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## Androgenesis in the African catfish, *Clarias gariepinus*

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*Androgénèse chez le poisson-chat africain, Clarias gariepinus.*

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### INTRODUCTION

Fish are important laboratory animals, used mainly in aquatic toxicology, developmental biology, immunology and genetics. In the European Community, numbers are still increasing due to EC-legislation directed towards the use of lower vertebrates, obtained from registered breeding and supplying organizations (Anonymous, 1986). In contrast to the situation in laboratory mammals, very little demands are made thusfar on laboratory fish by international standardization organizations. There are no commercial suppliers of laboratory fish, resulting in the use of fish with unknown genetic background in almost all cases. It was recently shown that parental effects are one of the major sources of variation in aquatic toxicity testing using *Oncorhynchus mykiss* as test organism (Dave, 1993).

Inbreeding, followed by hybridization of two inbred (homozygous) lines is one of the classical methods to obtain isogenic (standardized) animals. In a variety of fish species, homozygous animals can be obtained using gynogenesis (all-female inheritance) or androgenesis (all-male inheritance) in only one generation (Ihssen *et al.*, 1990). In androgenesis, the female genome is inactivated. This can be achieved by the use of ionizing radiation such as gamma-rays, or UV irradiation. UV has lower penetrating abilities than gamma-rays (Thorgaard, 1983), leading to less damaging side effects to the eggs. In androgenesis in common carp, *Cyprinus carpio*, we found that UV irradiating eggs, while stirring in a synthetic

ovarian fluid leads to highly efficient inactivation of the female pronucleus (Bongers *et al.*, 1994). In principle, restoration of diploidy can be induced by either physical shocks (*i.e.* temperature, pressure) or chemicals. All treatments have a destructive effect on microtubuli and thus inhibit nuclear division. In androgenesis, heat and pressure shocks have been used successfully in *C. carpio* (Bongers *et al.*, 1994) and in *O. mykiss* (Parsons and Thorgaard, 1985) respectively.

In the present study, an attempt was made to develop a protocol for induced androgenesis in the African catfish, *Clarias gariepinus*. Research on gynogenesis was already done by Volckaert *et al.* (1994). Since *Clarias gariepinus* is being used in a wide variety of experimental research areas, the importance of developing isogenic strains is obvious. Also, the suitability for aquaculture has been established (De Kimpe and Micha, 1974; Richter, 1976). In generating homozygous broodstock and subsequent selection for production characteristics, isogenic strains can be developed in a breeding program.

### MATERIALS AND METHODS

#### Broodstock and collection of gametes

Broodstock (550-2 800 g) were maintained in 800 l rectangular tanks with recirculation water of 25°C. They were fed catfish pellets (Meerval Grower nr. 7, 45% crude protein, Provimi b.v., Rotterdam, the

Netherlands) at a daily ratio of 0.5% of their body weight.

Eggs were obtained after treating the females with one injection of carp pituitary suspension (4 mg cPS/kg body weight). Eggs were handstripped 11 hours after the injection ( $T=25^{\circ}\text{C}$ ) and were stored at room temperature. Males were sacrificed and the testes were removed. Milt collected was diluted 1:1 in physiological saline (0.9% NaCl) and stored on ice. Before starting the experiments, sperm was checked for motility (Hoogendoorn and Vismans, 1980). In every experiment, pooled milt of two males was used.

### Irradiation, fertilization and heat shocking

Irradiation was done using a VL-6 UV-lamp (7.5 W, 254 nm) in a UV-CN6 darkroom (Ankersmit, the Netherlands). The total (cumulative) energy ( $\text{mJ}/\text{cm}^2$ ) was recorded using a VLX-3W sensor (Ankersmit, the Netherlands), which was positioned at the same (fixed) distance from the UV-source as the eggs. The intensity was kept constant at  $1.1 \text{ mW}/\text{cm}^2$ . During irradiation, egg-samples (150-200 eggs) were put in a petri dish (diameter: 10 cm) and 5 ml of a synthetic ovarian fluid (OF) was added to allow stirring during irradiation. The petri dish was then placed slightly tilted on a mechanical stirrer (Schüttler MTS 2). The ovarian fluid consisted of: 4.11 g BSA fraction IV per litre, 3.8 mmol  $\text{Na}_2\text{HPO}_4$ , 118.0 mmol NaCl, 12.7 mmol KCl, 0.7 mmol  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 2.7 mmol  $\text{CaCl}_2$ , 5.5 mmol Tyrosin and 5.5 mmol Glycin in distilled water. In this ovarian fluid, eggs do not become adhesive and are not activated (Bongers *et al.*, 1994). During stirring at 300 rpm, eggs were able to roll in the fluid. After irradiation, egg samples were immediately mixed with 200  $\mu\text{l}$  of sperm suspension and fertilized by adding water of  $30.0^{\circ}\text{C}$ . They were transferred to baskets (diameter: 10 cm) with a mesh-bottom (mesh-size 0.5 mm) and incubated at  $30.0^{\circ}\text{C}$  in the dark.

