

Cytogenetic and histological studies of the brook trout, *Salvelinus fontinalis* (Mitchill), and the Arctic char, *S. alpinus* (L.) hybrids

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Abstract Although brook trout and the Arctic char hybrids are able to reproduce, individuals with decreased fertility or even fish that are unable to produce any gametes have been also described. Abnormal gonadal development and disturbances in the gamete production in the char hybrid offspring may be triggered by the odd chromosome number and disturbances in their pairing during meiosis. To verify this hypothesis, cytogenetic examination and the gonadal histology analysis of the brook trout x Arctic char hybrids were carried out. Diploid chromosome number in the studied char (F_1) hybrids varied from 82 to 84 ($FN = 99-102$). Among 28 hybrids, 12 males, three females, nine intersex individuals and two sterile specimens were described. In the case of two individuals, gonads were not found. Diploid chromosome numbers in the males and intersex individuals varied from 82 to 84. Chromosome numbers in the females were 82 and 83 chromosomes. Two sterile fish exhibited karyotypes composed of 82 and 84 chromosomes. Predominance of the ovarian component in the intersex gonads and gonadal sex ratio distortion towards the males suggested hybrid females had problems with gonadal differentiation. However, the lack of the clear relationship between chromosome number and gonadal development in the studied hybrids did not support our hypothesis that odd chromosome number may be responsible for such reproductive disturbances in the hybrid individuals. We have presumed that sterility and intersexual development of the gonads may be caused by interactions between brook trout and Arctic char genes on the sex chromosomes and autosomes rather than unpairing of the parental chromosomes.

Keywords Chromosomes · Gonads · Intersex · Reproduction · Sex differentiation

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Introduction

The main aim of the fish interspecific hybridization approach is to produce progenies exhibiting better characteristics when compared to the parental species. Interspecific hybrid fishes have been proposed to be utilized in aquaculture and stocking programs to increase growth rate, combine valuable traits of two species, support disease resistance and produce mono-sex progenies (Bartley et al. 2001). Sometimes, interspecific crosses result in progenies exhibiting decreased reproductive capacity or even sterility that is related to the problems with gonadal development caused by the incompatibility of the parental sets of chromosomes. Production of the sterile fish may reduce the overall costs of the fish rearing as such individuals do not experience decline in growth, survival and meat quality quite frequently observed at the early sexual maturation of the normal fish. Moreover, sterile specimens minimize the risk of unwanted reproduction. So far, several sterile interspecific hybrids like tiger trout (*Salmo trutta* × *Salvelinus fontinalis*), sunshine bass (*Morone chrysops* × *M. saxatilis*), gilthead seabream (*Sparus aurata*) × red seabream (*Pagrus major*) have been described and proposed to be utilized in aquaculture (Bartley et al. 2001). Recently, special attention has been dedicated to the brook trout (*Salvelinus fontinalis*) × Arctic char (*Salvelinus alpinus*) hybrids as their meat is highly appreciated by consumers in several places in Europe (Gross et al. 2004). Although brook trout and Arctic char hybrids are able to reproduce, individuals with decreased fertility or even fish that are unable to produce any gametes have been also described. Diploid chromosome number in the brook trout is invariably 84, while Arctic char exhibits chromosome number that may range from 78 to 84 (Phillips and Ráb 2001). Thus, reproductive problems including abnormal gonadal development and disturbances in the gamete production in the brook trout and Arctic char hybrids can be caused by the odd chromosome number and suppression in their pairing during meiosis. To verify this hypothesis, cytogenetic examination and the gonad histology analysis of the brook trout × Arctic char hybrids were carried out.

Materials and methods

Brook trout eggs and Arctic char spermatozoa were collected from the broodstocks kept at 6–8 °C at the Department of Salmonid Research, Inland Fisheries Institute in Olsztyn, Rutki, Poland. Individuals from the brook trout broodstock are characterized by a diploid chromosome number ($2n$) and a chromosome arm number, FN (Fundamental Number), which are 84 and 100, respectively (Ocalewicz et al. 2004). In the Arctic char individuals from Rutki broodstock, diploid chromosome number varies from 81 to 82 (FN = 100) (Pomianowski et al. 2012). Brook trout eggs collected from four females were inseminated with the spermatozoa from one Arctic char male. Inseminated eggs were incubated at 6–8 °C under routine programme conditions performed at the Departments of Salmonid Research, Rutki.

