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Impaired gonadal development in the sea trout (*Salmo trutta*) × Atlantic salmon (*Salmo salar*) F1 hybrid females

by

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Abstract

Despite large karyotype differences between parental species, the hybrid progeny of the sea trout (*Salmo trutta*) females (2n = 80, FN = 100) and the Atlantic salmon (*Salmo salar*) males (2n = 58, FN = 74) may be viable and even fertile. As hybrids would pose threat to natural populations of parental species through backcrossing, gonadal development in the sea trout × Atlantic salmon hybrids has been studied in the present work. Moreover, cytogenetic investigation was performed to identify any changes in the number and structure of chromosomes that may affect gonadal differentiation and gamete production in the hybrid progeny. Eight males, two intersex individuals and one female were described among the two-year-old hybrids. Some of the hybrid males were already spermiating during the study. Predominance of the testicular component in the gonads was observed in both intersex individuals. Prevalence of males and the presence of intersex individuals together with only one female may suggest disturbances in the gonadal development only in females. Homogeneity of the chromosome number (69) and the chromosome arm number (87) in the hybrid individuals analyzed in this study suggests that unpaired chromosomes disturbed the development of ovaries but not testes.

Key words: hybrids, introgression, sex chromosomes, sex differentiation, telomeres

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Introduction

Atlantic salmon (*Salmo salar*, Linnaeus 1758) and the brown trout (*Salmo trutta*, Linnaeus 1758), including its anadromous form known as the sea trout, are sympatric species in their natural distribution in Europe. Atlantic salmon and brown trout hybridize under natural and control conditions. In the regions where the species co-occur, hybrids have been found in the freshwater and the sea catchments (Adams et al. 2013) with the highest hybridization rates observed in some rivers in Sweden (Jansson, Ost 1997). Even though the percentage of hybrids in the natural populations rarely exceed 1% (Hurell, Price 1991). The re-stocking practices using domesticated fish or their escapes from the fish farms may significantly increase the hybridization rates (Youngson et al. 1993; Castillo et al. 2008; Jansson, Ost 1997). Although both Atlantic salmon and sea trout females were found to be the maternal species of the hybrids (MacGovan, Davison 1992; Garcia-Vazquez et al. 2001), hybridization between these two species has been observed to be asymmetric. In southern Europe, for example, all hybrids caught in nature came from the crossing of salmon females and trout males. In hybrids from North America, on the other hand, the sea trout is the maternal species (MacGovan, Davidson 1992). A higher survival rate of hybrids produced using salmon eggs and trout spermatozoa has been proven under culture conditions. Performance of such hybrids was similar or even better than the performance of the parental species (MacGovan, Davidson 1992). On the contrary, hybrids obtained in the course of insemination of trout eggs with salmon spermatozoa exhibited a very high mortality rate (Galbreath, Thorgaard 1995). Moreover, female salmon × male trout hybrids hatch before female trout × male salmon hybrids (Alvarez, Garcia-Vazquez 2011). Both types of hybrids are able to reproduce, however, when backcrossed with Atlantic salmon, offspring of the female trout × male salmon hybrids showed a significantly reduced survival rate in comparison with the survival of the backcross progeny of reciprocal hybrids (Garcia-Vazquez et al. 2004). Higher mortality rates of female trout × male salmon hybrids may result from the nucleocytoplasmic conflict, i.e. conflict between trout egg cytoplasm and salmon chromosomes. In some salmonid fish hybrids, such nucleocytoplasmic conflict leads to the rearrangements of paternal chromosomes within the telomeric regions, fragmentation of chromosomes and their final elimination (Fujiwara et al. 1997). Although closely related, sea trout and Atlantic salmon differ greatly in the chromosome numbers. The diploid chromosome number in brown trout may vary from 78 to 84 and Atlantic salmon karyotype is composed of 54-58 chromosomes (Phillips, Rab 2001). In hybrids, unpaired chromosomes may disturb meiosis, impair gonadal development and disturb sexual maturation which in turn may decrease the performance of hybrids. Hybrids of the *Salmo* species backcrossed to brown trout usually give unviable offspring (Garcia-Vazquez et al. 2004) what poses a real risk to the trout populations in the regions where this species is abundant. As potentially threatening to the populations of the parental species, *Salmo* hybrids should be carefully studied for the gonadal development. Thus, karyological and histological examination of the sea trout × Atlantic salmon hybrids has been performed in the presented research to assess the potential impact of unpaired chromosomes on the gonadal development and the ability of these hybrids to reproduce.