Heat shocks were administered by transferring the eggs from the incubation system to a water bath of  $41.0^{\circ}\text{C}$  for 1 minute. These shock parameters were chosen on the basis of preliminary experiments.

#### Experiment 1

In this experiment, it was attempted to determine the optimal UV-dose to eliminate the female genome. Eggs received a total (cumulative) UV-dose of 5, 25, 50, 100, 125, 150, 200, 250 or  $300 \text{ mJ}/\text{cm}^2$ , corresponding to irradiation-durations of 4.5 to 270 seconds.

#### Experiment 2

This experiment was designed to determine the optimal onset of the heat shock to induce diploidy. Eggs received a UV-dose of  $125 \text{ mJ}/\text{cm}^2$ , and were shocked 16, 19, 21, 23, 25, 27, 29, 31, 33, 38 or 44 minutes after fertilization.

In both experiments, a normal fertilization was carried out to check the quality of the eggs (normal control). Also, a mechanical stirring control was performed where eggs were mechanically stirred in ovarian fluid (without irradiation) to test for negative side-effects of the stirring. In experiment 1, egg samples were irradiated in ovarian fluid at a dose of  $150 \text{ mJ}/\text{cm}^2$  without stirring to test for the effect of only UV-exposure (exposure control). In experiment 2, the efficiency of the UV-treatment was tested by irradiating egg samples ( $125 \text{ mJ}/\text{cm}^2$ ) without subsequent heat shock (UV control).

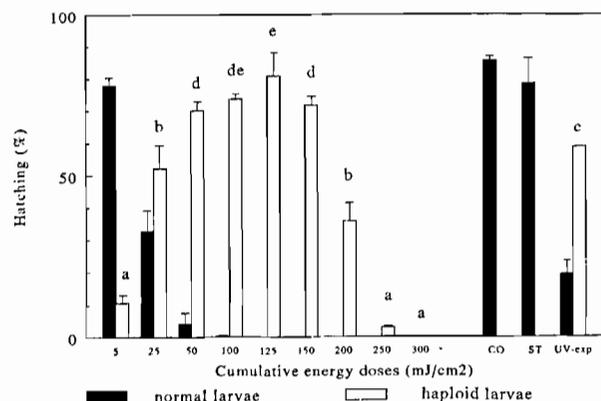
### Parameters recorded and statistical analysis

Egg samples (controls and treatments) were carried out in duplicate. At 24 hours after fertilization, percentages of normal larvae, deformed larvae and non-developed eggs were recorded. All percentages were expressed as percentage of the total number of incubated (treated) eggs.

Yields of larvae were compared using Duncan's multiple range test ( $P < 0.05$ ) after arcsin transformation. All analyses were carried out using BMDP computer programs (Dixon *et al.*, 1988).

## RESULTS

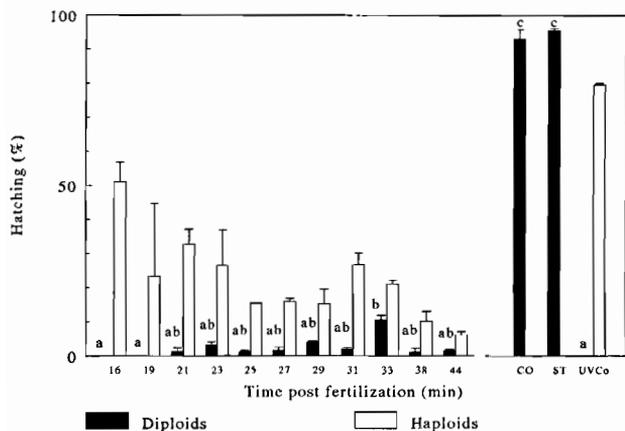
Figure 1 shows the relation between the cumulative energy dose and hatching percentage of normal and deformed (haploid) larvae. The highest yield of haploid larvae (81.0%) was obtained when eggs were irradiated with a total UV-dose of  $125 \text{ mJ}/\text{cm}^2$ . All hatchings showed the classical symptoms of the haploid syndrome (Gervai *et al.*, 1980). Hatching of normal diploids decreased at this dose to zero. Higher UV dosages decreased hatchability significantly, with



