Radiation induced heat shocked gynogenesis in mrigal *Cirrhinus mrigala* Hamilton

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**ABSTRACT**

Gynogenesis was induced in mrigal *Cirrhinus mrigala*, by subjecting eggs fertilized with UV-irradiated sperm through heat shock. The sperm was inactivated through UV-irradiation with 580 μW/cm² at an exposure of 2 minutes at a constant distance of 13.5 cm lamp-sperm. Gynogenetic diploidy was induced by restoring second polar body by sub-lethal heat shock at 39 to 40°C for 1 to 2 minutes, initiated 4 minutes after fertilization. Heat-shock regimes with the constant dose of UV-irradiation were optimized at 39°C for 2 minutes, applied 4 minutes after fertilization. The triploids in the controls were induced at low rates ranging from 0 to 70% and haploids ranged from 50 to 100% with 0 to 8% survival rates. Gynogenetics had poor survival rates which varied from 2 to 50% of which some individuals even could be triploids or normal diploids due to presumed inappropriate heat-shock and UV-irradiation. Survival rate of 30 to 60% was recorded in the control.

**Introduction**

Gynogenesis is the development of embryos from eggs without genetic contribution from the paternal side. Though it is an infrequent way of reproduction, incidence of artificial gynogenesis in frog, induced by irradiated (genetically inactive) sperm has been known since 1911 (Purdom, 1983; Chourrout, 1984).

Production of diploid gynogenetic individuals requires the combination of sperm inactivation and diploidization of the maternal chromosome set. Ultraviolet irradiation for sperm inactivation has been used by many workers (Hertwig, 1911; Chourrout, 1984 and Shah, 1984). Induced diploid gynogenesis can be achieved by the manipulation of chromosome sets and various physical and chemical treatments which disrupt metaphase of cell division, have been used in many species of fish (Purdom, 1983; Shah, 1984; Chourrout, 1984; Hussain, 1992). Every mature ovu-
lated fish eggs are at metaphase-II of meiosis and fertilization of an egg by an irradiated spermatozoon is expected to result in the production of a haploid embryo. In experimental diploid gynogenesis, the diploidization of the eggs is possible by subjecting the same to extreme sublethal heat, cold or pressure shock which can induce the condition for retaining the chromosomes normally destined to be contained within the second polar body (Purdom, 1983).

Gynogenesis offers a means for reproducing fish of one sex, permitting complete elimination of reproduction and allowing the undesirable fish to be eliminated (Chao and Liao, 1990). Unisex populations can be used in fisheries management in situations where reproduction is undesirable or must be limited (Shah, 1984). Since male inheritance is excluded in gynogenesis, offsprings are exclusively females except when the females are of heterogametic sex or undergo sex inversions, both of which are rare. Gynogenetically produced fish can be all-female if the females in the species concerned is homogamatic i.e., XX type. The determining mechanism present in the species employed can therefore be found out through gynogenesis. Moreover, gynogenetics are useful in studies of karyotypes and mutagenesis.

Gynogenetic offsprings are highly homozygous since they receive maternal genes and are useful in genetic studies and selective breeding schemes (Golovinskaya, 1969). Chao and Liao (1990) reported that gynogenesis greatly increases the efficiency of selection of desirable traits. Gynogenetic diploid offsprings can be homozygous for most gene pair because they bear gynogenomes in their genetic materials except for crossed over. They can produce 50 to 100% inbred individuals in a single generation. Purdom (1983) is of the opinion that one generation of gynogenesis was equivalent to seven generations of full sib mating and that induced diploid gynogenesis was a potential method in the production of inbred lines of fish which can subsequently be crossed to produce hybrid vigor or heterosis. Streisinger et al. (1981) found that hybrids between inbred lines showed increased vigor and that more vigorous lines could be produced by further selection.

Gynogenesis or parthenogenesis has been induced in many species of fish such as common carp, *Cyprinus carpio* (Golovinskaya, 1969); brown trout, *Salmo trutta* (Purdom, 1969); grass carp, *Ctenopharyngodon idella* (Stanley, 1976); Indian major carps, rohu *Labeo rohita* and catla, *Catla catla* (John et al., 1984); tilapia, *Oreochromis niloticus* (Shah, 1984 and Mair et al., 1991); cyprinid loach, *Misgurnus anguillicaudatus* (Suzuki et al., 1985; Chao and Liao, 1990).

In the present study efforts were made to produce radiation induced heat shocked gynogenesis in mrigal, *Cirrhinus mrigala*. The renewed prospective role of the species in the culture system through genetic manipulation was sought for and thus the objective of the study was to produce inbred lines of the species and thereby to hybridize them for producing hybrid vigor. Further gynogenesis could also provide an insight into the sex-determining mechanism of the species.

