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Short communications

ANDROGENESIS OF IDE, *LEUCISCUS IDUS* (L.), USING CHUB, *LEUCISCUS CEPHALUS* (L.), EGGS

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ABSTRACT. The focus of this study was the agdrogenesis of ide, *Leuciscus idus* (L.), using eggs from other species. Chub, *Leuciscus cephalus* (L.), oocytes were genetically inactivated using UV irradiation. The eggs used in the experiment were obtained from dark-colored chub females, whereas milt was taken from yellow-colored (recessive marker) ide males. The highest yield of ide androgenesis was noted when eggs were exposed to a thermal shock 3 hrs 00 min after activation. Androgenetic origin (haploid or diploid embryos) was checked using a recessive color marker ("blond").

Key words: INTERSPECIFIC ANDROGENOTES, GENOME MANIPULATION, COLOR MARKER, CYPRINIDS

Artificial androgenesis is a method for obtaining an organism without the contribution of maternal chromosomes. In this genome manipulation, the female genome in the oocyte is inactivated using ionizing radiation (Lin and Dabrowski 1998, Kucharczyk 2002). The duplication of the paternal chromosomes is done by applying an environmental shock to suppress the first cleavage in genetically inactivated eggs (Masaoka et al. 1995, Kucharczyk et al. 1998). Androgenesis can be used for the production of homozygous (inbred) lines, nucleo-cytoplasmatic hybrid between different species, the study of sex determination, or the preservation of endangered species (Arai et al. 1992, Bongers et al. 1994). Ionizing radiation such as gamma (γ), X, or UV has been widely used to induce androgenesis in fishes (Scheerer et al. 1986, Fujikawa et al. 1993). The application of gamma or X radiation is technically difficult, because of safety issues (Arai et al. 1992)

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and residual chromosome fragments in the oocyte. UV irradiation has been used for the genetic inactivation of cyprinid oocytes (Kucharczyk et al. 1998, Kucharczyk 2002). The aim of the present study was to examine the possibility of obtaining ide, *Leuciscus idus* (L.), androgenotes from chub, *Leuciscus cephalus* (L.), eggs.

The study was conducted in May 2008. Wild chub females spawners were caught in Mazurian lakes (northern Poland), and the yellow form of ide (orfe) was obtained from Oleśnica Fish Farm (southern Poland). The fish were transported to the hatchery and kept in 1000 dm^3 tanks at a controlled temperature (10-15°C for orfe and 18-20°C for chub) and photoperiod (14L: 10D) (Kujawa et al. 1999). Spawners received injections of Ovopel (Unic-Trade, Hungary) to induce gamete maturation (Kucharczyk 2002, Krejszeff et al. 2008).

In each experiment, milt was collected from a few males. The quality of the sperm was expressed as the percentage of motile spermatozoa. Motility was estimated by microscopic (500X) observation of sperm activated with 0.5% NaCl. Samples of sperm with 70-80% (or more) motile spermatozoa were pooled and used for further treatments. The eggs were stripped from one female 16-18 hrs (chub) or 30-36 hrs (orfe) after the last hormone injection.

During irradiation, chub eggs were kept in Petri dishes in artificial ovarian fluid composed for common carp, *Cyprinus carpio* (L.) (Bongers et al. 1994). The dishes with eggs were placed on a shake table with a cycle of ~1 s, which allowed the eggs to roll in the fluid. A UV lamp (30 W, 6.4 W m^{-2}, Philips, The Netherlands) was switched on at least 30 min before the onset of irradiation. Before the irradiation of the oocytes, control samples of eggs (100-150 eggs in each sample) were fertilized with a small volume (0.05 ml) of sperm (egg quality control group C – chub eggs inseminated with ide semen; group K – ide eggs inseminated with ide milt). Non-irradiated eggs treated in ovarian fluid were also fertilized using the same volume of milt (ovarian fluid quality control group D1). Experimental groups of chub eggs were fertilized with 0.05 ml of orfe sperm after a UV irradiation exposure time of 9 min (the dose of UV irradiation was 3456 J m^{-2}). The eggs were exposed to a thermal shock (36°C; 5 min duration) at 2 h 40 min, 3 h 00 min, 3 h 20 min, 3 h 40 min, and 4 h 00 min. UV-treated eggs that were not exposed to the thermal shock were fertilized using orfe sperm (irradiation quality control group I). After the conclusion of the experiment, eggs from additional control groups were fertilized (treatment in ovarian fluid control group D2). The whole

procedure was conducted in darkness to avoid genetic photo-reactivation (Kaastrup and Hørlyck 1987). Before the application of the shock, the eggs were kept at a temperature of 15°C. After the experiment, the eggs were incubated in a laboratory recirculating system at 17°C. All control and experimental groups were in duplicate.

