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

TABLE 1

Chromosome counts and relative red blood cell nuclear volumes of eleven rainbow trout

Fish	Tissue	<56	56	57	58	59	60	61	62-69	70-79	80-85	86	87	88	89	90	91	>91	Relative rbc nuclear volume (± S.E.)
G174	White blood cells (WBC)	—	—	—	—	—	—	—	1	2	4	4	4	11(1)	34(17)	2	1	—	187.0 ± 6.5
G176	WBC	—	—	—	—	—	—	—	—	2	3	—	2	6	10	11(1)	4	3	207.2 ± 7.1
G176	Kidney	—	—	—	1	—	—	—	3	6	6	1	1	2	—	2(1)	1	1	—
G176	Gill	—	1	—	—	—	—	—	—	—	—	2	—	—	1	1	1	—	—
G176	Intestine	—	—	—	—	—	—	—	—	—	1	1	—	1	1	1	—	—	—
G176	Ovary, mitotic cells	1	—	—	—	—	—	1	3	1	1	1	1	—	—	2	1	—	—
R131	WBC	—	—	—	—	—	—	—	—	1	5	1	—	—	3(1)	6(1)	1	—	164.7 ± 4.4
R126	WBC	—	—	—	—	—	—	—	—	—	3	1	—	3	5(1)	—	—	—	134.2 ± 4.5
R134	WBC	—	—	—	—	—	—	—	1	1	—	1	1	1	1	—	—	—	189.0 ± 5.6
G182	WBC	2	—	—	7(1)	15(8)	1	—	—	3	4	—	2	1	5(2)	1	—	—	177.0 ± 7.1
G175	WBC	3	—	2	1	6	16(10)	—	—	—	—	—	—	—	—	—	—	—	120.9 ± 3.9
G177	WBC	3	1	2	1	—	—	1	—	—	—	—	—	—	—	—	—	—	87.6 ± 2.7
G180	WBC	1	—	1	5	18(10)	—	—	—	—	—	—	—	—	—	—	—	—	65.1 ± 2.5
R129	WBC	—	—	1	—	8(5)	—	—	—	—	—	—	—	—	—	—	—	—	129.8 ± 2.6
G181	WBC	1	—	2	3	15(10)	1	1	—	—	—	—	—	—	—	—	—	—	96.7 ± 2.5

* Numbers of cells that could be identified as having the diploid (104) or triploid (156) number of chromosome arms are shown in parentheses.

chromosome arms have also been observed in other rainbow trout populations (OHNO *et al.* 1965; THORGAARD 1976). Although chromosome arm number determination was quite difficult in cells of some of the triploid individuals, there also appeared to be a Robertsonian rearrangement difference between the fish with 89 and 90 chromosomes. It seems likely that one, or both, of the parents of family 267 was heterozygous for a Robertsonian rearrangement.

Observation of chromosome preparations from kidney, gill, intestine and ovary of fish G176 confirmed that this individual was triploid (Table 1). Although most cells from the kidney and ovary had hypo-triploid chromosome numbers, determination of chromosome arm numbers, where possible, revealed that the variation probably resulted from chromosome loss during the preparation procedure and not from the intraindividual Robertsonian variation that has been reported in some salmonid fish (OHNO *et al.* 1965; DAVISSON, WRIGHT and ATHERTON 1973; and others). The low level of apparent Robertsonian variation within individual fish observed in this study (Table 1) is similar to that observed in some other studies in rainbow trout (THORGAARD 1976, 1977) and probably resulted from counting errors. There was no evidence of diploid cells in any of the triploid individuals; the only cell with a diploid number, in fish G182, had a karyotype more consistent with chromosome loss from a triploid cell.

Because red blood cell nuclear volumes were used by ALLEN and STANLEY (1978) in identifying some sterile brook trout as mosaics of polyploid and diploid cells, we examined the red blood cell nuclear volumes of the diploid and triploid trout in family 267 to test the accuracy of this indirect method of ploidy determination. All the triploid individuals had higher mean red blood cell nuclear volumes than any of the diploid individuals (Table 1). However, triploid fish R126 had a mean nuclear volume very close to those of the largest diploids. There was considerable variation in nuclear volume both within individual fish and among individuals of a given ploidy; this may have partly resulted from differences in the degree of cell flattening on the slides. Triploid individuals had many cells with nuclear volumes similar to those found in the diploid individuals (Figure 1). An "artificial mosaic" made up of an equal mixture of blood from diploid fish G175 and triploid fish G176 had the largest within-sample variation of the 12 samples (Figure 1).