Materials and methods

Eggs were collected from the sea trout females from broodstock kept at the Department of Salmonid Research, Inland Fisheries Institute in Olsztyn, Rutki, Poland. Atlantic salmon spermatozoa were obtained from the male from broodstock kept in the "*Aquamar*" Fish Farm in Miastko (Poland) and transported to Rutki. Sea trout eggs were inseminated with the Atlantic salmon spermatozoa in November 2011. Inseminated eggs were incubated at 6-8°C under routine program conditions performed at the Departments of Salmonid Research, Rutki.

For the histological examination of gonads, sea trout × Atlantic salmon F_1 hybrid individuals were sampled three times. For the preliminary examination of the gonadal development, eight hybrid individuals were sacrificed in July 2013 (I). Four months later (November 2013), twelve hybrid individuals were sampled for both histological analysis and chromosomal examination (II). In December 2014, ten hybrids were dissected for the assessment of their sexual and gonadal development at the spawning time.

For the preliminary analysis, fragments of gonads were removed, placed on microscopic slides and squashed with coverslips. Histological verification of the gonadal development in hybrids sampled in November 2013 was performed according to the method described by Ocalewicz et al. (2014) with small modifications. Fragments of gonads were sampled and fixed in Bouin's solution. Fixed tissues were then dehydrated in alcohol, fixed in xylene and embedded in paraffin blocks. Slices of 4-5 µm thickness were cut using a rotational microtome, model RM 2155 (LEICA Microsystems, Wetzlar, Germany), and stained with haematoxylin and eosin (HE method) (Zawistowski 1986). Description of shape, size and the type of germ cells presented in gonads were made with a LEICA DM 3000 transmission light microscope and micro image computer analysis software LEICA QWin Pro (LEICA Microsystems AG, Heerbrugg, Switzerland). The nomenclature of cells and cellular structures of gonads was used according to Brown-Peterson et al. (2011).

Cytogenetic analysis was performed on hybrid individuals sampled for histology (n = 12) and sea trout (n = 4) and salmons (n = 8) originated from broodstocks used in the experiment. Metaphase plates were obtained from cell suspensions of the cephalic kidney according to Ocalewicz et al. (2008). For chromosomal localization of the telomeric DNA sequences, the fluorescence *in situ* hybridization (FISH) procedure with a telomere PNA (peptide nucleic acid) probe conjugated with FITC (fluorescein isothiocyanate) (DAKO, Denmark) was performed according to the manufacturer's protocol. Chromosomal DNA was denatured at 84°C for 5 minutes under the coverslip in the presence of the PNA probe. Hybridization took place in the darkness at room temperature for at least 60 minutes. After the hybridization procedure, chromosomes were counterstained with 25 μl (1.5 μg ml^{-1}) of DAPI (4', 6-diamidino-2-phenylindole) in antifade solution Vectashield. Metaphase plates were analyzed under a Zeiss Axio Imager A1 microscope equipped with a fluorescent lamp and a digital camera. The images taken were electronically processed using the Band View/FISH View software (Applied Spectral Imaging).

Results

Chromosome numbers of the sea trout and the Atlantic salmons from the studied broodstocks were 2n = 80 (FN = 100) and 2n = 58 (FN = 74), respectively (Figure 1a, 1b). In all cytogenetically studied sea trout × Atlantic salmon hybrids, the diploid chromosome number was 69 and the chromosome arm number was 87 (Figure 1c, 1d). Fluorescent signals after PNA-FISH with the telomeric probe were observed only at the ends of all sea trout, Atlantic salmon and the hybrid chromosomes (Figure 1).

