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PROGRAM IN GENETICS AND CELL BIOLOGY
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Dr. Robert J. Behnke
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Dear Bob,

We have been out of touch for a while now (since the meeting in Maine). I have been teaching Genetics here at Washington State and doing some research on producing polyploid trout. A paper is scheduled to come out in the July issue of TAFS on that work; a copy of the manuscript is enclosed. This year we have produced a large number of triploid trout (which should be sterile) and will stock them in lakes along with controls to study their growth and survival. One approach to the problem.

My plans for next year are still a bit "up in the air." I just got a fellowship to go work in Seattle at NMFS and may do that starting in October. The job here is temporary and the budget squeeze here is squeezing me out. Actually, Seattle might be a good thing because I could step up the pace of my research quite a bit.

I am finally putting together a manuscript on the chromosome variation in rainbow, and want to cite your monograph. What is the proper citation? I already have a copy of most of the relevant part, but would like to get a copy of the complete monograph if you have any extras or know where I could ask.

Best wishes, and keep in touch.

Gary Thorgaard

ADULT TRIPLOIDS IN A RAINBOW TROUT FAMILY

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ABSTRACT

Six triploid individuals were found in a full-sib family of 11 adult rainbow trout (*Salmo gairdneri*) from a domesticated hatchery stock. The triploid individuals were normal in size and external appearance, had underdeveloped gonads, and showed no evidence of 3n/2n chimerism or mosaicism. *XXY* triploids were males, suggesting that the *Y* chromosome is male determining in trout. Because they may avoid production losses associated with sexual maturation in normal fish, triploid trout and salmon could potentially be useful in fish culture.

TRIPLOIDY is tolerated to very different degrees among different groups of vertebrates. Mammalian triploids are inviable and apparently never survive long past birth, although viable diploid/triploid chimeras have been found in some species (CHU, THULINE and NORBY 1964; NES 1966; DUNN, MCENTEE and HANSEL 1970; NIEBUHR 1974). Triploidy also substantially reduces viability in chickens (BLOOM 1972; MONG *et al.* 1974), but some triploid individuals do survive to adulthood (OHNO *et al.* 1963; ABDEL-HAMEED and SHOFFNER 1971).

Triploids are much more viable in lower vertebrates. Triploidy has been observed in unisexual species and in hybrids between unisexual and bisexual species of fish, amphibians and reptiles, and occasionally in bisexual species and their interspecific hybrids in fish and amphibians (references in CUELLAR and UYENO 1972; GOLD and AVISE 1976; ALLEN and STANLEY 1978). Triploidy has also been experimentally induced in fish and amphibians, using thermal shocks and other treatments applied shortly after fertilization (FANKHAUSER 1945; VALENTI 1975; TOMPKINS 1978; and others).

Among salmonid fish, triploid individuals have been found in rainbow trout (CUELLAR and UYENO 1972; GRAMMELTVEDT 1974), brook trout (ALLEN and STANLEY 1978) and rainbow trout × brook trout hybrids (CAPANNA, CATAUDELLA and VOLPE 1974). There has been interest in the potential use of triploids in fish culture and management (PURDOM 1972, 1976; GJEDREM 1976). Attempts to induce polyploidy in salmonids have been discussed by SVARDSON (1945), LINCOLN, AULSTAD and GRAMMELTVEDT (1974). REFSTIE, VASSVIK and GJEDREM (1977) and SMITH and LEMOINE (1979). REFSTIE, VASSVIK and GJEDREM (1977) reported producing embryos that were a mosaic of polyploid and diploid cells in

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Atlantic salmon and in rainbow trout, using a cytochalasin-B treatment shortly after fertilization. SMITH and LEMOINE (1979) reported producing similar mosaic embryos and fry in brook trout after an early colchicine treatment.

In this report, we describe six adult triploids found among 11 individuals in a full-sib rainbow trout family. Possible causes for the high incidence of triploids in this family and the relationship of sex chromosomes to sex determination in trout are discussed.