History of family 267: Family 267 was the product of an artificial mating made on September 22, 1976, between a female weighing 1155 g and a male of 911 g. The water temperature at the hatchery that day ranged between 10° and 11.6°; the temperature during the preceding month ranged between 10° and 14.4°. Records indicate that early survival in family 267 was not substantially different from that in related families or in other families produced on the same day; 59.2% of the 2431 eggs hatched, and 50.2% survived to the initiation of feeding about two weeks later. The only notable difference was that a higher proportion of fish died between hatching and initiation of feeding in family 267 (15.2%) than in the nine other families whose records were examined (range 0 to 3%). This could reflect a reduced viability of triploid fish in family 267 or might simply have been a coincidence.
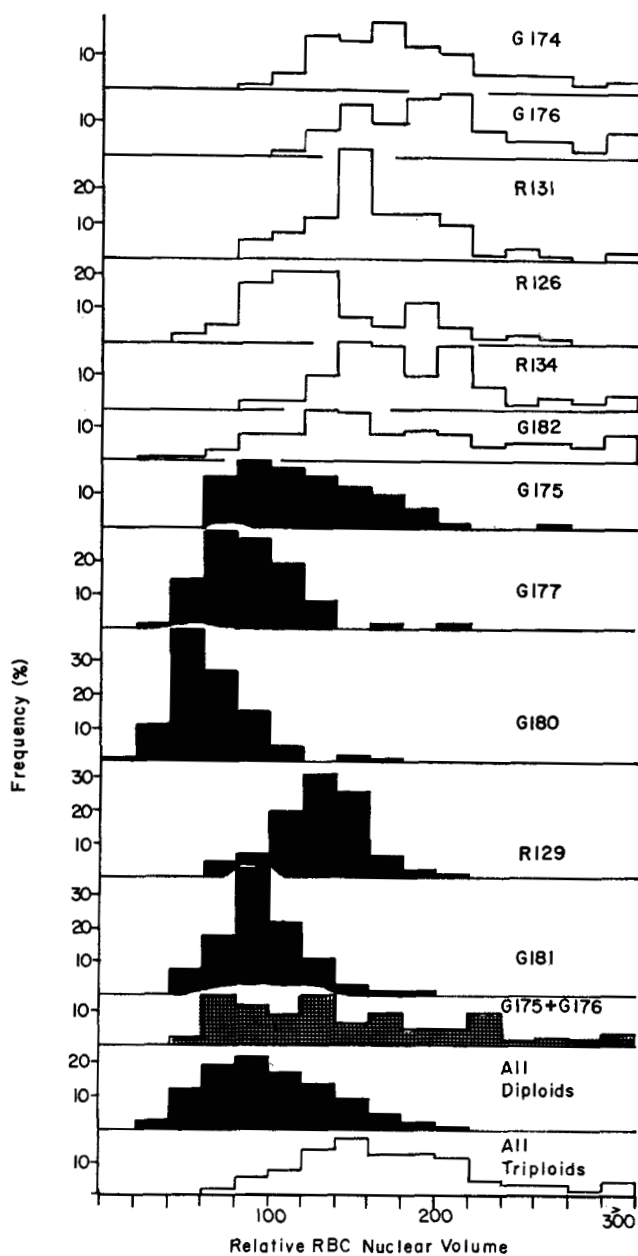
FIGURE 1.—Frequency distributions of relative red blood cell nuclear volumes of diploid and triploid rainbow trout. Triploid fish are represented by open bars, diploid fish by filled bars, and one mixture of blood from a diploid and a triploid (G175 + G176) by cross-hatched bars. Mean relative rbc nuclear volumes (± standard error) for diploid and triploid individuals are shown in Table 1; mean (± s.e.) for all diploids was 100.0 ± 2.2, for all triploids 176.5 ± 2.0, for G175 + G176 mixture 156.4 ± 8.0.

Fish in family 267 were randomly culled during their first year; at one year of age the heaviest one-fourth of the remaining fish were kept to be reared to maturity as part of a selection program for increased weight at one year of age. It is possible that this nonrandom sampling could have changed the proportion of triploids remaining in family 267, although triploids showed no evidence of being larger than diploids of the same sex at two years of age (Table 2).