### Materials and methods

**Stock of fish and gonadal materials:** The fishes used were from the freshwater station, Bangladesh Fisheries Research Institute, Mymensingh. The eggs and sperms were obtained from the females and males which were induced to breeding condition by pituitary gland injection.

**Ultraviolet irradiation of sperm:** In order to induce gynogenesis, sperms were irradiated with UVG-11 ultraviolet lamp with an output capacity of 660 µw/cm². The milt was diluted 10 times with Ringer's
solution (GIBCO) in a petridish resulting a thickness of about 0.1-0.2 mm and irradiated at a distance of 13.5 cm from the lamp filter for 2 minutes.

**Fertilization:** In order to ascertain that the diploid individual produced are really of maternal origin, and to optimize the experimental procedure, such assay had to contain three types of control, one involving the treatment of UV (UV-irradiation only without heat shock); one involving the effect of heat shock (only heat shock without UV-irradiation) and the one on normal fertilization without irradiation and heat shock (no UV-irradiation and no heat shock). Therefore, eggs were divided into 4 groups and each group containing approximately 3 ml of eggs. Artificial fertilization was initiated by mixing the eggs and sperms in petridish and excess milt was washed off.

**Application of heat shock:** The fertilized eggs were taken in a plastic strainer and dipped in a hot water bath of required temperature and removed.

**Egg incubation and rearing of progeny:** Eggs were incubated in hatching jar with 6 l capacity at 26°C (± 1°C) with a water flow rate of 1 litre/minute. After hatching, the spawns were left in a trough (250 x 35 x 20 cm) and kept there for 5-7 days feeding with finely ground boiled egg yolk.

**Ploidy determination:** In the present study, ploidy of each group was determined following the method of Kligerman and Bloom (1977), Krasznai (1985). The embryos were kept at 0.01% colchicine solution for about three and half hours. Then they were transferred to 0.4% KCl solution and kept for 30 minutes. After that they were put in 3:1 methanol acetic acid solution for 30-45 minutes before being subjected to staining. Staining was done with 2-3% aceto-orcein and kept for 24 hours in a refrigerator at 4°C. After that they were taken out and squashed on a slide with lactic acid and examined under microscope. From each sample drawn out of a batch, at least 10 preserved embryos were taken for ploidy determination and degree of ploidy induced was expressed as percentage.

**Results**

Table 1 shows the results on the radiation induced heat shocked gynogenetic experiments in *Cirrhinus mrigala*. Heat shock was given to the eggs fertilized with UV-irradiated sperm. To optimize the UV-irradiation dose for sperm inactivation and different parameters of heat shock treatment for the retention of second polar body of eggs, 7 different attempts were made at different combinations of the parameters of temperature time regimes with a constant irradiation dose at 2 minutes exposure at a distance of 13.5 cm lamp-sperm. From the observations, it appears that the heat-shock regimes took a fewer trials to stick at the temperature of 39 to 40°C for 1 to 2 minutes, starting 4 minutes after fertilization; the important reference in this regard being used from the studies of John *et al.* (1984).

Out of 7 different attempts made, only 6 attempts resulted in the induction of gynogenetics at the rate of 30 to 100% with a poor survival of 0 to 40% whereas 30 to 60% survival was observed in control. Triploidy and haploidy in the controls were induced at the rate of 0 to 70% and 50 to 100%, respectively. The survival rates in the haploid groups ranged from 0 to 8% (Table 1).

In the three attempts at 40°C for 2 minutes, commencing 4 minutes after fertilization, gynogenetics were induced at the rate of 30 to 80% but with poor survival of 2 to 50%. The triploidy and haploidy were induced at the rate of 50 to 60% and 50 to 100% respectively.

In the other three attempts made at 39°C for 1 and 2 minutes and initiated 4
TABLE 1. Observations of gynogenetic experiments in the female of Cirrhinus mrigala by heat shock treatment; sample of eggs (3 ml) = 2200; Duration of irradiation = 2 minutes; lamp-sperm distance = 13.8 cm.

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<th>Gynogenesis (2n)</th>
<th>Remarks</th>
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<td>Normal Control</td>
<td>Triploid (3n)</td>
<td>Haploid (n)</td>
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<td>Temperature exposed (°C)</td>
<td>Duration of heat shock (min.)</td>
<td>Time after fertilization (%)</td>
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minutes after fertilization, gynogenetics were induced at the rate of 80 to 100% with the survival rate at 0 to 40%. Out of the three attempts, one attempt with heat shock for 1 minute induced gynogenetics at 100% with 40% survival but that did not induce any triploidy in the control, though haploidy was induced at the rate of 100% with 0% survival. The other two attempts with the duration of heat shock for 2 minutes, gynogenetics was induced at 80%, one having survival of 0% and the other of 30%. From the observations made, it appeared that the heat shock regimes could be used at 39°C for 2 minutes, applied 4 minutes after fertilization for induction up to 90% gynogenetics where triploidy and haploidy could be induced at the rate of 40 to 70% and 80%, respectively.