MATERIALS AND METHODS

The rainbow trout used in this study were raised at the Fisheries Biology Research Facility, University of California, Davis, as part of an ongoing quantitative genetics study and selective breeding program (GALL 1975). The strain used at the hatchery (RTD) originated as a cross of two domesticated rainbow trout broodstocks, which themselves probably originated from fish from the McCloud River in northern California (BUSACK and GALL 1979). Trout at the U.C. Davis hatchery are reared as full-sib families, and a limited number of fish from each family are raised to maturity. Individual trout were identified in this study by plastic anchor tags (Floy Tag Co., Seattle, Washington).

Chromosomes of 79 rainbow trout were examined; chromosome preparations of 53 adult fish were made from white blood cell cultures (THORGAARD 1976), and those of 26 young fish and one adult were made by direct preparation from body tissues after colchicine injection (KLIGERMAN and BLOOM 1977). Slides were stained with undiluted Giemsa for six min, transferred directly to 0.06 M NH_4OH for two min, rinsed in tap water, destained for 10 sec each in acetone and 1:1 acetone:xylene, and cleared in xylene before examination.

Red blood cell nuclear volumes from five diploids, six triploids, and one mixture of blood of a diploid and a triploid were compared. Blood smears were stained for two min with undiluted Giemsa, flooded with tap water for three min, and rinsed in tap water. Outlines of 100 nuclei from each of the 12 samples were traced, using a camera lucida. Lengths and widths of the tracings were measured, and nuclear volumes were calculated as four-thirds ab^2 (the volume of a perfect ellipsoid), where a = one-half the length, and b = one-half the width. The calculated volumes were then scaled relative to the overall mean nuclear volume of diploid individuals, which was assigned a value of 100. All tracings and measurements of lengths and widths were done using coded samples to avoid experimental bias..

RESULTS

During a survey of chromosome differences among rainbow trout from the RTD stock, one triploid (fish G174 from family 267) was found among 14 individuals from ten different families. This led us to examine the chromosomes of the other fish in family 267.

Chromosome numbers and red blood cell nuclear volumes in family 267: Among the 11 fish in family 267, chromosome preparations from white blood cell cultures showed that five were diploid with 59 or 60 chromosomes, and six were triploid with 89 or 90 chromosomes (Table 1). The karyotype of fish G177, with 60 chromosomes, consisted of 44 metacentric and submetacentric, two subtelocentric, and 14 acrocentric chromosomes, and appeared identical to that reported in other hatchery rainbow trout strains (SIMON and DOLLAR 1963; CUELLAR and UYENO 1972; FUKUOKA 1972). The difference between the diploids with 59 and 60 chromosomes appeared to be associated with a Robertsonian rearrangement. These differences in chromosome number among fish with the same number of

Polyploidy Induced by Heat Shock in the Rainbow Trout

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Abstract

Polyploidy was induced in rainbow trout, Salmo gairdneri, when fertilized eggs were immersed in 36 C water for 1 minute. Triploids were produced if the eggs were treated 10 minutes after fertilization; tetraploids were produced by treatments 5 hours after fertilization. Sterile triploid trout and salmon potentially could grow and survive better than normal fish at sexual maturation. Heat-shock techniques should also be useful in the production of gynogenetic diploid trout and salmon for biological studies and breeding programs.

Introduction

Induced polyploidy has been a widely applied and sometimes useful tool in plant breeding (Allard 1960; Dewey 1980) but it has not yet found wide application in animal breeding. Polyploid fish frequently are viable, however, as evidenced by examples of spontaneous polyploidy (Gold and Avise 1976; Allen and Stanley 1978; and others). Induction of polyploidy has also been reported in fish by cytochalasin B (Refstie et al. 1977; Allen and Stanley 1979), colchicine (Smith and Lemoine 1979), and high and low temperature (Swarup 1959; Purdom 1972; Valenti 1975; Chourrout 1980; Gervai et al. 1980; Lemoine and Smith 1980) treatments of eggs shortly after fertilization. Triploids are produced by treatments blocking the second meiotic division or extrusion of the second polar body of the egg, which normally occur shortly after fertilization; tetraploids are produced by treatments blocking the first mitotic division.