Characteristics of triploid trout: The diploid fish in family 267 were typical of mature, adult rainbow trout in external appearance, size and gonad development (Table 2). Diploid males were smaller than diploid females, but this has also been observed in other rainbow trout strains, including one (Hot Creek; RTH) of the two strains that gave rise to the Davis strain (GALL and GROSS 1978). Males had a heteromorphic pair of subtelocentric chromosomes as reported in other rainbow trout (THORGAARD 1977).

The three triploid males in family 267 were normal in external appearance and, like the diploid males, showed the male secondary sexual characteristics associated with sexual maturation (large head/body ratio, deep body and dark coloration) when examined in September, 1978. Male triploids had partially developed testes similar in appearance to those of normal, developing males (Table 2); the testes were thick at the anterior end and very thin at the posterior end. Two of the three male triploids clearly had an *XXY* sex chromosome complement (Figure 2).

The three triploid females in family 267 were also normal in external appearance and, like the diploid females, did not show male secondary sexual characteristics. The single triploid female that survived a power failure at the hatchery in September 1978 (fish G176) was silvery in color and did not, when sacrificed in November, 1978, have the spawning coloration (moderately dark background with rosy strip along the side) normally associated with sexual maturation in both male and female rainbow trout. It also had noticeably pinker flesh than its diploid sibs and had large deposits of fat around the internal organs. Fish G176 continued to gain weight between September and November, 1978, unlike its diploid sibs that spawned during this period (Table 2).

All three triploid females had a *XXX* sex chromosome complement and had very small, string-like gonads (Table 2). Chromosome preparations from the ovaries of fish G176 showed many cells at early stages of the first meiotic prophase. These cells showed unusual meiotic pairing configurations (Figure 3), which have also been found in triploid human ovaries (LUCIANI *et al.* 1978). No cells were observed beyond the pachytene stage of the first meiotic prophase.

Search for other triploids: The observation of a high frequency of triploid individuals in family 267 led us to examine the chromosomes of other fish at the hatchery. One other triploid, a female, was found among 20 cousins of family 267. It was one of five fish examined that were double first cousins to family 267; the sire was a full sib of the dam of 267 and the dam was a sib of the sire of 267. No triploids were found among nine fish examined from a family identified at the hatchery as having many fish that were not ready to spawn during the fall season. On sacrifice in November 1978, it was apparent that some of these fish

TABLE 2

Characteristics of six triploid and five diploid rainbow trout

Fish	Ploidy	Sex chromosome complement	External sex phenotype	Weight (g) 7/11/78	Weight (g) 9/7/78	Weight (g) 11/10/78	Gonad development
G174*	3n	XXX	F	926	1000	—	Undeveloped, string gonads
G176†	3n	XXX	F	976	1067	1457	Undeveloped, string gonads
R131*	3n	XXX	F	—	750	—	Undeveloped, string gonads
R126*	3n	XXY	M	—	714	—	Partially developed testes
R134*	3n	?	M	—	517	—	Partially developed testes
G182*	3n	XXY	F	747	882	—	Partially developed testes
G175*	2n	XX	F	893	1022	—	Normal female, full of eggs
G177†	2n	XX	F	1167	1292	1246	Normal female, spawned 11/2/78
G180†	2n	XX	F	988	1173	1171	Normal female, spawned 11/2/78
R129*	2n	XY	M	—	893	—	Normal male, ready to spawn
G181†	2n	XY	M	620	739	690	Normal male, spawned 11/2/78

