vertebral deformities found in farmed rainbow trout. We report on an X-ray characterization and a genetic study of a sample of 45 individuals from a rainbow trout broodstock population in the Faroe Islands, which was found to be affected by externally visible rib deformities. 41 fish were from 36 progeny families of 23 sires and 33 dams and 4 fish were of unknown family origin. These fish were from a family-based selection program, year-class 2005, produced from 144 females and 72 males, and the total number of families was 252. In the X-ray characterization both rib deformities and vertebral deformities were found. Only 8 fish were found to be unaffected: 15 fish had both rib and vertebral deformities, 20 fish had only rib deformities and 2 fish had only vertebral deformities. The abnormal ribs were observed as anomalously short and thin bones located inside the abdominal wall. Some had their ends pointing towards the skin, and in the most serious cases, they were perforating the skin. The numbers of rib and vertebral deformities in each individual were assigned rib and vertebral deformity scores. We estimate the proportions in year-class 2005 of deformed fish, of fish with rib deformities and of fish with vertebral deformities, to be 0.82 ± 0.11, 0.78 ± 0.12 and 0.38 ± 0.14, respectively. The 45 individuals were genotyped at nine microsatellite marker loci to investigate potential inbreeding problems. The actual and effective numbers of alleles, $n_a = 5.8$ and $n_e = 4.2$, the heterozygosity, $H_o = 0.718$, and the inbreeding coefficient, $F_{is} = 0.034$, are similar to the corresponding quantities for Danish strains. The heterozygosity and the inbreeding coefficient indicate a low inbreeding level. A phylogenetic tree and pairwise-relatedness estimates were also constructed from the genotype information and compared with the information from the family-based selection program for rainbow trout. We investigate the dominant-mutation hypothesis and show that genotype counts inferred from the deformity scores are consistent with Hardy–Weinberg equilibrium. From this model, we predict phenotypic segregation ratios of the deformity traits, which are in close agreement with the experimental observations. Finally, we discuss both environmental and genetic causes and conclude in favor of the dominant-mutation mechanism as the most likely cause of the observed deformities.

1. Introduction

Fish in their natural wild environment or produced by aquaculture can be affected by various deformities and sometimes a very high incidence of deformities is found, e.g., an incidence of 81% of spinal deformities was found in a recent study of sea bass (Bardon et al., 2009).

Regarding skeletal deformities, the salmonids are known to be affected by gill-cover defects, jaw deformities and most importantly by spinal deformities (Branson and Turnbull, 2008).

In general, the causes of these deformities are complex and not fully understood. Known factors affecting the incidence of skeletal deformities in salmonids are: diet composition, toxins, timing of vaccine treatments, specific infections, vitamin and mineral deficiencies, egg incubation temperature to first feeding, temperature from first feeding as well as genetic factors (Gjerde et al., 2005; Lall and Lewis-McCrea, 2007; Branson and Turnbull, 2008).

Deformities in farmed fish can cause both economical losses due to a lower disease resistance, higher mortalities and lower processing yields, as well as ethical concerns regarding fish welfare. Therefore, it is desirable to keep the deformities at the lowest possible levels. Hence, an increased understanding of deformities in farmed fish and how they can be reduced is much desired.

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In late 2005, rainbow trout in a Faroese fish farm were reported as being affected by a problem with the ribs. The bones gave rise to small skin erosions that could be seen or palpated on the side of the fish. Initial X-ray inspection by the veterinarian of the fish farm revealed unusual and deformed ribs.

The Faroese fish farms are regulated by the Faroese Food and Veterinary Agency and are regularly inspected. However, the rib deformity problem was, so far, unknown, and the nature of the phenomenon remained an open issue.

When the rib deformity problem was discovered, the suspicion was that these deformities could be caused by inbreeding, due to a repeated use of broodstock from the local rainbow trout population, without introducing new fish from other populations. The genetic relatedness of the broodstock had not been investigated with molecular tools.

To investigate potential inbreeding problems, the Aquaculture Research Station of the Faroes (Fiskaaling) ordered a genetic analysis to be performed of samples from the affected fish farm. These samples were already in stock at the Faroese Food and Veterinary Agency, but without any information on deformities connected to them. This analysis used the same nine microsatellite markers as were used in a study by Silverstein et al. (2004) of rainbow trout strains in the USA. However, the genetic analysis performed by the Agency did not indicate inbreeding problems: $H_o = 0.760$ and $F_{is} = -0.006$ (Christiansen, D., personal communication, 2006).