Egg survival was calculated as the percentage of hatched embryos. The ploidy of the embryos was determined by observing the haploid syndromes and using color markers. Differences in the hatching success and survival of ide embryos were analyzed using analysis of variance (ANOVA) and tested with the post hoc Duncan's multiple range test ($P < 0.05$).

The survival of free embryos in all the experimental groups was much lower than in the control groups (Table 1). Blond colored haploid embryos exhibited morphological abnormalities, which are referred to as "haploid syndrome" (i.e., stunted body, poorly formed retina, etc.). The highest androgenesis yield was noted when eggs were exposed to the shock 3 h after activation.

TABLE 1

Results (mean survival of hatched larvae ± SD) of inducing ide, *Leuciscus idus*, androgenetic development using chub, *L. cephalus,* eggs. Male ide were yellow (orfe) and female chub were wild colored

Groups/Time of shock application	2 N – diploid"black"	1 N – haploids "yellow"	Androgenotes "yellow"
C	82.0 ± 2.1	0.0 ± 0.0	0.0 ± 0.0[a]
D1	77.5 ± 1.8	0.0 ± 0.0	0.0 ± 0.0[a]
I	0.2 ± 0.1	5.1 ± 0.1	0.0 ± 0.0[a]
2 hr 40 min	0.1 ± 0.1	0.2 ± 0.1	0.3 ±0.1[b]
3 hr 00 min	0.1 ± 0.1	0.1 ± 0.1	1.9 ± 0.2[d]
3 hr 20 min	0.1 ± 0.1	0.2 ± 0.1	0.8 ± 0.1[c]
3 hr 40 min	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1[b]
4 hr 00 min	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.0[a]
D2	74.3 ± 2.2	0.0 ± 0.0	0.0 ± 0.0[a]

Data in the Andregenotes column with the same index letter did not differ statistically in the yield of the androgenesis process

The low hatching rate of ide embryos from genetically inactivated oocytes has been observed in many fish species. Data similar to that in the present work were reported for rainbow trout, *Oncorhynchus mykiss* (Walbaum), (Scheerer et al. 1986); brook trout, *Salvelinus fontinalis* (Mitchill), (May et al. (1988); loach, *Misgurnus anguillicaudatus* (Cantor), (Arai et al. 1992, Masaoka et al. 1995); crucian carp,

Carassius auratus (L.), (Fujikawa et al. 1993); common carp (Bongers et al. 1994); common bream, *Abramis brama* (L.), (Kucharczyk et al. 1998); muskellunge, *Esox masquinongy* Mitchill, (Lin and Dabrowski 1998).

The high level of survival to the eyed-egg stage in the control group (D) and treated groups suggests that stirring is not harmful to the eggs. A similar observation was made by Bongers et al. (1994). The results of the current study show that UV treatment inactivated the nuclear DNA in chub oocytes. The androgenic origin (haploid or diploid) of the embryos was checked using a recessive color marker ("blond"). The dose of UV irradiation applied was 3456 J m^{-2}, at which almost 100% of the embryos obtained were haploid at a hatching rate of over 5%. These doses were higher than those obtained at optimum UV oocyte treatment for common carp (2500 J m^{-2}) by Bongers et al. (1994) and for northern pike (660-1320 J m^{-2}) by Lin and Dabrowski (1998), and were similar to those reported by Kucharczyk et al. (1998) for common bream (2700-3500 J m^{-2}). The results obtained indicate that it is possible to produce viable ide androgenotes using oocytes of other species. This procedure might be useful in restoration programs, especially in combination with the application of cryopreserved semen (Bongers et al. 1994, Kucharczyk 2002).

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STRESZCZENIE

ANDROGENETYCZNE POTOMSTWO JAZIA, *LEUCISCUS IDUS* (L.) UZYSKANE Z WYKORZYSTANIEM IKRY KLENIA, *LEUCISCUS CEPHALUS* (L.)

Celem niniejszej pracy było wywołanie rozwoju androgenetycznego jazia z wykorzystaniem oocytów innego gatunku. Oocyty klenia zostały inaktywowane genetycznie przy użyciu promieniowania ultrafioletowego. Ikra użyta w doświadczeniu pochodziła od ciemno ubarwionych samic podczas gdy nasienie od ryb żółto ubarwionych (recesywny marker). Najwyższą wydajność procesu androgenezy osiągnięto po zastosowaniu szoku termicznego w 3 godz. 00 minut po aktywacji. Pochodzenie androgenetyczne zostało potwierdzone markerem barwnym, zarówno u haploidów jak i diploidów.