Theor Appl Genet (1985) 71:119–121

Residual paternal inheritance in gynogenetic rainbow trout: implications for gene transfer

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Accepted January 30, 1985
Communicated by R. Riley

Summary. Pollen irradiation has recently been widely investigated as a method for differential gene transfer in plants. Using an albino color marker in rainbow trout (*Salmo gairdneri*), we have investigated whether irradiated sperm might be used in an analogous manner for gene transfer in fish. Our results indicate that paternal chromosome fragments are genetically active in gynogenetic offspring, but that these fragments may be lost during mitotic cell division, producing mosaic fish.

Key words: Trout – Sperm – Irradiation – Gene transfer – Gynogenesis

Introduction

Diploid gynogenesis, which involves the production of individuals with both chromosome sets from the female parent, has been investigated as a method for sex control and the production of inbred lines in fish (Cherfas 1981; Streisinger et al. 1981; Chourrout 1982, 1984; Thorgaard 1983; Guyomard 1984). In gynogenesis, sperm is typically treated with UV or gamma radiation and used to activate egg development. A temperature or pressure treatment is then used to block second polar body extrusion or first cleavage and produce gynogenetic diploids.

Several studies have demonstrated the presence of chromosome fragments (presumably of paternal origin) in gynogenetic offspring after activation with gamma-irradiated sperm (Chourrout and Quillet 1982; Onozato 1982; Chourrout 1984). If these paternal chromosome fragments were expressed in the offspring, a possibility raised by Ijiri (1980, 1983), and could be stably inherited, this incomplete gynogenesis might be used in gene transfer to facilitate the introduction of traits such as disease resistance from one fish species or strain to another. Similar methods have been proposed for gene transfer in plants using irradiated pollen (Pandey 1983; Werner et al. 1984; Davies 1984; Sanford et al. 1984a, b).

In this study we have used an albino color marker in rainbow trout (*Salmo gairdneri*) to investigate whether irradiated sperm might be used for gene transfer in fish. It appears that paternal chromosome fragments are genetically active in gynogenetic offspring but that these fragments are frequently lost during mitotic cell division in the developing embryo.

Materials and methods

To test for paternal gene expression in gynogenetic trout, we fertilized albino rainbow trout eggs with irradiated sperm from normally pigmented males, heat-shocked the eggs to produce gynogenetic diploids, and looked for pigmentation among the offspring. Because albinism in rainbow trout is a recessive trait (Bridges and von Limbach 1972), pigmentation in the gynogenetic offspring should indicate the presence of paternal chromosomal material carrying the functional pigmentation gene.

Albino rainbow trout eggs were obtained from the Egan (Utah) State Fish Hatchery and sperm from normal rainbow trout was obtained from the Spokane Trout Hatchery, Spokane, Washington. Sperm was irradiated using the ^{60}Co gamma source at the Washington State University Nuclear Radiation Facility. Ten minutes after fertilization in 10 °C dechlorinated tap water, the eggs were heat-shocked for 10 min at 29 °C to induce retention of the second polar body and produce gynogenetic diploid offspring (Thorgaard et al. 1983). The eggs were then placed in a recirculating water system at 10 °C to continue development. Chromosome preparations of 23-day-old embryos from control and treatment groups were made as previously described (Thorgaard et al.

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1981) to test for the presence of chromosome fragments. Survival of embryos was monitored at 9 and 41 days after fertilization and the proportion of survivors showing eye pigmentation was monitored at 41 days.

Results

Fertilization with irradiated sperm followed by no heat shock resulted in gynogenetic haploids which showed early development (day 9) but died before the alevin stage (day 41) (Table 1). The few individuals which did survive to day 41 in these groups were apparently spontaneous gynogenetic diploids; none of these individuals showed eye pigmentation. The low proportion of individuals developing to day 41 after fertilization with irradiated sperm and the albino phenotype of the survivors demonstrated that the sperm chromosomes were extensively damaged. All of the offspring fertilized with non-irradiated sperm showed eye pigmentation at day 41 (Table 1).

Table 1. Survival and pigmentation of gynogenetic and control rainbow trout embryos. No heat shock was applied to the fertilized eggs

Sperm radiation treatment	No. of eggs treated	Proportion of embryos surviving to day		Proportion of survivors showing eye pigmentation day 41
		9[a]	41	
2.7×10^4 R	1,696	0.33	0	–
5.4×10^4 R	2,228	0.58	0.004	0
8.1×10^4 R	1,997	0.35	0.006	0
1.08×10^5 R	1,153	0.61	0.009	0
1.35×10^5 R	1,155	0.42	0.003	0
None	1,895	0.57	0.45	1.00

[a] Fraction of embryos developing at 9 days estimated on samples of 54–80 eggs per treatment

Table 2. Survival and pigmentation of gynogenetic and control rainbow trout embryos. Eggs were heat-shocked 10 min after fertilization to induce retention of the second polar body

Sperm radiation treatment	No. of eggs treated	Proportion of embryos surviving to day		Proportion of survivors showing eye pigmentation day 41
		9[a]	41	
2.7×10^4 R	3,369	0.13	0.005	0
5.4×10^4 R	2,767	0.29	0.120	0.025
8.1×10^4 R	3,783	0.32	0.077	0.021
1.08×10^5 R	3,969	0.30	0.141	0.009
1.35×10^5 R	3,786	0.25	0.089	0
None	2,213	0.63	0.384	0.985

