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Research Article

Androgenesis: The Best Tool for Manipulation of Fish Genomes

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Abstract: Androgenesis involves the development of an organism with only paternal chromosomes. The induction of androgenesis using diploid sperm, highly effective UV-irradiation of egg, dispermy using heterologous eggs, the possibility of producing supermales for all male production and the recovery of cryopreserved sperm are recent technological developments in androgenesis in fish. However, few researchers have successfully produced viable diploid androgens in fish. The authors made an attempt to induce androgenesis in the common carp (*Cyprinus carpio* L.) and the results are discussed along with the potential applications of androgenesis in conserving fish genomes and current knowledge on inducing androgenesis in fish.

Key Words: Androgenesis, Common carp, Genome conservation.

Androgenez: Balık Genomlarının İşlemede En İyi Araç

Özet: Androgenez bir organizmanın sadece paternal kromozomlarla üretilmesidir. Balıklarda androgenezin son teknik gelişmelerinin arasında diploid sperm ile androgenezi sağlamak, etkisi yüksek bir yöntem olan yumurtayı ışınlara maruz bırakmak, heterolog yumurtalar ile dispermi, yalnız erkek eldesi için süper erkek üretimi ve dondurulmuş olarak muhafaza edilmiş sperm eldesi vardır. Ancak çok az araştırmacı balıklarda yaşayabilir diploid androjeni başarıyla üretebilmiştir. Bu çalışmada *Cyprinus carpio* L'de androgenez sağlanmaya çalışılmıştır ve sonuçlar androgenezin balık genomlarını korumakta olası uygulamaları ile balıklarda androgenezi sağlamaktaki güncel bilgiler ile birlikte tartışılmaktadır.

Anahtar Sözcükler: Androgenez, Sazan, Genom Koruması

Introduction

Genetic engineering provides the latest tools for the improvement of fish stocks through chromosome manipulation techniqes: gynogenesis (all maternal), androgenesis (all paternal) and polyploidy (triploidy and tetraploidy). Chromosome manipulations in fish have been recently reviewed by Pandian (1) and Khan (2). Androgenesis involves the development of an organism which has chromosomes of only paternal origin. Androgens can be produced in two ways, the most common of which is to fertilize irradiated eggs with normal sperm. This produces a haploid androgen and at first cleavage a shock is given to prevent cell division, and the two haploid (N) nuclei fuse to form a diploid (2N) nucleus. Androgens have been produced by this technique and are highly inbred. Examples include androgenetic rainbow trout by Parsons and Thorgaard (3 and 4), Scheerer *et al.* (5) and Thorgaard *et al.* (6), brook trout by May *et al.* (7), and grass carp by Stanley (8) and Stanley and Jones (9). The second technique that can be used to produce androgens is to fertilize eggs whose genetic material has been destroyed by irradiation with sperm from a tetraploid male. Consequently, the sperm pronucleus is diploid rather than haploid, which means the ensuing zygote will also be diploid. Thorgaard *et al.* (6) produced androgenetic rainbow trout using this technique; these androgens are far less inbred and survival is much higher.

UV-light or gamma rays have been used to inactivate the maternal genetic material. Haploid androgenesis has been induced in several teleost species by Parsons and Thorgaard (3), Thorgaard *et al.* (6), May *et al.* (7), Purdom (10) Arai *et al.* (11) using ^{60}CO irradiation of unfertilized eggs. The doses used were between 10^4 and 10^5 roentgen (R). Thorgaard *et al.* (6) produced diploid androgenetic rainbow trout by the normal route and also by fertilizing gamma ray inactivated eggs with diploid spermatozoa from previously generated tetraploid males.

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The comparison of the two methods showed clearly that fertilization by haploid spermatozoa followed by pressure shock at first mitosis was followed by a much lower rate of survival (0.8%) to hatching and to first feeding than the method using diploid spermatozoa (43%). The author's opinion was that the low survival rate of androgenetic diploids was a feature of the shock treatment, rather than of inactivation of the egg genetic material.

Successful androgenesis in carp was first reported in 1990 by Grunina *et al.* (12) by applying 25-30 KR of X-ray irradiation to the eggs and later 2-3 min of heat shock at 40.5-41°C at 1.7-1.9 τ_0 (τ_0=21 min at 22.5°C or 20 min at 23°C). Mutation in the colour of males (b_1b_2 orange) was used as a marker, since they exhibited poor pigmentation at the larval stage. Six to nine percent of the treated eggs developed into larvae without pigmentation, representing androgenetic diploids. However, biparental diploids indicating incomplete inactivation of the female genetic material also appeared among the survivors. Half of the androgenetic progeny was necessarily XX female, while the other half was YY male.

