The observed increase of GSH-Px activity in erythrocytes of Down's syndrome patients could be due to various causes. The simplest explanation would be to ascribe it to a gene dosage effect due to the linkage of the locus for GSH-Px to chromosome 21. However, enzymes known not to be linked to this chromosome were also reported to be elevated in the erythrocytes of Down's syndrome patients[5-9,15,20], suggesting a more complex basis for this phenomenon. In any case, the elevated activity of GSH-Px, a protective, H_2O_2-removing enzyme, may be of importance for the physiology of the Down's syndrome erythrocyte, which is subjected to increased generation of H_2O_2 due to the increased activity of superoxide dismutase[19].

Table 2. GSH-Px activity in erythrocytes of patients with Down's syndrome (units per g hemoglobin)

No.	Sex	Karyotype	Activity
1	M	47, XY, +G	53.45
2	M	47, XY, +G	51.15
3	M	47, XY, +G	49.06
4	M	47, XY, +G	57.30
5	M	47, XY, +G	50.90
6	M	47, XY, +G	55.12
7	M	47, XY, +G	53.45
8	M	47, XY, +G	48.95
9	M	47, XY, +G	53.27
10	M	47, XY, +G	50.38
		Mean	52.30
		SD	0.85
11	F	47, XX, +G	52.06
12	F	47, XX, +G	56.20
13	F	47, XX, +G	51.36
14	F	47, XX, +G	52.80
15	F	47, XX, +G	47.38
16	F	47, XX, +G	53.26
17	F	47, XX, +G	53.27
18	F	47, XX, +G	52.93
19	F	47, XX, +G	56.90
		Mean	52.88
		SD	0.91
20	F	46,XX, −22, +t/21q22q/mat	49.20
21	M	46,XY, −22, +t/21q22q/mat	46.33

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2 Department of Biophysics, University of Łódź, Łódź (Poland).
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Induction of triploids in *Rhodeus ocellatus ocellatus* by cold shock treatment of fertilized eggs

K. Ueno and B. Arimoto[1]

Department of Fisheries, Faculty of Agriculture, Kinki University, Higashi-Osaka, 577 (Japan), 22 September 1981

Summary. Triploids were induced by cold shock in fertilized eggs of *Rhodeus ocellatus ocellatus*. The maximum percentage (95%) of triploidy was obtained from eggs treated at 5 min after fertilization. The triploids grew normally to adult size, and they were all sterile males.

Few studies have been reported on artificial polyploidy in fish, so the existing knowledge of the characteristics and techniques of production concerning polyploidy are not very useful in the practical breeding of fish. However, the morphological and physiological changes induced in fish by gene duplication could be useful for the improvement of fish breeding; such changes have recently proved to be helpful in plants. Treatment by low or high temperatures[2-5], X-rays[6] and chemicals[7] has been used to induce polyploidy in fish. The present paper deals with the necessary conditions of low temperature to induce triploid fish, their fertility and sex ratio.

For the subject fish, the rose bitterling, *Rhodeus ocellatus ocellatus* was chosen for this experiment because this acheilognathine fish has no chromosomal polymorphisms within the same species or among the different tissues in the body of one fish[8]. Also, it grows into an adult within 1 year under the climatic conditions prevailing in Japan. The parent fishes for the spawning of eggs were obtained from the lower stream of the Yodo River (Akagawa-cho, Osaka Prefecture), and raised in laboratory aquaria until the eggs matured. The eggs were obtained from several fully mature females using the stripping method. A sufficient number of eggs were immediately inseminated by sperm which was obtained from several males.

For the cold shock treatment, the eggs were exposed directly to cold water which was maintained at a temperature of 0-0.2 °C for 1 h beginning within 1 min after

fertilization or 1.5, 3, 5, 7, 9, 11, 14, 17 or 20 min after fertilization. Then the treated eggs were kept at room temperature (23–28 °C) until they hatched. Chromosome preparations from the kidney tissues of diploid and triploid fish were made using the usual air-drying method. The sex of the fish was determined from their nuptial colorations and gonads when they became of adult size. Fertility was determined by comparing the growth of the gonads with that of the control diploid.

Triploid fish were successfully obtained by cold shocking of the fertilized eggs. Figures 1B and 1C show the metaphase spreads of fish obtained by cold treatment given 5 and 17 min after fertilization, respectively. Figure 2B shows the chromosome arrangement of the fish shown in figure 1C. This karyotype is clearly seen to be euploid by comparison with the diploid karyotype (figs 1A and 2A). That is, the triploid karyotype is $3n=72$, and the homologous chromosomes were grouped in threes. In the present study, no haploids or mosaics as have been reported by Swarup[2] and Remonine and Smith[9] were obtained. The triploids were induced when the eggs were treated from 1 min up to 17 min after fertilization. Almost all of the eggs (95%) treated at 5 min after fertilization became triploid. The relationship between the starting time of the cold treatment after fertilization and the successful induction of triploidy is shown in detail in figure 3.