There has been considerable interest recently in the possible application of induced polyploidy in fish culture and management. The interest has especially focused on trout and salmon; triploid trout

appear to be sterile (Allen and Stanley 1978; Thorgaard and Gall 1979) and could potentially avoid the growth depression and survival losses associated with sexual maturation in normal fish (Purdom and Lincoln 1973; Purdom 1976; Gjedrem 1976). This report describes simple techniques for inducing triploidy and tetraploidy in the rainbow trout by heat shock.

Methods

Rainbow trout eggs and sperm were obtained from the Spokane Trout Hatchery of the Washington Department of Game between December 1979 and February 1980 and shipped to Washington State University on ice. Fertilization of the eggs, heat-shock experiments, and subsequent incubation took place in a 10 C cold room. The eggs were fertilized in 10 C dechlorinated tap water. After several minutes the fertilized eggs were rinsed in fresh water and allowed to incubate in covered petri dishes containing a thin layer of water (Bevan 1963).

Fertilized eggs were heat-shocked 10 minutes or 5 hours after fertilization. Eggs were immersed in a hot-water bath at the appropriate temperature for 1 minute, cooled in a 10 C bath, and then returned to the petri dishes for incubation. Eggs from single-pair matings were divided and treated at different temperatures to study the effect of varying treatment temperature on induction of polyploidy. Embryos normally showed obvious eyes ("eyed" stage) about 15 days after fertilization and hatched about 28 days after fertilization. Dead (white) eggs were removed from the petri dishes daily. The proportion of eggs remaining with viable embryos 20 days after fertilization was taken as a measure of relative survival after the heat-shock treatments.

Chromosome preparations were made from embryos 10-25 days after

fertilization. Embryos were dissected out of the egg shell in 0.9% NaCl and transferred to tissue culture medium or phosphate-buffered saline solution containing 25 µg/ml colchicine for 4 hours. After the colchicine treatment, chromosome preparations were made by the solid-tissue technique of Kligerman and Bloom (1977). Counts of chromosomes from three or more metaphase spreads per individual were used to determine the ploidy of the individual embryos. Chromosome numbers of rainbow trout vary due to centric fusions and fissions, but hatchery strains typically have about 60 chromosomes (references in Thorgaard and Gall 1979). Triploid and tetraploid individuals thus have about 90 and 120 chromosomes, respectively.

Results

Heat-shock treatments of eggs 10 minutes after fertilization induced triploidy (Fig. 1). The incidence of triploidy increased with shock temperature (Fig. 2), but survival was reduced at 37-38 C. The variability in results among crosses may have been due to differences in susceptibility to temperature shock of eggs with different degrees of ripeness (Lincoln et al. 1974) or to other individual to individual differences.

Heat shocks 5 hours after fertilization induced tetraploidy in about 10% of the embryos (Figs. 1 and 2); the proportions of tetraploid embryos were similar at the different shock temperatures, but survival was reduced at the higher temperatures. Triploidy was found in some embryos treated at 36 and 37 C, and in a few control embryos. Spontaneous triploids have been found previously among rainbow trout (Cuellar and Ueno 1972; Grammeltvedt 1974; Thorgaard and Gall 1979). The triploid embryos in lots treated 5 hours after fertilization might

represent spontaneous triploids, or possibly cases in which the second meiotic division or the second polar body extrusion had not been completed by 5 hours after fertilization. There have been several reports of induced polyploid-diploid mosaicism in trout and salmon (Refstie et al. 1977; Smith and Lemoine 1979; Allen and Stanley 1979; Lemoine and Smith 1980). We did not find evidence for a high frequency of polyploid-diploid mosaic individuals after our treatments.

Lots that contained high proportions of triploid embryos are now being reared at the Spokane Trout Hatchery for future study. Many individuals from lots containing high proportions of triploid embryos survive, but there was an increased incidence of fish with curved spines in these lots. This might have been caused by the heat-shock treatment itself or it might have been associated with triploidy.