For the cytogenetic and histological analysis, twenty-eight hybrid individuals were sampled after 8 months of rearing (H1–6) and after 15 months of rearing (H13–H34) (Table 1).

Metaphase spreads were obtained from the cell suspensions of the cephalic kidney according to Ocalewicz et al. (2008). Chromosomes were stained with buffered Giemsa (10 %) and 4, 6-diamidino-2-phenylindole (DAPI) for visualization and description of the chromosomal morphology. Metaphase plates were analysed under a Zeiss Axio Imager A1 microscope equipped with a fluorescent lamp and a digital camera. Images were captured,

Table 1 Results of the karyological and histological examination of the brook trout (*Salvelinus fontinalis*) × Arctic char (*Salvelinus alpinus*) F₁ hybrids (H)

Fish	Chromosome number (2 <i>n</i>)	Chromosome arm number (FN)	Gonadal histology
H1	84	100	Gonads not found
H2	84	100	Intersex
H3	83	100	Male
H4	83	100	Male
H5	84	100	Male
H6	84	100	Male
H13	83	100	Intersex
H14	82	99	Male
H15	84	100	Male
H16	82	99	Intersex
H17	83	100	Female
H18	83	100	Intersex
H19	82	100	Female
H20	82	99	Intersex
H21	83	100	Male
H22	83	99	Male
H23	84	101	Intersex
H24	84	99	Male
H25	82	99	Sterile
H26	83	100	Male
H27	83	100	Intersex
H28	84	100	Gonads not found
H29	84	100	Sterile
H30	84	100	Male
H31	84	102	Intersex
H32	83	100	Male
H33	82	99	Female
H34	–	–	Intersex

and the electronic processing of the images was performed using Band View/FISH View software (Applied Spectral Imaging).

Histological analysis of the karyologically studied hybrid individuals was carried out to confirm gonadal sex of the fish. Fragments of gonads were collected and fixed in Bouin's solution. Subsequently, the tissues were dehydrated in alcohol, fixed in xylene and embedded in paraffin blocks. Slices of 4–5 μm thick were cut using a rotational microtome model RM 2155 (LEICA Microsystems, Wetzlar, Germany) and stained with haematoxylin and eosin (HE method) (Zawistowski 1986). Histological analyses of cross-sections for the shape, size and the type of germ cells present in gonads were made with LEICA DM 3000 transmission light microscope and micro image computer analysis software LEICA QWin Pro (LEICA Microsystems AG, Heerbrugg, Switzerland). The nomenclature of germ cells and cellular structures was used according to Hliwa et al. (2002) and Schulz et al. (2010).

Results

Diploid chromosome number in the studied F_1 char hybrids varied from 82 to 84 ($FN = 99–102$) (Fig. 1). Low number and poor quality of the metaphase spreads made us unable to properly evaluate chromosomes number and morphology in one specimen (Table 1). Among 28 hybrids, three females, 12 males, nine intersex individuals and two asexual or sterile specimens were described. In the case of two individuals, gonads were not found (Table 1) (Fig. 2). The three females were at the early vitellogenic stage of the gonadal development, and previtellogenic and early vitellogenic oocytes were observed in their gonads. Both types of the oocytes were surrounded by the layers of the follicular cells. Previtellogenic oocytes observed at the periphery of the ovarian lamellae exhibited many nuclei located at the margin of the nuclear membrane (Fig. 2a).

