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Short communications

SPONTANEOUS GYNOGENESIS IN IDE, *LEUCISCUS IDUS* (L.)

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ABSTRACT. The aim of the study was to obtain information regarding the spontaneous formation of gynogens in ide, *Leuciscus idus* (L.). Oocytes obtained from orfe and activated with the milt of dace, *Leuciscus leuciscus* (L.), were used for the study. Several control groups were established to determine the quality of gametes and the quality of the diluted semen. Ploidy in the offspring was determined by analyzing the number of nucleoli in the cells. Spontaneous diploid individuals appeared in groups in which the biological quality of the oocytes was poor. The percentage of these individuals was not large, and the average was a maximum of 0.4%.

Key words: GYNOGENS, GENOME MANIPULATION, BIOTECHNOLOGY

One of the methods of obtaining fish with certain desirable characters is gynogenesis (Chourrout et al. 1980), through which offspring are produced with maternal genetic material. This is a two-step process. First, the genetic material in the sperm is destroyed with X, γ, or UV irradiation (Varadaraj 1993). The resulting sperm is only able to activate eggs. The activated egg begins to develop into a haploid zygote. If the embryo is to develop normally, it must be subjected to an environmental shock, either thermal, chemical, or pressure (Komen et al. 1988). There are two moments during embryonic development when the desired effect can be achieved, namely the creation of a diploid embryo. The first period occurs shortly after egg activation when the second polar body, which connects to the cell nucleus, can be retained thus creating the diploid structure of the future organism. The second period occurs later during the first mitotic division. Thanks to such steps, the paternal parent does not participate in the transfer of

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genes. Fish thus produced are 100% maternal products. The great benefit of this is the possibility of creating recessive homozygotes, and a breeding line that is highly inbred. It is also possible to produce fish with superior characters responsible for condition, growth, or resistance to parasites and diseases (Kaastrup and Hørlyck 1987).

The quality of the gametes, especially those of the female, is especially important during genome manipulation studies. During genetic engineering work it has been demonstrated more than once that gynogenetic or androgenetic specimens can sometimes occur spontaneously. The aim of the present study was to obtain information about the formation of spontaneous gynogens of ide, *Leuciscus idus* (L.), using the semen of dace, *Leuciscus leuciscus* (L.), while attempting to link this information to the biological quality of the oocytes.

Dace spawners with natural coloring were obtained from the Marózka River (north Poland). Orfe were purchased from a pond fish farm OZ PZW Halinów (central Poland). The spawners were handled and stimulated hormonally in accordance with the methods described by Kucharczyk (2002). Semen was collected with a syringe from individual males. The eggs obtained were placed in dry plastic containers. The sexual products were held in a cool place until manipulation was performed. Semen with sperm motility exceeding 60% or higher was grouped into one sample. The milt was diluted in 0.85% NaCl at a ratio of 1:9, which caused the sperm to become immobilized. Portions (2.5 ml) of diluted semen were moved to Petri dishes. The thickness of the semen layer was approximately 1 mm. A UV (30 W, 6.4 W m^{-2}, Philips, the Netherlands) lamp was turned on for at least 30 minutes prior to the beginning of exposure. The Petri dishes containing the semen were placed on a shake table (frequency of movement about 1 s). One minute of semen exposure to UV irradiation was equal to a dose of 384 J m^{-2}. For gynogenesis, the semen was subjected to UV irradiation for nine minutes. Several control groups were designated during the experiment (Kucharczyk 1999): the group for determining the biological quality of the eggs (K) from one species (ide), in which the eggs were fertilized with semen that was neither diluted nor irradiated (0.05 ml per egg sample); the group for determining the biological quality of eggs fertilized with dace semen (C) that had neither been diluted nor irradiated; the group for determining the quality of the diluent comprised of eggs fertilized with diluted dace semen (0.5 ml per egg sample) that had either not been irradiated prior to the experiment (DP) or after irradiation (DK). The control group for determining the effect of

inactivating the genetic material of the sperm (I) was comprised of eggs activated with dace sperm that had been diluted and irradiated (0.5 ml per egg sample). Samples of approximately 100-150 eggs were mixed with semen that had been diluted and irradiated. The eggs were incubated at a temperature of 12°C in a closed recirculating system. The experiment was conducted in two replicates.