Since irradiation by gamma or X-rays inflicts more injuries than desired as shown by Thorgaard (13), the first step was to replace it by UV-irradiation. Bongers *et al.* (14) were the first to claim 100% elimination of the genome from *Cyprinus carpio* eggs. To facilitate more homogenous distribution, eggs of *Cyprinus carpio* were immersed in synthetic ovarian fluid and exposed to UV-irradiation at a dose of 250 mJ/cm^2. Irradiated eggs were later heat shocked (40°C, 2 min and 26-30 min after fertilization) to restore diploidy. The ratio of androgenetic diploids identified by the absence of the dominant black colour of the females ranged from 7.2-18.3%. No biparental diploids exhibiting a black colour were produced by applying the optimum UV-dose (250 mJ/cm^2) followed by the heat shock.

To irradiate the eggs over the animal pole, Arai *et al.* (15) exposed *Misgurnus anguillicaudatus* eggs to a UV source from the upper and bottom sides. Bongers *et al.* (14) achieved the highest (54%) yield of androgenetic haploids because of the superior quality of gametes and genomic inactivation techniques. Bongers *et al.* (14) showed that the homozygous male parent yielded significantly more normal and fewer deformed fry than the heterozygous male. In contrast to this, Sheerer *et al.* (5 and 16) reported that a large number of viable androgenetic diploids of *Oncorhynchus mykiss* were obtained when inbred and outbred sperm were used. Bongers *et al.* (17) also demonstrated that UV (175 mJ/cm^2) may be a cause of the high frequency of deformed androgenetic hatchlings.

To reduce the homozygosity, Grunina *et al.* (18) explored the possibility of dispermic activation and generation of diploid androgens. They successfully activated the genetically inactivated eggs of *Acipenser baeri* using *A. ruthenus* sperm. They also used genetically inactivated eggs of the hybrid *Cyprinus carpio* female and sperm from *Carasius auratus.* Colour was used as a marker for confirming the genetic purity of the androgens. They claimed a higher survival rate for these hybrid androgens. Cherfas *et al.* (19) generated a biparental androgenetic carp by activating inactivated hybrid eggs using hybrid carp sperm (2N). Haploid androgens have been produced by UV-irradiation (430-540 Jm^2) of eggs in *Oreochromis niloticus*, and diploidization of the haploid genome was achieved by suppression of first mitosis by giving a pressure shock. In a series of experiments, only 0.5% diploid androgens were produced by Myers *et al.* (20).

Preliminary investigations on androgenesis in common carp have been carried out in India as well by Pooniah *et al.* (21) and putative androgens were produced in some experiments with a very low yield of 2% viable larvae. Nagoya *et al.* (22) have induced androgenesis using super ^{60}CO gamma irradiation and hydrostatic pressure shock in amago salmon (*Oncorhynchus masou*). Androgenetic haploids were produced when eggs were irradiated prior to fertilization, with an optimal dose of 450 Gray. The optimum timing of hydrostatic pressure shock (650 Kgf/cm super 2, 6 min) was determined to be 7.5 h after insemination at 10°C, and later DNA fingerprinting was used to confirm the clonal nature of androgenic diploids. The current information on the induction of androgenesis is presented in the Table.

Androgenesis has also been induced in a variety of other ways. Ymazaki (23) observed haploid androgenetic embryos resulting from normal fertilization of overmature salmon eggs. Briedis and Elinson (24) induced haploid androgenesis in frog embryos by applying pressure or D_2O treatments to inhibit male pronucleus movement and thus prevent syngamy. The androgenetic

Table: Summary of methods used to induce haploid and diploid androgenesis in fish

