# ANDROGENETIC DEVELOPMENT OF BROOK TROUT (*SALVELINUS FONTINALIS* MITCHILL), ARCTIC CHAR (*SALVELINUS ALPINUS* L.) AND THEIR HYBRIDS

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**Background**:

Interspecies androgenesis is thought to be useful biotechnological method in restoration of extinct or endangered fish species. Unfortunately, generally very high mortality of androgenetic fish during embryogenesis and after hatching is still a limiting factor in application of such approach in species protection programmes. The low survival of androgenetic fish have complex background and seems to be mainly triggered by the quality of eggs, manipulations performed on the oocytes and zygotes and homozygosity for the lethal alleles, among others. Apart from the inappropriate androgenesis parameters, conflict between cytoplasmic factors like maternal mRNAs deposited in the oocytes during oogenesis and male chromosomes may be responsible for the failure of interspecies androgenesis. Therefore, efficient recovery of maternal nuclear DNA in the course of interspecies androgenesis demands the use of fish species which their hybrid progenies are viable and fertile. Moreover, female hybrid individuals when matured might be promising donors of eggs for the recovery of sperm nuclear DNA from one or both parental species. The primary goal of this study was to evaluate suitability of eggs derived from brook trout x Arctic char hybrids for androgenetic development of brook trout and Arctic char individuals.

**Methods:**

In order to destroy maternal nuclear DNA, part of the brook trout, Arctic char and hybrid oocytes were exposed to 42 000 R of X-ray radiation. Left oocytes were kept for the control variants of the experiments. Batches of irradiated and untreated oocytes were further divided into several batches and inseminated independently with semen collected from brook trout, Arctic char and hybrid males. High pressure shock (7,000 psi for 4 min) was applied 420 minutes post fertilization to the part of the irradiated and inseminated eggs in order to double the haploid chromosomes set in the zygotes during their first mitotic division. Fertilized irradiated as well as untreated eggs which were not subjected to the high pressure shock were left to develop as androgenetic haploid control and normal diploid control variants, respectively. Diploid androgenetic batches consisted of *c.* 1 100 eggs, haploid androgenetic control batches, *c.* 350 eggs and normal diploid batches, *c.* 300 eggs. Survival of embryos was checked at their eyed-stage. Live larvae were counted just after hatching and at the swim-up stage (after five weeks of rearing). Survival of androgenetic progenies after swim-up stage was monitored continually. Randomly chosen androgenetic brook trout and hybrids, and their siblings from the control variant of the experiments were karyologically studied after nine months of rearing to confirm efficiency of the androgenesis conditions.

**Results and Discussion:**

In all variants of the experiment diploid androgenetic embryos survived up to the eyed stage with the highest rate (39. 25 %) in the case of androgenetic hybrid embryos developing in the enucleated hybrid eggs, and the lowest rate in the interspecies androgenetic variant where enucleated Arctic char eggs were inseminated with brook trout semen (0. 21 %). Apart from the latter experimental variant, androgenetic brook trout, Arctic char and hybrids hatched. Twenty seven (1, 63%) and 107 (6, 15%) live androgenetic brook trout hatched from the brook trout and hybrid eggs were observed at the swim-up stage, respectively. The only androgenetic Arctic chars that survived to the swim-up stage were individuals hatched from the hybrid eggs (5) and brook trout eggs (3). The most successful in terms of survival rates were androgenetic hybrids that hatched from the hybrid eggs (248 individuals - 14, 17%), brook trout eggs (56 specimens - 6, 15%) and Arctic char eggs (6 individuals - 0, 29%). Viable progenies were obtained in all diploid control variants. Unfortunately, during next months of rearing survival of androgenetic progenies decreased dramatically especially among androgenetic hybrid fish. After nine months of rearing, the only survived androgentic fish were brook trout and hybrids: Sf x Sf (10 specimens), H x Sf (10 specimens), H x H (5 specimens), Sf x H (10 specimens), P x H (2 specimes).

Table 2. Survival (% ± SD) of haploid and diploid androgenetic and control brook trout (Sf), Arctic char (Sa) and the hybrid (H) embryos and larvae.

| Androgenetic variant | Eyed embryos | | Hatched larvae | | Swim-up larvae | |
|---|---|---|---|---|---|---|
| | control | 2n - andro | control | 2n - andro | control | 2n - andro |
| Sf x Sf | 92,82 ±1,15 | 15,98 ±0,85 | 86,25 ±0,36 | 2,13 ±0,11 | 86,25 ±0,36 | 1,63±0,04 |
| H x Sf | 94,80 ±1,27 | 35,86 ±1,63 | 91,48 ±2,90 | 6,77 ±0,31 | 91,48 ±2,90 | 6,15±0,51 |
| Sa x Sf | 76,71 ±3,17 | 0,21 ±0,29 | 68,12 ±4,57 | 0 | 68,12 ±4,57 | 0 |
| Sf x H | 94,88 ±3,28 | 19,05 ±1,67 | 93,13 ±2,79 | 4,68 ±0,58 | 93,13 ±2,79 | 3,17±0,13 |
| H x H | 94,45 ±1,58 | 39,25 ±1,04 | 90,66 ±1,24 | 16,80 ±0,50 | 90,66 ±1,24 | 14,17±0,04 |
| Sa x H | 57,55 ±4,00 | 14,86 ±1,10 | 49,06 ±2,67 | 1,18 ±0,37 | 49,06 ±2,67 | 0,88±0,38 |
| Sf x Sa | 75,47 ±4,09 | 9,96 ±1,39 | 71,49 ±4,44 | 0,18 ±0,10 | 71,49 ±4,44 | 0,18±0,10 |
| H x Sa | 76,55 ±0,53 | 19,68 ±2,93 | 72,27 ±0,54 | 0,64 ±0,13 | 72,27 ±0,54 | 0,29±0,10 |
| Sa x Sa | 55,57 ±1,56 | 4,71 ±0,67 | 43,57 ±1,51 | 0,13 ±0,18 | 43,57 ±1,51 | 0 |

Results of karyological research exhibited diploid chromosome arm number equaled 84 in the androgenetic brook trout and 82 and 84 in the androgenetic hybrids. Apart from the doubled paternal chromosomes, remnants of maternal nuclear DNA in form of chromosome fragments intraindividually variable in number and size were observed in most of the analyzed fish. The highest numbers of X-ray induced chromosome fragments (0-13) were observed in the androgenetic brook trout and hybrids hatched from the hybrid eggs.

**Conclusions:**

1. The use of enucleated brook trout x Arctic char hybrid eggs to restore DNA from the brook trout sperm cells is possible.
2. It has not worked out very well in the case of Arctic chars because:
   2.1. quality of gametes matters! Even medium quality gametes should be avoided when induce androgenesis in fish.
   2.2. even slight interspecies differences during fish ontogeny must be taken into consideration and androgenetic conditions adjusted.
3. High survival rates of androgenetic fish during the first weeks after hatching do not assure high survivability during next months of rearing.