Histological analysis of the male gonads exhibited testicular tissue with seminal vesicles and spermatogenic cells at the different stages of spermatogenesis including spermatogonia, primary spermatocytes and secondary spermatocytes (Fig. 2b). Gonads of the mosaic type with larger ovarian component and considerably smaller and randomly distributed testicular portions were observed in all of the intersex specimens. The female



Fig. 1 Karyotypes of the brook trout \times Arctic char hybrids after DAPI fluorochrome staining showing variable chromosome numbers; $2n = 82$, $NF = 99$ (H33 female) (a), $2n = 83$, $NF = 100$ (H13 intersex) (b), $2n = 84$, $NF = 99$ (H24 male) (c) and $2n = 84$, $NF = 102$ (H31 intersex) (d). Bar equals 10 μm

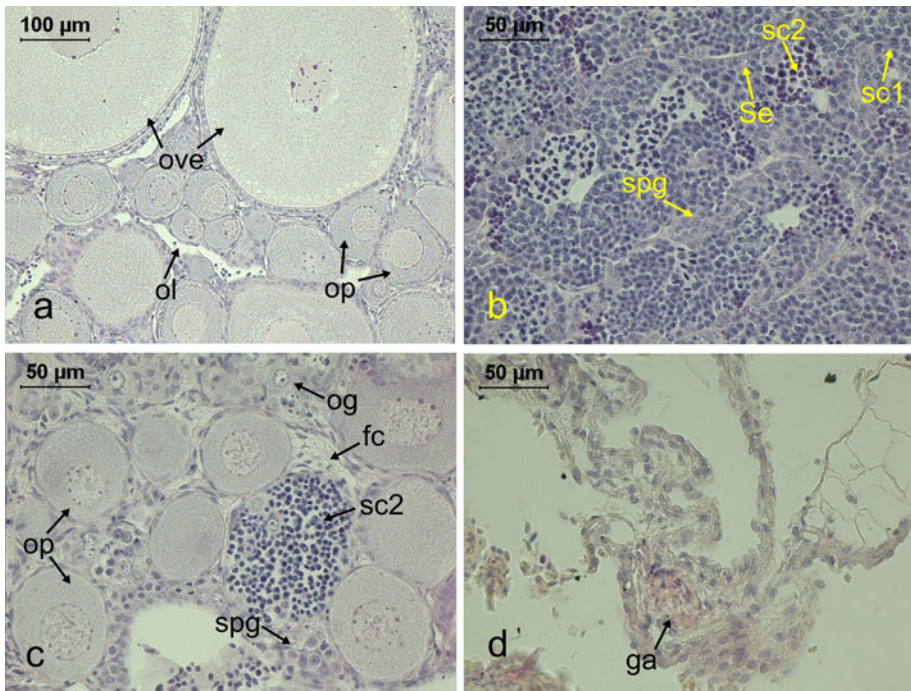


Fig. 2 Haematoxylin and eosin-stained gonad sections of the brook trout (*Salvelinus fontinalis*) × Arctic char (*Salvelinus alpinus*) F₁ hybrid female (H17) (a), male (H21) (b), intersex (H34) (c) and sterile (H29) (d) specimens sampled 15 months after hatching. *fc* follicular cells, *ga* gonadal anlage, *og* oogonia, *ol* ovarian lamellae, *ove* early vitellogenic oocytes (during endogenic growth), *op* previtellogenic oocytes, *spg* spermatogonia, *sc1* primary spermatocytes, *sc2* secondary spermatocytes, *Se* Sertoli cells

gonadal component contained oogonia and previtellogenic oocytes, while male component comprised spermatogonia and spermatocytes. Follicular cells were observed to surround both, oocytes and male components in the intersex gonad (Fig. 2c). The gonadal tissue in the two sterile specimens was reduced to the portion of the gonadal anlage (Fig. 2d).

Diploid chromosome numbers in the males and intersex individuals varied from 82 to 84 (FN = 99–102). Chromosome numbers in the females were 82 (FN = 99–100) and 83 (FN = 100) chromosomes. Two sterile individuals: H25 and H29 exhibited 82 (FN = 99) and 84 (FN = 100) chromosomes (respectively) in their somatic cells. Two fish with no microscopically identified gonads showed karyotypes composed of 84 chromosomes (FN = 100) (Table 1).