Species	Inactivation of female genome	Diploidization treatment	Genetic marker	Androgenetic yield%	References	Remarks
Salmonidae						
Oncorhynchus masou	^{60}Co; 50-60 KR	-	-	35 haploids at day 40, 0 hatching	Arai *et al.* (11)	-
Oncorhynchus mykiss	^{60}Co; 30 KR	-	Colour	26.7 survival at day 20, 0 hatching	Parsons and Thorgaard (3)	Maternal DNA fragments at high doses (50 KR)
O. mykiss	^{60}Co; 36 KR	Pressure	Colour	Hatching 32-38 diploids	Parsons and Thorgaard (4)	-
O. mykiss	^{60}Co; 36 KR	Pressure	Isozymes	Hatching 7.2-7.9 diploids	Scheerer *et al.* (5)	Inbred sperm source
O. mykiss	^{60}Co; 40 KR	Pressure	-	Hatching 1	Thorgaard *et al.* (6)	Diploid sperm source
O. mykiss	^{60}Co; 40 KR	Pressure	-	Hatching 12	Thorgaard *et al.* (6)	Tetraploid sperm source
O. mykiss	^{60}Co; 36 KR	Pressure	Isozymes, colour	Hatching 2-3 diploids	Scheerer *et al.* (16)	Outbred sperm source
Salvelinus fontinalis	^{60}Co; 88 KR	Pressure	Allozymes	37 diploids at eyed stage, ? hatching	May *et al.* (7)	-
Cyprinidae						
Cyprinus carpio	X-ray; 25-30 KR	Heat shock	Colour	Hatching 9 diploids and 12 haploids	Grunina *et al.* (12)	Insufficient elimination of female genome
C. carpio	UV; 100-250mJ/cm^2	Heat shock	Colour	Hatching 8-19 diploids and 52 haploids	Bongers *et al.* (14)	Incubation in ovarian fluid during irradiation
C. carpio	UV	Dispermy	-	-	Grunina *et al.* (18)	Goldfish sperm; Hybrid eggs
C. carpio	UV	Heat shock	-	Diploids 2	Pooniah *et al.* (21)	-
Misgurinus angullicaudatus	UV; 100-7500 ergs/mm^2	Pressure	Colour	Hatching 6	Masaoka *et al.* (31)	Egg irradiation in Ringers
M. angullicaudatus	UV; 7500 ergs/mm^2	-	Allozymes	Hatching 8	Arai *et al.* (15)	100% elimination of egg genome; 4N sperm

Table (contunied)

Cichlidae						
Oreochromis niloticus	UV	Heat shock	Colour	Hatching 3	Myers *et al.* (20)	Fresh/ cryopreserved sperm of same or related species
Others						
Platichthys flesus	^{60}Co; 67 KR	Cold shock	-	No diploids ? haploids	Purdom (10)	-
Ambystoma mexicanum	UV; 47 mJ/cm^2	Pressure	Colour	Hatching 2.4 diploids	Gillespie and Armstrong (46)	Hatching in one out of four trials
Acipenser ruthenus	UV	Dispermy	-	Hatching	Grunina *et al.* (18)	Sperm of *A. baeri*

origin of these embryos was explained by the reliance of cell division on the centrioles, which are brought into the egg by the sperm. Gervai *et al.* (25) observed haploid embryos when attempting to optimize cold shock treatments for inducing triploidy in the common carp (*Cyprinus carpio*). However, these occurred following cold shocks given at an earlier stage than the optimal time for inducing triploidy, and the haploids were of both maternal and paternal origin.

Stanley and Jones (9) crossed female common carp with male grass carp and obtained diploid androgenetic grass carp as well as F_1 hybrids. Liu *et al.* (26) transferred haploid androgenetic blastula nuclei (enucleated *Paramisgurnus dabryanus* eggs were fertilized with loach, *Misgurnus anguillicaudatus,* sperm), into enucleated *P. dabryanus* eggs and obtained both haploid and diploid androgenetic embyros, a few of which survived to adulthood. The spontaneous diploidization observed in these two studies would remove the need for further treatment of the eggs, but they seem unlikely to have wider applicability.

Once androgenetic diploids are produced, it is important to have proof that the egg did not contribute genetically to the embryo. This may be obtained by several methods. Perhaps the simplest method for androgenesis studies is the use of irradiated eggs from a related species; however, the possibility of an incompatibility between egg cytoplasmic constituents and the paternal genome in androgenesis might limit the usefulness of foreign eggs for androgenesis studies. However, Stanley and Jones (9) found that androgenetic grass carp which was developed from carp eggs fertilized by grass carp sperm, were morphologically normal. The study of nuclear cytoplasmic compatibility in androgenesis may provide some interesting insights to development.