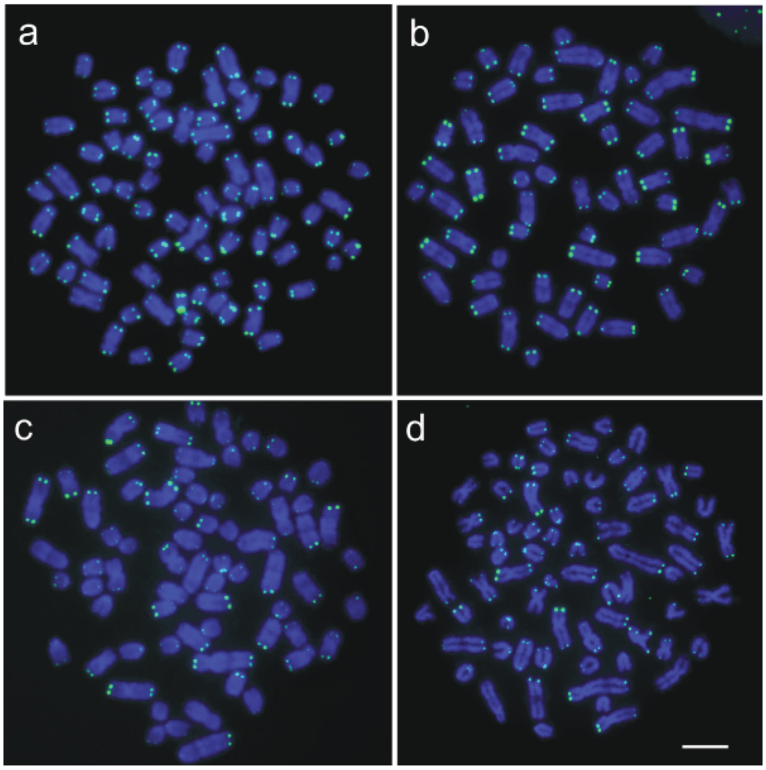

Figure 1

Metaphase spreads of the sea trout (2n = 80) (a), Atlantic salmon (2n = 58) (b) and sea trout × Atlantic salmon hybrid female (c) and hybrid male (d) (2n = 69) after fluorescence *in situ* hybridization with the fluorescein-conjugated telomere PNA probe. Chromosomes were counterstained with DAPI. Bar represents 5μm.

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Microscope analysis of the squashed gonadal tissue enabled the identification of four males and four intersex individuals with significant predominance of the testicular component and few oocytes among fish sampled in July 2013.

Among cytogenetically and histologically examined hybrid individuals (November 2013), eight males, two intersex individuals and one female were described. In one specimen, gonads were not found. Predominance of the testicular component was observed in the gonads of both intersex individuals. Gonads in both intersex individuals exhibited a mosaic type with a larger testicular component filled by spermatogonia, primary and secondary spermatocytes and considerably smaller and randomly distributed ovarian tissues. In the ovarian tissue, intersex gonads revealed the presence of a few atretic cortical oocytes (Figure 2a). Histological analysis evidenced that the studied male specimens were at different stages of spermatogenesis. In three specimens, histological analysis revealed an early stage of spermatogenesis with numerous spermatogonia, primary and secondary spermatocytes (Figure 2b). The spermatogenesis process was shown to be more advanced in five males, which were already spermiating during the examination. Their gonads contained mostly spermatids and spermatozoa (Figure 2c). Histological analysis of the female gonads showed ovarian tissue with regular ovarian lamellae containing oogonia, few cortical alveolar oocytes and single atretic cortical oocytes (Figure 2d).

Seven males, two females and one intersex were observed among the three-year-old fish. All males show two, properly developed testes ready for spermiation (Figure 3a). Both females had only few greenish-yellow eggs that looked overripe (Figure 3b).