[a] Fraction of embryos developing at 9 days estimated on samples of 58–95 eggs per treatment

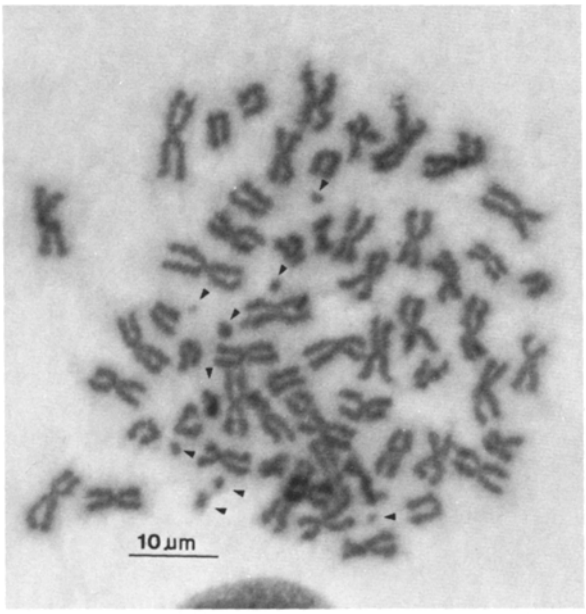

Fig. 1. Metaphase spread from a gynogenetic diploid (2n = 58) rainbow trout embryo with nine chromosome fragments (indicated by *arrows*). Sperm was irradiated with 8.1×10^4 R from a ^{60}Co source before fertilization

Fertilization with sperm irradiated at doses above 5.4×10^4 R followed by a heat shock resulted in a much better survival to the alevin stage (Table 2). Chromosome analysis of 4–5 embryos from each of these groups confirmed that they had near-diploid chromosome numbers. Fertilization with non-irradiated sperm followed by heat shock caused production of triploid offspring; 5/5 embryos tested were triploid. The few albino offspring in this group (Table 2) may represent gynogenetic diploids induced by the heat shock.

A low proportion of the survivors in the groups fertilized with irradiated sperm and heat-shocked showed eye pigmentation. The proportion of pigmented individuals decreased with increasing radiation dose (Table 2). Chromosome analysis of 4–5 embryos per group revealed numerous chromosome fragments in the 2.7×10^4 R and 5.4×10^4 R lots, varying numbers among embryos in the 8.1×10^4 R lot (Fig. 1), and few or no fragments in embryos from the high radiation lots. These results are similar to those described by Onozato (1982). No chromosome fragments were observed in control diploid or triploid embryos.

Thirteen embryos showing eye pigmentation survived to the initiation of feeding (approximately day 50). All of these individuals showed evidence of mosaicism for albino and pigmented tissue, suggesting that the chromosome fragment carrying the pigmentation gene was being lost during mitotic cell division. An eight-month-old rainbow trout showing mosaicism is shown in Fig. 2.

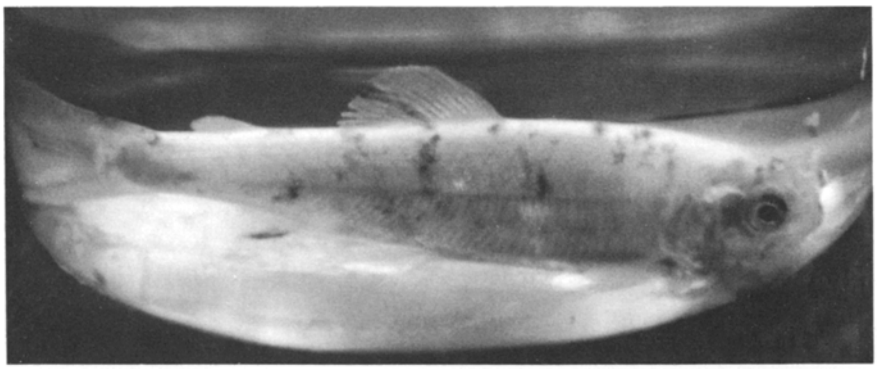

Fig. 2. Gynogenetic rainbow trout showing mosaicism for albino and pigmented tissue. Sperm was irradiated with 5.4×10^4 R from a ^{60}Co source before fertilization

Discussion

Our results demonstrate that paternal genes on chromosome fragments may be expressed in gynogenetic trout but that the fragments are frequently lost during mitotic cell division. The instability of the chromosome fragments is consistent with studies of chromosome-mediated gene transfer (McBride and Peterson 1980; Klobutcher and Ruddle 1981), which have shown high rates of segregation and low rates of stable incorporation of chromosome fragments in somatic cells. Acentric fragments in somatic cells appear to be lost at rates of 10^{-2}–10^{-1} per cell per generation; fragments containing centromeres may have somewhat higher stability (McBride and Peterson 1980). Similar problems with chromosome fragment instability might explain the lack of success of gene transfer experiments in some plant species using irradiated pollen (Sanford et al. 1984a, b).

The mitotic instability of the chromosome fragments indicates that further study is needed before this technique could be used for gene transfer in fish. Even if this technique does not ultimately prove useful in gene transfer, it may be valuable in other types of genetic studies.

Acknowledgements. We thank personnel of the Utah Division of Wildlife Resources and the Washington Department of Game for providing rainbow trout eggs and sperm for these experiments. Supported by National Science Foundation grant PCM 8108787 and U.S. Department of Agriculture grant 82-CSRS-2-1058.

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