Constraints in inducing androgenesis

The production of viable diploid progeny by androgenesis is much more difficult than gynogenesis, for two reasons:

(a) It is quite difficult to achieve the elimination of the female pronucleus and polar bodies without damaging the cytoplasm, and the radiation may adversely affect the mitochondrial DNA, messenger RNA (mRNA) and other constituents besides chromosomal DNA. Much less research has been done on the inactivation of egg chromosomes to induce androgenesis. Arai *et al.* (11) also believed that the radiation treatment might have damaged egg cytoplasmic constituents in a study with masu salmon (*Oncorhynchus masou*). However, Purdom (10) found that androgenetic haploids were indistinguishable from gynegenetic haploids in plaice (*Pleuronectes platessa*). The mortality of the haploids has generally been assumed due to the presence of deleterious recessive lethal genes as shown by Purdom (10), Thorgaard (13) and Fankhauser (27).

Carter *et al.* (28) doubted the total elimination of an egg genome, since mitochondrial DNA and messenger RNA are present in large quantities in the egg as described by Gardner *et al.* (29). Myers *et al.* (20) reported that owing to protection by the mitochondrial membrane, mitochondrial DNA in eggs of *Oreochromis niloticus* suffered no damage from UV-irradiation. Irrespective of this, it was demonstrated by Bongers *et al.* (17 and 30) that radiation may disrupt maternal RNA and thereby the fate of individual cells and cell lineages, and affect differentiation, leading to deformed hatchlings. The intensity of the radiation to which the sperm are subjected is generally less than 10% of the UV dose administered in eliminating the egg genome, as shown by Bongers *et al.* (14) and Carter *et al.* (28). Masaoka (31) recorded up to 10% maternal genomic contamination in androgenetic *Misgurnus anguillicaudatus.*

(b) The production of diploid progeny is a difficult process, since there is no partner genome integrating, like polar bodies in gynogenesis. This is the reason that first mitotic division has to be inhibited in androgenesis.

Potential applications of androgenesis

Androgenesis has a very interesting and important application in the area of germplasm maintenance and the conservation of endangered species, as described by Thorgaard *et al.* (6), Thorgaard (32) and Myers (33). Androgenesis can be used to recover genes from stored populations of cryopreserved sperm that exist in germplasm resource centres. Unfortunately, the technology to cryopreserve fish eggs has proved elusive, as shown by Stoss (34). Using androgenesis, the genes from cryopreserved sperm could be recovered, even if the species becomes extinct, because the sperm could be used to fertilize irradiated eggs from closely related species. Cryopreservation and storage of diploid spermatozoa from tetraploid males would be a very useful tool in the coservation of genetic resources. Such a practice would enable the recovery of heterozygous diploid genotypes, even in the event of the extinction of a species or strain through the use of androgenesis. Protocols for successful generations of gynogens have been described for several species, but for androgenesis few have been reported. Hence, there is a need to catalyze research to describe protocols for the cryopreservation of sperm and the induction of androgenesis in fish.

Another application is to produce a monosex population in species with male homogamety. Gillespie and Armstrong (35) produced androgenetic diploid males in the axolotl (*Ambystoma mexicanum*), a male homogametic salamander, by suppressing first cleavage after UV-inactivation of egg chromosomes. Androgenesis may prove useful for the production of viable (YY) supermales in male heterogametic species, to establish brood stock for consistent production of all-male populations. Viable YY-supermales have been induced in a few cyprinids, cichlids and salmonids. In male heterogametic species, including carp and probably most salmonids, diploid androgenesis followed by suppression of first cleavage should lead to 50% XX and 50% YY offspring. The XX individuals would be homozygous females and YY individuals homozygous males with the potential of producing all male offspring when crossed with normal females. On the basis of studies with hormonally sex reversed fish, YY males are known to be viable in goldfish (*Carassius auratus*) and medaka (*Oryzias latipes*) as reported by Yamamoto (36 and 37), and in coho salmon (*Oncorhynchus kisutch*), as reported by Hunter *et al.* (38).

Androgenesis may not be the best method for the production of a monosex population, or sex control in populations to be released in nature because the offspring are inbred, or capable of reproduction (in contrast to triploids) and are genetically uniform and therefore able to establish an undesirable monoculture in nature, as shown by Streisinger *et al.* (39). However, androgenesis could be useful in the production of YY males for creating outbred monosex populations.

It also allows the possibility of rapid development of inbred lines for domesticated hatchery brood stock, but the potential is difficult to realize because the androgens produced, though 100% homozygous, suffer from high rates of abnormality and mortality. Inbreeding leads to an increased probability of homozygosity for harmful recessive alleles and a reduction in value for characters associated with reproduction or physiological efficiency known as inbreeding depression. Androgenesis is a very rapid system of inbreeding and, as expected, androgenetic fish show evidence of inbreeding depression.