Many tetraploid embryos were short and abnormal in appearance even in treated lots in which diploid embryos appeared normal. Any fertile tetraploids that survive to adulthood might be crossed to diploids to produce sterile triploids (Refstie et al. 1977).

Discussion

Our success and that of Chourrout (1980) demonstrate that heat shock is a practical method for inducing polyploidy in salmonids. Chourrout (1980) used heat shocks of longer duration (10 minutes versus our 1 minute) and lower temperature (27-30 C versus our 35-37 C) to induce triploidy in rainbow trout. Both treatments apparently prevented the second meiotic division or extrusion of the second polar body of the egg. The successful induction of triploidy in salmonids should make it possible to study the value of triploids in fish culture and management.

The exact time at which the heat shocks were applied did not appear to be very critical. The early treatment was first applied at 10 minutes post fertilization because extrusion of the second polar body was believed to take place soon after fertilization, but we also induced triploidy with treatments applied at 40 minutes post fertilization. Chourrout (1980) induced diploid gynogenesis in rainbow trout with treatments applied at various times during the first 80 minutes after fertilization. We initially applied the late treatment at 5 hours post fertilization, but have also induced tetraploidy with treatments at 5.5 and 6 hours post fertilization. The first cleavage furrow was apparent at about 7 hours post fertilization under our conditions.

The success of the heat shock techniques in inducing polyploidy is not restricted to the Spokane strain of rainbow trout. We have obtained similar results with treatments of fertilized eggs of sea-run rainbow trout (steelhead) from the Dworshak National Fish Hatchery, Ahsahka, Idaho.

Our results suggest a general approach for inducing polyploidy in fish by heat shock. Treatments applied shortly after fertilization at just below lethal temperatures may induce triploidy. Treatments shortly before the first cleavage division at just below lethal temperatures may induce tetraploidy. The techniques for making chromosome preparations from embryos described in this report should be useful in assessing the results of experiments.

Temperature-shock treatments of fertilized eggs also can be useful for producing gynogenetic diploids if the eggs are fertilized with radiation-inactivated sperm instead of normal sperm. Chourrout (1980) described the production of gynogenetic diploid rainbow trout in this

way after suppression of the second meiotic division or second polar body extrusion by temperature shock. These fish should be partially homozygous, with the degree depending on the amount of crossing-over that takes place in meiosis. The heat-shock treatment we describe for producing tetraploids should make it possible to produce completely homozygous gynogenetic diploids by blocking the first mitotic division. Any surviving homozygous fish should be XX females (Thorgaard 1977; Okada et al. 1979) which could then be used to found inbred lines of genetically identical individuals by gynogenesis. Such lines could be useful in basic biological studies and breeding programs (Purdom and Lincoln 1973; Stanley and Sneed 1974; Purdom 1976; Nagy et al. 1978; Streisinger et al., in press).