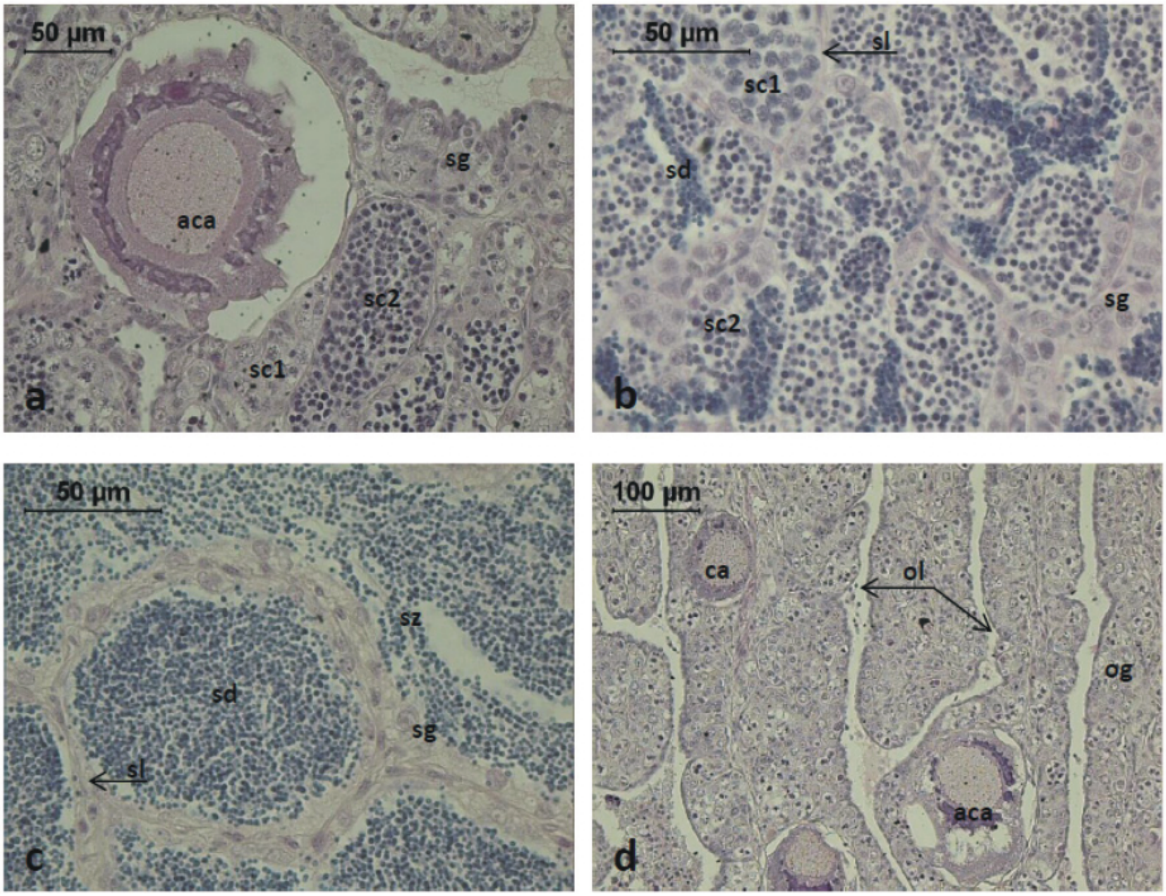

Figure 2

Cross-sections of gonads of the sea trout and Atlantic salmon hybrids: a – mosaic intersex; b – male at developing stage of maturation, c – male at spawning capable phase, d – female at developing stage of maturation. Descriptions: aca – atretic cortical alveolar oocyte, ca – cortical alveolar oocyte, og – oogonia, ol – ovarian lamellae, sg – spermatogonia, sc1 – primary spermatocytes, sc2 – secondary spermatocytes, sd – spermatids, sl – wall of seminiferous tubule, sz – spermatozoa

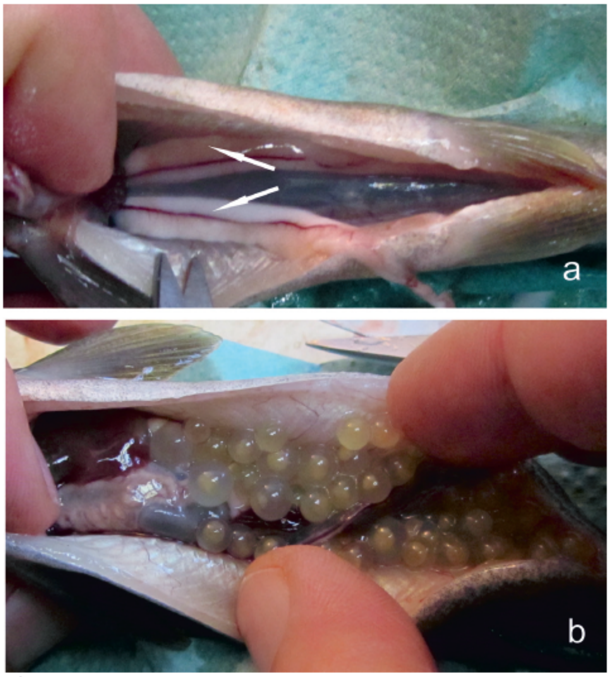

Figure 3

Dissection of the three-year-old adult sea trout × Atlantic salmon hybrid male (a) and female (b). Arrows indicate testes.