Ihssen (40) argued that the optimal breeding strategy in fish would be to maximize heterozygosity to achieve heterosis or hybrid vigour. The most effective means of maximizing heterozygosity is to cross divergent inbred lines. Traditional breeding systems for the development

of inbred lines of salmonids have proved to be time consuming and, therefore, cost prohibitive. Thus, the development of inbred lines, has, for the most part, being discontinued. Alternative approaches such as androgenesis may provide more effective mechanisms for the development of inbred lines.

Scheerer (5) showed that an outbred strain can also be restored from cyropreserved sperm through a two-step process, in which homozygous androgenetic diploids may be produced first, and different homozygous androgenetic diploids may then be crossed to restore heterozygosity and genetic variability. A major obstacle to the use of androgenesis for generation of inbred lines and for gene banking is the extremely low survival rate expected in androgenetic progenies because of the homozygosity for deleterious recessive genes.

One more application of androgenesis lies in monitoring the effects of mitochondrial genotype on performance, because mitochondrial DNA in animals is maternally inherited, as described by Avise and Lansman (41), and this is the case with androgenetic individuals in spite of egg genome inactivation. By using androgenesis, it would be possible to compare the performance of fish with identical nuclear genotypes and different mitochondrial genotypes. Legget (42) showed that this approach is analogous to the study of alloplasmic lines in plant breeding; this approach, however, requires many generations of back crossing while androgenesis can be done in a single generation.

According to Yan Shaoyi *et al.* (43), cytoplasm plays a major role in genetic inheritance. Yan Shaoyi *et al.* (43) have developed nucleocytoplasmic hybrids between enucleated eggs of common carp (*Cyprinus carpio*) and the egg nucleus of crucian carp (*Carrasius auratus*). The morphological characteristics of these hybrid fish showed that some features were inherited from the nucleus of the donor fish, such as barbs and pharyngeal teeth; some features, such as vertebrae, came from the cytoplasm of the host fish; and some features, such as the number of scales along the lateral line, were intermediate. Using this technique, it should be possible to compare the performance of fish with identical nuclear genotypes and different mitochondrial genotypes.

In view of the above, the present investigation on induction of androgenesis was taken up. The objectives were to determine the optimal conditions for the production of diploid androgens of common carp by finding the optimal levels with regard to (1) UV-irradiation dosage and (2) heat shock temperature, exposure time and post-insemination time.

Materials and Methods

Mature orange males and pigmented females weighing 1.0-1.25 kg were selected and induced by injecting ovaprim (0.3 ml/kg body wt). The eggs and sperms were collected by stripping method. Eggs were stripped in a petri dish containing 5 ml of synthetic ovarian fluid, the composition of which was same as that used by Bongers *et al.* (14). By keeping the eggs in synthetic ovarian fluid, a single layer was obtained and eggs did not become adhesive and were not activated.

UV-irradiation of eggs

Around 90-100 eggs spread on a petri dish forming a single layer were UV-irradiated (254 nm; Germicidal UV-lamp 6W) for different time periods ranging from 0.5 to 10 min. The distance between the lamp and the egg samples was 8 cm. During irradiation, eggs in the petri dish were kept in an ice tray, and a temperature of 4°C was maintained throughout the irradiation period to avoid damage caused by generation of heat during irradiation. Egg samples were manually stirred to ensure uniform irradiation. Males were stripped and their milt was diluted at 1:3 with ice cold physiological saline (0.9% Nacl). After irradiation, egg samples were immediately mixed with 0.25 ml of sperm suspension and fertilized by adding 10 ml freshwater. The exact time when the milt was added to fertilize the irradiated eggs was considered as zero time, which helped in determining the age of the embryo at the time off heat shock. Excess milt was washed of and the developing embryos were left at ambient temperature for further development and treatments.

Heat shock treatment

Fertilized eggs were divided into batches and heat shocks were administered by transferring the eggs from the incubation system to a thermoregulated waterbath. The heat shock parameters (temperature, post-insemination time and exposure time) were varied to determine the optimum conditions for inducing diploidy. After heat shock, developing embryos were transferred to aquaria filled with clean, aerated water. Water was

changed regularly, the survival rate was calculated and dead eggs were removed. Feeding of the hatchlings started on the third day, when the yolk sac was fully absorbed. Spawn was first fed on egg yolk and later on the live feed.