Acknowledgments

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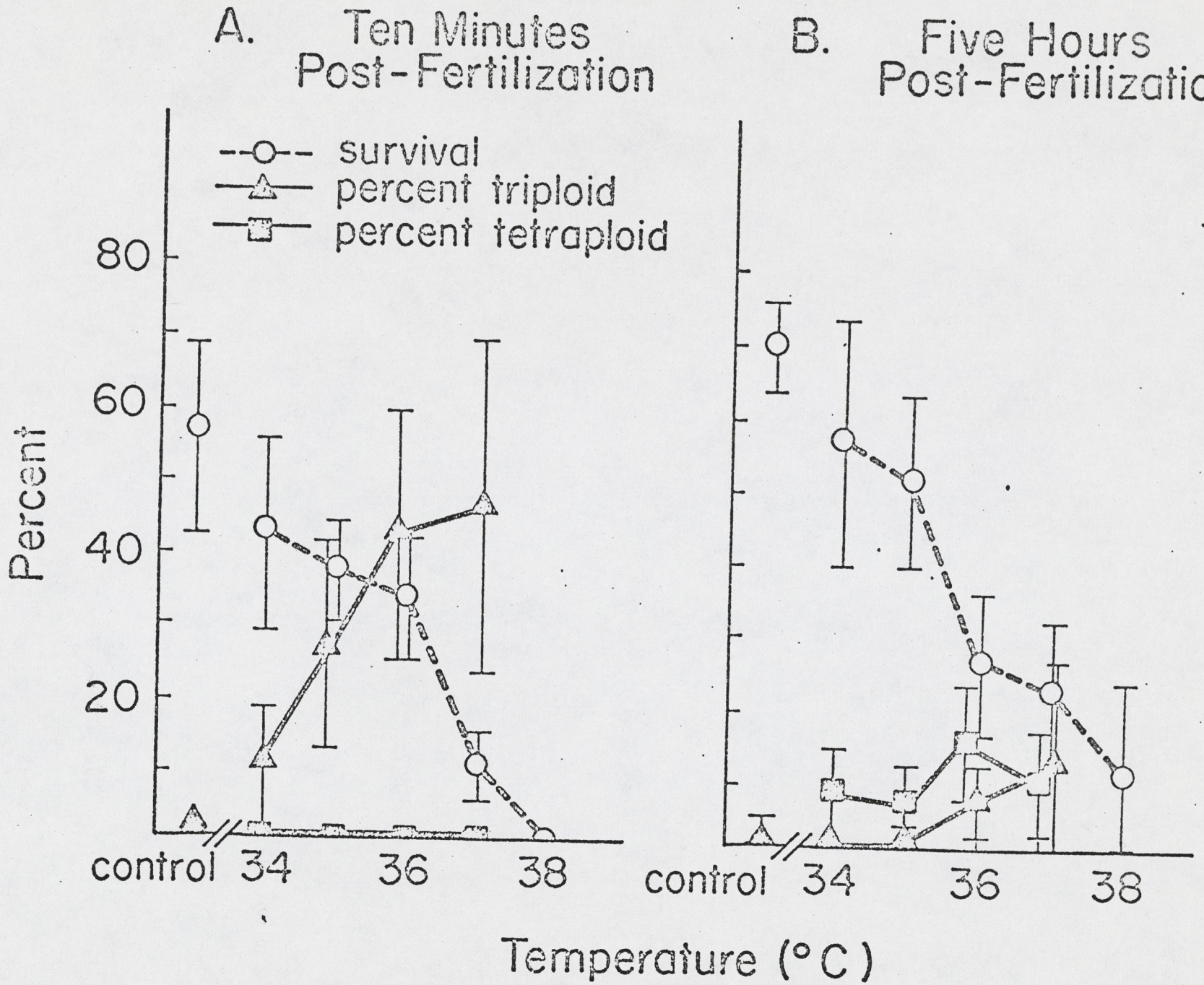
FIGURE CAPTIONS

Figure 1. Chromosome spreads from rainbow trout embryos demonstrating polyploidy induced by heat shock. (A) Triploidy induced by heat shock 10 minutes after fertilization; 92 chromosomes. (B) Tetraploidy induced by heat shock 5 hours after fertilization; 120 chromosomes.

Figure 2. Polyploidy and survival of rainbow trout after treatment at various heat-shock temperatures. (A) One-minute heat shock applied 10 minutes after fertilization. (B) One-minute heat shock applied 5 hours after fertilization. The values for percent survival and percent polyploids represent the average of the values obtained for 4 to 7 crosses (single-pair matings) at each temperature. Eggs from the crosses were divided and treated at different temperatures. Ploidy values are based on chromosome counts of 4-15 embryos from each cross treated at each temperature. Percent survival to 20 days post fertilization is based on 19-1,186 eggs (mean, 242) for each cross and shock temperature. Vertical bars are standard errors around means.

A
10 um

B


A. Ten Minutes Post-Fertilization
B. Five Hours Post-Fertilization
survival
percent triploid
percent tetraploid
Percent
80
60
40
20
control
34
36
38
Temperature (°C)

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