The hatching percentage recorded in gynogenetic groups were comparatively lower ranging from 30 to 50%; the corresponding hatching percentage obtained in the normal diploid controls ranged from 45 to 80%. The haploids in mrigal were all deformed being distinguished by short body with curved tail with a survival of 0 to 8%. The karyological investigations on the haploid (n), diploid (2n) and triploid (3n) complements resulted 25, 50 and 75 chromosomes, respectively, in mrigal.

Discussion

The techniques of artificial gynogenesis consists of in vitro fertilization of eggs by irradiated sperm with immediate physical shock of the fertilized eggs for diploidization by retention of the females chromosome set (Purdom and Lincoln, 1973). During the present study the diploidization of eggs was obtained by sub-
jecting the same to extreme sub-lethal temperature which affect the normal course of meiosis thereby retaining the chromosome set which normally disjoins to be the second polar body.

Heat shock treatment have been studied far less than cold shocks but were amongst the first agents to be used to produce polyploidy in fish. The induction of diploid gynogenesis by the use of cold shocks has succeeded in a variety of fish species but has not so far proved effective in salmonids (Lincoln et al., 1974). High temperature shocks, however, were effective and Chourrout (1980) was able to produce increased number of diploid gynogenome in trout. In the present study, the application of heat shock was followed as per the procedure of John et al. (1984) on the artificial gynogenesis in rohu, Labeo rohita and catla, Catla catla. They applied heat shock to the eggs fertilized with UV-irradiated sperm at 39°C for 1 minute, starting 4 and 8 minutes after fertilization and successfully induced diploid gynogenetics in L.rohita and C.catla. However, this was not fully fruitful in the present experiment and two minutes duration of heat shock was found to be more appropriate.

Suzuki et al. (1985) found that UV-irradiation of 0.12J/cm² for sperm inactivation in cyprinid loach resulted in high fertilization rate. Chao and Liao (1990) found that irradiation of sperm with UV light at 0.05 µW/cm² for 20-40 seconds (1-2J/cm² in total) while Hussain (1992) found 300-310 µW/cm² for 2 minutes at 4°C seemed to be optimum for inactivation. However, in attempting to optimize the UV-irradiation dose in the present study to de-nature the sperm, information of the study of John et al. (1984) in inducing gynogenetics in two Indian major carps, rohu, L.rohita and catla, C.catla was found most useful. They used UV-light (15 w) at a distance of 20 cm lamp-sperm. In the present experiment, UV-irradiation dose of 580 µW/cm² at a distance of 13.5 cm lamp-sperm was found to be effective for genetic inactivation of sperm of mrigal. The induction of gynogenetic haploids was higher, mostly ranging from 50 to 100% with a survival rate of 0 to 8%. Sumantadinata et al. (1990) observed the survival rate of embryos resulting from insemination of UV-irradiated sperm without heat shock to be about 52% with all embryo haploid. However, the surviving individuals from 5 to 7 days in the haploid control obtained in the present study were not haploid, but diploids produced due to improper irradiation and heat shock.

Variations in the level of success in inducing gynogenetics is reported by several workers. In the present study, it observed that the response of fish eggs to the induction of gynogenetics in mrigal varied from one combination of treatment parameters to another. Among 7 different trials (Table 1) diploid gynogenetics was induced from 30 to 100%. Though in the attempt, induction of 100% diploid gynogenetics and gynogenetic haploids was possible, triploidy was not induced in the control which indicate a gross possible error in the procedure. In two other attempts of triploid control being made with the same temperature-time regime, triploidy was induced from 40 to 70% and the percentage of gynogenetics induced was higher. Here it was not clear why the same temperature-time regime should have produced higher percentage of gynogenetics. However, all normal fry resulting from radiation induced heat shock treatment were confirmed to be diploid by their chromosomal complement and their appearance.

Reports on viability of gynogenetic fish are conflicting. Majority of workers have reported rather poor survival rate in different groups of fish (Shah, 1984). Purdom (1983) cited poor survival rates in gynogenetic common carp, other cyprinids and
loaches. Purdom (1969) in plaice, Chourrout (1980) in trout and Suzuki et al. (1985) in cyprinid loach reported similar low rates of survival. Report on high rates of survival of gynogenetic grass carp come from the studies of Stanley (1976). In the present study survival rate of the gynogenetics within five to seven days after hatching, ranged from 0-50%, whereas it was 30 to 60% in the controls. This poor survival rate in the gynogenetics may be explained by the fact that the process of reproduction might have increased chances of homozygous combination of recessive lethal alleles, thereby causing higher percentage of mortality. The results obtained on survival rates of gynogenetics in the present experiment are in consistence with many authors as discussed above however, with some exceptions.

References