Discussion

Although cytogenetic traits of the interspecific hybrids are supposed to be intermediate between two parental species, chromosome numbers observed in such specimens may differ from what is expected. Incompatibility between egg cytoplasm of one species and sperm genome of another species sometimes results in the uniparental chromosome elimination observed during early embryogenesis. Male chromosome elimination through the chromosome loss or partial deletion has been observed in the cross between female

masu salmon (*Oncorhynchus masou masou*, Brevoort, 1856) and male rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) (Fujiwara et al. 1997). Presumably, huge variation in the chromosome number observed among the brook trout x Arctic char hybrids studied here resulted from both inheritance of the chromosomal polymorphism observed in the parental individuals and *de novo* chromosome rearrangements (Ohno et al. 1965; Phillips et al. 1999). Variation in the chromosome number observed in the Arctic char from Rutki is attributed to the centric fusion or fission and known as Robertsonian polymorphism (Pomianowski et al. 2012). Therefore, it is reasonable to assume that the Arctic char male used in the experiment was heterozygous for the Robertsonian polymorphism ($2n = 81$) and provided gametes that differ in the chromosome number ($n = 40$ and $n = 41$). Moreover, the post-zygotic fission of one metacentric chromosome into two acrocentric chromosomes or non-disjunction of the sister chromatids might have been responsible for the increase of the chromosome number in the hybrid specimens exhibiting 84 chromosomes. Incompatibility between parental sets of chromosomes may result in reduced fertility or even sterility in the interspecific fish hybrids (Tave 1993; Hulata 1995). Odd chromosome number and the homologous chromosome pairing suppression may impair gonadal development and/or trigger production of the aneuploid gametes (LeGrande et al. 1984; Goudie et al. 1993). That is why decreased reproductive performance observed in the brook trout x Arctic char hybrids was first of all attributed to the karyological differences between parental species. Nevertheless, we did not find any link between chromosome number and development of the intersexual or sterile gonads in the hybrids studied here.

Sex differentiation process in fish can be modified by genetic, environmental, behavioural and physiological factors (Chan and Yeung 1983; Devlin and Nagahama 2002). Interspecific crosses may change both sex determination and gonadal differentiation processes in the fish hybrid progenies. Predominantly male offspring is produced when the Nile tilapia (*Oreochromis niloticus*) cross with the blue tilapia (*O. aureus*) as both species show different sex determination systems (Beardmore et al. 2001). The Nile tilapia has XX/XY system with the female being homogametic (XX), whereas blue tilapia has ZZ/ZW system with the male showing both sex chromosomes of the same type (ZZ). Hybridization between the Nile tilapia female and blue tilapia male yields all-male or almost all-male progenies (ZX) (Penman and McAndrew 2000). Anomalies during the anatomical sex differentiation have been found in the whitefish (*Coregonus lavaretus* L.) and peled (*Coregonus peled* Gmel.) hybrids where previtellogenic oocytes, vitellogenic oocytes and spermatogonial cells have been found in the course of the histological assay of the gonads (Demska-Zakes and Mamcarz 1996). In the present study, gonadal sex ratio distortion towards males and much bigger portion of the female component in the intersex gonads suggested hybrid char females had problems with gonadal differentiation. Similarly, ovarian development has been observed to be impaired in the Atlantic salmon (*Salmo salar*) x brown trout (*Salmo trutta*) hybrids (Youngson et al. 1992). Both parental char species of the hybrids studied here show genetic sex determination (Woram et al. 2003), but probably, their sex chromosomes are at different stages of the morphological differentiation. Indeed, brook trout putative sex chromosomes are two medium-sized metacentric chromosomes (Phillips et al. 2002; Ocalewicz et al. 2004), while Arctic char sex-linked microsatellite DNA sequences have been mapped to one of the largest metacentric chromosome pair (Kwitkowski 2007). Thus, interactions between brook trout and Arctic char sex chromosomes and genes from the sex chromosomes and autosomes rather than unpairing of the parental chromosomes may affect gonadal development in the hybrids.

Reduced fertility observed in the intersex fish may be related to the malformations of the gonoducts, disruption of the testicular lobule structure and inhibition of the

spermatogenesis (Nolan et al. 2001). Despite the disturbances in the gonadal development, intersex individuals are not sterile. In the case of the brook trout and Arctic char hybrids, interspecific cross followed by the triploidization seems to be a promising approach leading to production of the sterile offspring. Similar attitude has been successfully applied in the Atlantic salmon x brown trout hybrids (Galbreath and Thorgaard 1997).

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