All of the individuals hatched in group C had dark body pigment and were free of visible deformation. In group I, the hatched individuals were of various types and were classified as follows: diploids/aneuploids with dark pigmentation. The hatched embryos were either not deformed or had slight body deformations, in comparison to the larvae hatched in group C. Individuals with yellow body pigmentation and typical haploid body deformation were classified as haploids, while individuals with no body deformation and yellow pigmentation were classified as spontaneous diploids.

The number of nucleoli in the cells was analyzed in 8 to 10 individuals from group I (Kucharczyka et al. 1999). There are two nucleolar organizer regions (NOR) located in the chromosomes of ide and four in dace. The occurrence of more than two nucleoli in cells is evidence of the creation of an inter-species hybrid between ide and dace (Kucharczyk 2002). The analysis of the number of nucleoli indicated there were differences among the various groups of examined fish (Table 1). The most were confirmed in hybrid individuals (inter-species cross between ide and dace).

TABLE 1

Number of nucleoli on tested fish

Group	number	mean±SD	range
Hybrids	40	2.3±0.2	2.17-2.43
Haploids	40	1.00±0.0	1.00-1.01
Spontaneous diploids	40	1.7±0.1	1.64-1.77

Spontaneous gynogenesis occurred especially when the biological quality of the eggs was lower (Fig. 1). The highest percentage of such fish were confirmed when survival in the control group (C) was at about 30%. Along with the decrease of embryo survival in the control group, the share of spontaneous diploids increased (Fig. 1). The occurrence of very few diploid individuals among the gynogene ide individuals is probably linked to the quality of oocytes. However, precisely identifying the mechanism by which gynogenes occur requires further study.

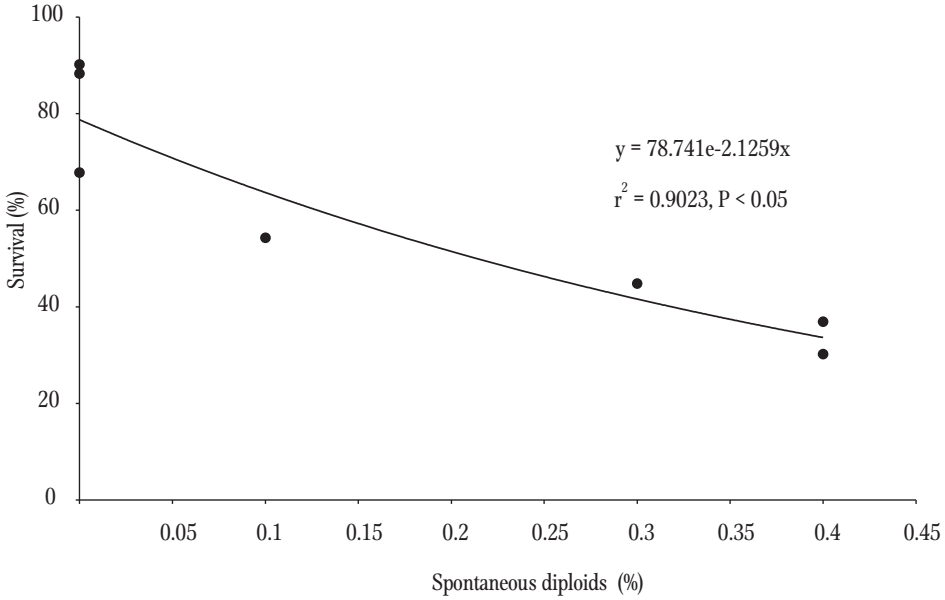

Fig. 1. Relationship of embryo survival in control group C and the percentage of spontaneous diploids in group I.

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STRESZCZENIE

SPONTANICZNA GYNOGENEZA U JAZIA *LEUCISCUS IDUS* (L.)

Celem pracy było zebranie informacji związanej z powstawaniem spontanicznych gynogenetów u jazia. Wykorzystano do tego celu oocyty pozyskane od złotej orfy, które aktywowano pozbawionym materiału genetycznego nasieniem jelca. Wyodrębniono kilka grup kontrolnych w celu określenia jakości gamet użytych w eksperymencie oraz jakości rozcieńczalnika nasienia. Ploidalność potomstwa określano poprzez analizę ilości jąderek w komórkach. Uzyskane wyniki wskazują, że spontaniczne osobniki diploidalne pojawiały się w grupach o niskiej jakości biologicznej oocytów. Odsetek takich osobników był niewielki i wynosił maksymalnie 0,4%. Prawdopodobnie powstawanie nielicznych spontanicznych gynogenetów jest związane z jakością oocytów, jednakże wyjaśnienie tego mechanizmu wymaga dalszych badań.