Experimental design

In experiment 1, eggs were stripped from females and divided into 10 groups. Two groups served as control and the remaining eight groups were irradiated for different durations: as 0.5, 1, 2, 3, 4, 6, 8 and 10 min. The distance between the UV tube and the sample was 8 cm. In experiment 2, irradiated eggs were fertilized with normal sperm and then heat shocked. Heat shocks were performed at 38, 40, 42 and 45°C for exposure times of 1, 2 and 3 min at each temperature. The intervals between insemination and heat shock were, variously, 26, 28, 30 and 32 min. The non-irradiated eggs (control A) were fertilized with normal milt, and irradiated eggs (control B) were inseminated with normal milt without heat shock.

Confirmation of ploidy

The number of nucleoli per nucleus was determined following Phillips *et al.* (44) in conjunction with morphological examination for ploidy identification. The larvae were sacrificed for such examination.

Nucleoli couting

Nucleoli counting is a simple and inexpensive alternative which is applicable to fish for ploidy identification. The method involves silver staining of cells and determining the maximum number of nucleoli per cell. The standard method of Phillips *et al.* (44) was followed for nucleoli counting.

Morphological appearance

Haploids are expected to suffer from haploid syndrome as described by Ijiiri and Egami (45), while androgens will be normal.

Results and Discussion

Fertilization of the non-irradiated eggs with sperm resulted in a survival rate of 52% at hatching, which indicated a good quality of gametes. When eggs were exposed to UV-rays for a duration of 0.5-10 min, almost all the embryos succumbed to death. Developing embryos succumbed to death 6 h after fertilization, the eggs first turned white and then died within 6 h after fertilization. Although some of the eggs were transparent, there was no development. However, at UV-irradiation durations of 2.0 and 4.0 min, only 2% of the eggs hatched and were normal. Maximum survival at the embryonic stage (12 h after fertilization), at hatching and at the feeding stage was 9, 2 and 2% respectively. It appears that these eggs escaped the radiation and developed normally. The feeding efficiency and growth rate of the irradiated hatched larvae were normal and comparable to normal diploids (non-irradiated eggs x sperm).

In diploid androgenesis, the UV-irradiated eggs (0.5-10 min) were fertilized with normal sperm and later heat shocked at temperatures of 38, 40, 42 and 45°C, and with exposure times of 1, 2 and 3 min and 26, 28, 30 and 32 min after fertilization. All of these combinations failed to produce diploid androgens. At all tested temperatures, exposures and post-fertilization ages, 100% mortality was observed 6 h after fertilization. The gastrula stage was found to be the most critical period in the developing embryos.

The fact that survival of androgenetic diploids can be extremely variable among experiments, as shown by Parsons and Thorgaard (4) and Scheerer *et al.* (5), seems more consistent with female-specific or treatment-specific effects than the general effect of homozygosity, which is primarily responsible for low survival rates.

Grunina *et al.* (12) reported a maximum of 12% hatched androgenetic haploids in common carp after irradiation with X-ray (dose: 25-30 KR). Hardly any biparental diploids hatched at this X-ray dose. After an application of heat shock (40.5-41°C, 2-3 min), there were persisting amounts of biparental diploid among androgenetic offsprings. Bongers *et al.* (14) also reported a maximum of 53.9% hatched androgenetic haploids in common carp after irradiation at a UV dose of 250 mJ/cm^2. The same author reported a survival rate of 7.2 to 18.3% putative androgenetic diploids after application of heat shock (40°C, 2 min) at 26, 28 or 30 min after fertilization. Parsons and Thorgaard (4) and Scheerer *et al.* (5 and 16) reported a complete absence of biparental diploids after ^{60}Co-irradiation in combination with pressure shocks in *O. mykiss.* Gillespie and Armstrong (35) used UV-irradiation to produce androgenetic *Ambystoma mexicanum.* They also found biparental diploids after irradiating eggs with a UV dose of 47 mJ/cm^2. Pooniah *et al.* (21) have reported a low yield

(2%) of putative androgens in common carp. The present results are inconclusive but provide baseline data for further trials on successful induction of androgenesis in common carp.

Conclusion

Androgenesis may prove useful for the production of (i) viable (YY) supermales in male-heterogametic species, (ii) inbred isogenic lines and (iii) conservation of germplasm. When crossed with a normal female, a supermale can produce all-male progenies. Androgenesis has successfully induced viable YY males in a few cyprinids, cichlids and salmonids. However, the technology has been successful in relatively few species as compared to gynogenesis.

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