Makino and Ojima[10], in their report on refrigeration experiments with carp eggs, confirmed the cytological evidence showing that cold treatment prevented the 2nd meiotic division from going to completion and caused chromosome duplication in the treated eggs due to a retention of the haploid set of chromosomes normally going to the 2nd polar body, leading to the formation of a diploid egg nucleus. Then it was concluded that the union of the diploid egg nucleus thus produced with the haploid sperm nucleus would give rise to a triploid zygote. The results of the present experiment suggest that the effect of cold treatment in preventing the 2nd meiotic division is exhibited immediately after fertilization when the egg nucleus has begun to divide (early anaphase II) to just before the time when the polar body is extracted (late anaphase II). The hatching rate of eggs treated at the low temperature was slightly lower (64–80%) than that of control eggs (96%), but the hatched fry grew favorably, showing no observable abnormalities, and reached the adult stage 6 months after being hatched. It was difficult to distinguish between the triploids (33 fish: average $\pm$ standard deviation, 36.2 ± 6.6 cm) and diploids (22 fish: average $\pm$ standard deviation, 38.5 ± 7.4 cm) by their body lengths. This result is identical with that found for artificial triploidy in stickleback *(Gasterosteus aculeatus)* by Swarup[2] and amphibia by Fankhauser[11].

During this experiment, very interesting facts were obtained concerning the sex ratio. Although both sexes were found in the diploidy which developed normally among the cold-treated eggs, all individuals showing triploidy

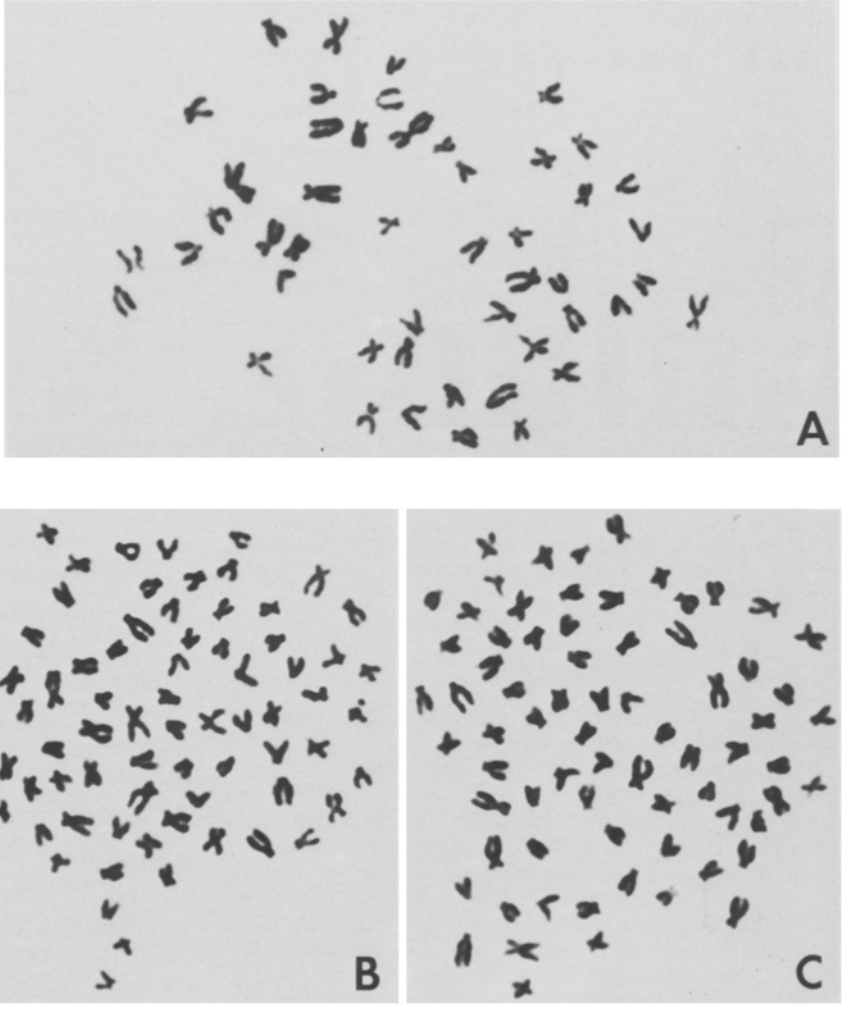

Figure 1. Metaphase spreads from kidney cell of diploid (*A:* 48 chromosomes) and artifical triploid (*B* and *C:* 72 chromosomes) *Rhodeus ocellatus ocellatus.* B and C show the metaphase spreads of individuals obtained by cold treatment given 5 and 17 min after fertilization, respectively.