Discussion

Interspecific hybrids of salmonid fish species have been recorded in nature. Although hybrids are expected to be sterile, fertile progeny of crosses among *Salmo* species or *Salvelinus* species have been observed. Co-existence of the fertile hybrid individuals and the parental species may lead to the introgression which was documented in the case of e.g. brook trout (*Salvelinus fontinalis*, Mitchill 1814) and Arctic charr (*Salvelinus alpinus*, Linnaeus 1758) from eastern Quebeck, Canada (Bernatchez et al. 1995) or Atlantic salmon and brown trout in the north of Spain (Castillo et al. 2007; 2008). Interestingly, all *Salmo* hybrids observed in some European regions were progeny of the female salmon and the male brown trout (Alvarez, Garcia-Vazquez 2011). Artificially performed reciprocal crosses of these two species resulted in higher mortality of the female trout × male salmon hybrid offspring during the embryonic development and immediately after hatching (Galbreath, Thorgaard 1995; Álvarez, Garcia-Vazquez 2011). Furthermore, many hatched trout × salmon hybrids exhibit external malformations (Álvarez, Garcia-Vazquez 2011). It is believed that such asymmetric maintenance of hybridization is related to the mismatch between gametes, which favors only one direction of the cross, the strong paternal effect on the embryonic development and some incompatibility between trout cytoplasm and salmon nuclear DNA (Álvarez, Garcia-Vazquez 2011). Conflict between the egg cytoplasm and the sperm nucleus resulting in the uniparental chromosome elimination makes the hybrid offspring of the female masu salmon (*Oncorhynchus masou masou*, Brevoort, 1856) and male rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) inviable (Fujiwara et al. 1997). Moreover, some of the presumed rainbow trout chromosomes identified in the karyotype of such hybrids missed the telomeric DNA sequences on one or both arms suggesting deletions within these regions (Fujiwara et al. 1997). Neither chromosome loss nor deletions within the telomeric chromosomal regions were observed in the hybrid individuals examined during this study, which rather proves against the theory on incompatibility between the sea trout egg cytoplasm and the Atlantic salmon chromosomes. Irrespective of the maternal species, hybrid offspring of *Salmo trutta* and *S. salar* show equal genomic contribution of the haploid set of chromosomes from each of the parental individuals (Perez et al. 1999; present study). Nevertheless, segregation of chromosomes in the hybrids differs depending on the maternal species. Hybrid females obtained in the course of crossing between female trout and male salmon produced haploid gametes, while female progeny of male trout and female salmon were found to produce unreduced eggs (Garcia-Vazquez et al. 2004).

The observed increase in the number of intersex specimens and very limited number of females may suggest that hybrid females had problems with the gonadal differentiation. The impaired ovarian development has been also reported for the captured adult *Salmo salar* × *S. trutta* hybrids (Youngson et al. 1992). Histological anomalies during the anatomical sex differentiation have been found in hybrids of European whitefish (*Coregonus lavaretus*, Linnaeus 1758) and peled (*Coregonus peled*, Gmelin 1789) reared in the controlled conditions (Demska-Zakes, Mamcarz 1996) or in the brook trout × Arctic charr hybrids (Ocalewicz et al. 2014). These alterations included the occurrence of both testes and ovaries in the same individual or the existence of mosaic gonads. The mosaic structure of gonads was noted in two hybrids of the sea trout × Atlantic salmon examined in this study. Single, properly developed oocytes (cortical) and atretic cortical alveolar oocytes were set in testis tissue in gonads morphologically resembling a typical feature of testes. Similar predominance of the testicular component and degeneration of maturing oocytes or disrupted differentiation of ovaries were observed in *C. peled* × *Coregonus nasus*, Pallas 1776 hybrids (Bogdanova 2002). Nevertheless, the results of our experiment showed that hybrid progeny of the sea trout females crossed with the Atlantic salmon males survived until the maturation stage and the hybrid males had properly developed testes able to produce spermatozoa in the second year of life.

Lack of the chromosomal differences between the studied hybrid specimens suggested that if unpaired chromosomes impair the gonadal development it might be the development of ovaries. Sex chromosomes in the salmonid fish species are at different stages of the morphological differentiation and they usually do not show homology between species (Woram et al. 2003; Phillips et al. 2011). In Atlantic salmon, a large metacentric chromosome has been identified as a sex chromosome (Artieri et al. 2006), while in the brown trout, the sex determining gene(s) is located on a small subtelocentric/acrocentric chromosome (Li et al. 2011). Thus, the disturbed development of gonads in hybrid females may result from the conflict between trout and salmon sex chromosomes or particular genes responsible for the female sexual development in both parental species. The strong effect of sex chromosomes on the hybrid unviability or sterility has been describe in various organisms (Bhattacharyya et al. 2014).

Viable and fertile hybrid offspring may cross with the parental species and lead to the genetic introgression between these two species. In the case of sea trout x Atlantic salmon hybrids, the consequences might be even more serious. Males of such hybrids may reduce the spawning success of the brown trout and the sea trout backcrossing with brown trout females and produce unviable progeny (Garcia-Vazquez et al. 2004).

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Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

All authors were involved in designing and performing the experiments. KO and PH were also involved in data analysis, writing and editing the manuscript.

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