**Figure 1.** – Relation between the UV-dose administered ( $\text{mJ}/\text{cm}^2$ ) and hatching of normal and androgenetic haploid larvae. Groups with a common superscript do not differ significantly ( $p < 0.05$ ) regarding the amount of haploid larvae. CO: Normal fertilization. ST: Mechanical stirring control. UV-exp: Exposure control (irradiation of eggs at  $150 \text{ mJ}/\text{cm}^2$  without stirring).

total mortality at 300 mJ/cm<sup>2</sup>. The normal control yielded 85.7% normal larvae, indicating good egg quality. The mechanical stirring control showed the absence of negative side effects of the stirring in ovarian fluid by yielding 78.5% normal larvae, which was not significantly different from the normal control. In the exposure control, 58.7% androgenetic haploids and 19.2% diploids hatched. This indicated increasing efficiency when eggs were stirred during irradiation.

The yield of androgenetic diploids after heat shocking (41.0°C, 1 min) irradiated eggs is presented in figure 2. A maximum yield of 10.5% normal diploids was obtained when eggs were shocked at 33 minutes after fertilization. Absence of normal diploids in the UV control confirmed inactivation of the female genome. In all heat shocked groups, high numbers of haploids hatched. The normal control and the mechanical stirring control yielded 93.1 and 95.6% normal larvae respectively.



**Figure 2.** – Hatching of androgenetic diploid and haploid larvae after heat shocking (41.0°C, 1 min) UV-irradiated eggs (125 mJ/cm<sup>2</sup>) at various times post fertilization. Groups with a common superscript do not differ significantly ( $p < 0.05$ ) regarding the amount of diploid larvae. CO: Normal fertilization. ST: Mechanical stirring control. UVCo: UV Control (irradiation of eggs at 125 mJ/cm<sup>2</sup> without heat shock).

## DISCUSSION

High yields of androgenetic haploid *C. gariepinus* (81.0%) were obtained by UV-irradiating eggs while stirring in a synthetic ovarian fluid. The stirring did not negatively interfere with hatching but increased the efficiency of the UV treatment (see exposure control, fig. 1). Similar results were obtained in common carp, *C. carpio* using the same method (Bongers *et al.*, 1994). Heat shocks (41.0°C for 1 min, 33 min post fertilization) proved to be effective in restoring diploidy (10.5% androgenetic diploids). Large numbers of eggs were not affected by the heat shock treatment and remained haploid. Increasing the

severity of the heat shock (higher shock temperature and/or duration) resulted repeatedly in nearly total mortality (unpubl. data). Komen *et al.* (1991) showed that heat shocks were only effective at the metaphase of the first mitotic division in gynogenetic common carp. In their experiments, peak survival occurred during a limited interval (28-32 min) post fertilization. When applied earlier, heat shocks were relatively ineffective, resulting in high hatching percentages of haploids. In our experiments, survival of putative androgenetic diploids occurred when shocks were administered 21-44 minutes after fertilization (fig. 2). This might indicate a great variety in developmental speed of *C. gariepinus* eggs within one batch. Currently, we are investigating the effect of lowering the pre-heat shock incubation temperature to reduce this variability.

When gamma-rays are used to eliminate the female genome, hatchlings could be contaminated with chromosomal fragments as found by Disney *et al.* (1987) after <sup>60</sup>Co irradiating sperm of *O. mykiss*. When UV is used, this is less likely to happen since UV does not have a fragmentation effect on chromosomes (Thorgaard, 1983). Although at the optimal UV dose no normal (biparental) diploids hatched, we still need to verify complete absence of the maternal genome in diploid androgenetic offspring using genetic markers at the DNA-level.

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