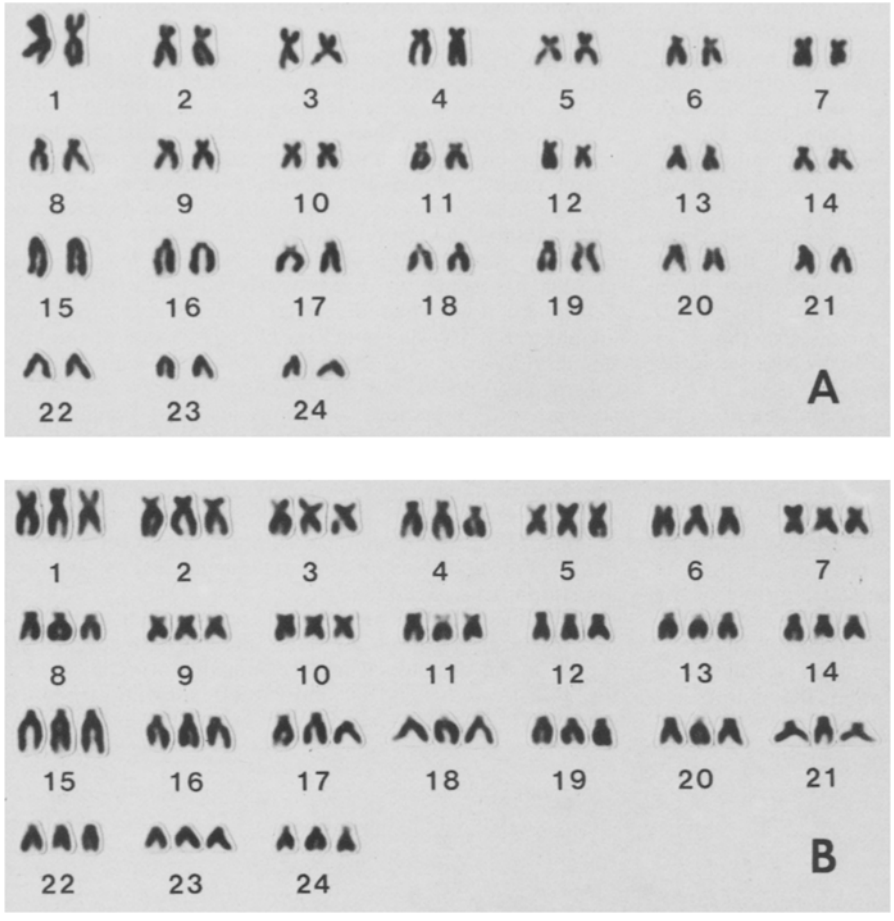

Figure 2. Chromosome arrangements of diploid (A) and artificial triploid (B) *Rhodeus ocellatus ocellatus* derived from metaphase spreads shown in fig. 1 (A and C). Triploid is made up from 24 sets of triple chromosomes.

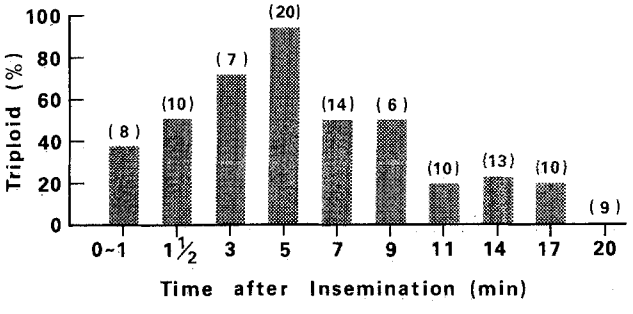

Figure 3. Frequency of triploid obtained from cold-treated eggs. Eggs subjected to cold water of from 0 to 0.2 °C for a duration of 1 h. A numeral in a parenthesis represents the number of individuals analyzed.

were males. The testes of the triploids showed very poor development compared to those of the diploid ones. It is very probable that these triploids were sterile.

From these studies the following conclusions were drawn concerning the use of triploids in fish breeding. Low temperature treatment is an effective method for obtaining triploids. It is also considered to be a practical method due to the simple procedures and low cost. The anisoploids such as those obtained in this experiment are generally known to be sterile, and this particular fact seems to be applicable in the breeding of fish. The fish *Tilapia* breeds repeatedly all the year round in tropical ponds. If uncontrolled they quickly produce overpopulation and their growth is stunted. Control breeding of such cultured fish is assumed to be possible by making use of triploids. Since fish hybrids are usually sterile, they are considered not to disturb the ecosystem even when transplanted[12]. Because they are not fertile, triploids are also suited for this purpose, and they have no hybridity. The triploids of the rose bitterling produced in this study were all male. If such a phenomenon were observed with cultured fish of species in which the growth rate of the males differs from that of the females, a higher yield could be obtained by monosexual culture.

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