In the present study we report on both vertebral deformities and rib deformities found in families of the breeding program that stocked the farm (Baeverfjord, G., personal communication, 2006). The objectives of this work are: 1) to report the characterization of the observed deformities in the broodstock population, 2) to investigate the genetic variation and relatedness of the affected broodstock and 3) to suggest and investigate a hypothesis of a dominant-mutation mechanism as a possible cause for the observed deformities.

2. Material and methods

2.1. Population origin and breeding design

The rainbow trout juveniles used by the fish farm were produced at Fiskaaling in a family-based breeding program, which was started in 2003 from a small population of rainbow trout originally imported in 1966–67 from Brøns, Denmark, as described in a Faroese book on the industry history (Jacobsen, 2006). Before this program started, they were used in a phenotypic selection program consisting basically of a simple system with two separate groups of fish, to avoid crossing of close relatives.

Fish from the 2000, 2001 and 2002 generations are the parent generation of the 2005 generation (Hansen, A., personal communication, 2009). 144 females and 72 males were used, and the total number of families was 252. Fish from each mating were reared in separate tanks until they reached tagging size, approximately 5–10 g, and thereafter in communal tanks. The fish tags used were supplied from Trac ID Systems (trac-id.com).

The family information consists of the sire, dam and the family number from the selection program. For each of these numbers, the values 000 were used for the fish of unknown family origin. The family (fam) number equals the tank number, and it was used to look for possible tank effects, which were not found.

2.2. Visual screening

Fiskaaling's rainbow trout broodstock is kept at the land-based station in Skopun on the Island of Sandoy. There, 94 individuals from year-class 2005 were randomly selected and investigated externally for deformities, then classified in two groups, as being affected (fish ID 1–50) or not affected (fish ID 51–94) by rib deformities. From this, we estimated that about 50% of the fish were visibly affected by rib deformities.

The visual inspection and classification were led by Fiskaaling's veterinarian. For genetic analysis, dorsal fin clips were collected from these individuals and stored in ethanol at 4 °C until use. The investigated fish were subsequently frozen and, for practical reasons, only 45 of the 94 fish were selected for analysis in the Nofima Marin X-ray laboratory in Sunndalsøra, Norway. About 50% of the 45 fish were selected from each of the visibly affected and not affected fish groups to represent the proportion of rib deformities in the original sampling: fish with numbers between 1 and 21 were classified by Fiskaaling as being affected by the problem, whereas fish with numbers between 51 and 74 were classified as not visibly affected. These 45 fish were gutted, with a mean weight of approximately 2 kg.

Later, a scanning of the fish tags from these 45 fish and a look-up in the database of the selection program revealed that 41 fish were from 36 progeny families of 23 sires and 33 dams. Five pairs of fish were siblings, i.e. the fish with the following pairs of IDs: (8, 11), (3, 71), (2, 12), (14, 61) and (19, 54) and 4 fish were of unknown family origin since their fish tags were either unreadable or lost.

The 21 visibly affected fish were from 16 families and included two pairs of siblings and three of the fish of unknown families. However, as seen in the subsequent X-ray analysis, many fish visually classified as unaffected were in fact also affected by deformities.

A picture of a rainbow trout with a rib bone penetrating the skin surface is shown in Fig. 1. In most cases, the deformities were not clearly visible to the human eye, but they were found by careful inspection while gently bending the fish and touching the skin to feel the deformities.

2.3. X-ray screening

Radiography of frozen fish was done in a semi-digital system, with a Shimadzu Mobile Art Eco (Shimadzu Corporation, Tokyo) standard X-ray source combined with an FCR Profect image plate reader and FCR Console (Fuji Medical Inc., Japan). For detailed imaging of deviant ribs, sections of the abdominal wall and cutlets were cut and imaged by means of an IMS Giotto mammography system X-ray source (model number 6020/3, IMS Giotto, Bologna, Italy). In order to facilitate demonstration of small structures, various adjustments of radiation dose were tested and image enhancement was done post-exposure by manipulation of brightness and contrast in order to visualize details.

The fish were screened by X-ray imaging. The presence of extra radio-dense structures towards the ventral end of the ribs was noted in a high proportion of the fish, but the nature of these structures was difficult to determine with standard X-ray imaging.

Fig. 1. A rainbow trout broodstock fish with a rib bone penetrating the skin surface (arrow).