* These fish died during a power failure at the hatchery, 9/10/78. Observations on gonad development in these individuals were made that day by CHRISTOPHER PATIN.
† These individuals were sacrificed 11/10/78.
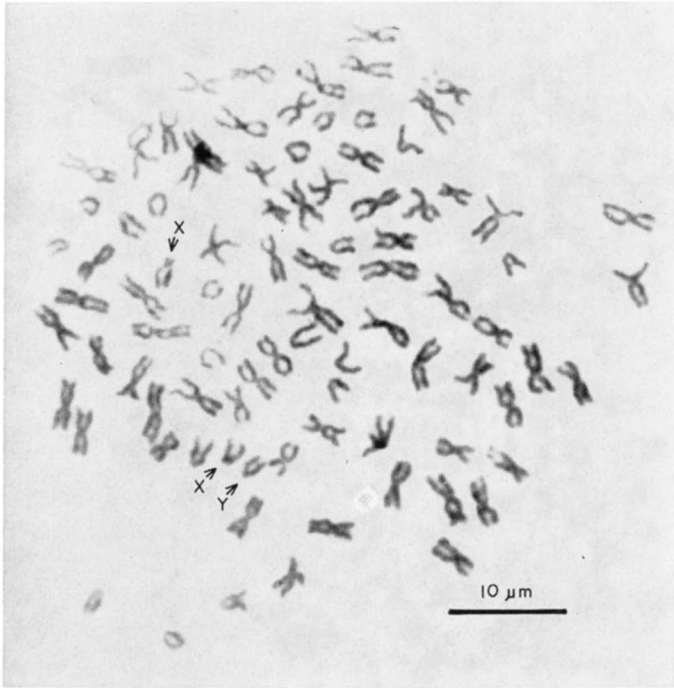
FIGURE 2.—Metaphase spread from an *XXY* triploid male rainbow trout (fish G182), with 67 metacentric and submetacentric, three subtelocentric and 19 acrocentric chromosomes. *X* and *Y* chromosomes are indicated by arrows.
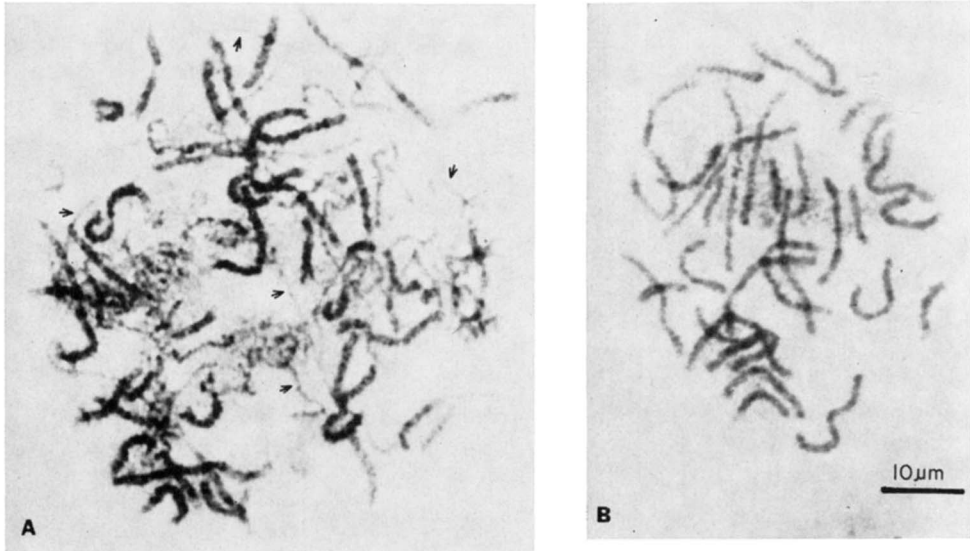

FIGURE 3.—(A) Pachytene oocyte from triploid fish G176, showing disturbed meiotic pairing and univalents (indicated by arrows). This is contrasted with the regular bivalent pairing seen in a pachytene oocyte from a diploid individual (B).

would have spawned in the future, but one diploid individual had string gonads similar to those found in the triploid females. This demonstrates that not all adults with string-like gonads are necessarily triploid.

On November 2, 1978, female G177 and male G181 from family 267 were crossed to produce family 250, and female G180 from family 267 was mated to an unrelated diploid male to produce family 249. Examination of gill, kidney and intestine chromosome preparations from 2.5-month-old progeny showed no triploids among 12 fish from family 250 and none among 14 fish from family 249.

<div style="text-align:center">DISCUSSION</div>

Origin and cause of triploidy: The observation of six triploid individuals in a family of 11 adult rainbow trout is rather surprising, and suggests that the spontaneous frequency of triploid individuals may sometimes be very high in single-pair matings of lower vertebrates. FANKHAUSER (1945) reported that the proportion of triploid larvae in salamanders varied greatly among different matings; the highest proportion observed was eight spontaneous triploids in a family of 29. RICHARDS and NACE (1977) found that the proportion of diploid ova produced varied among females in *Rana pipiens* and could be as high as 35 percent in some cases.

Spontaneous triploids in fish probably result from the fertilization of an unreduced ovum with a normal sperm (CUELLAR and UYENO 1972; GOLD and AVISE 1976). This is probably also the mechanism for production of triploids in chickens (MONG *et al.* 1974; ZARTMAN and SMITH 1975) and salamanders (FANKHAUSER 1945). Triploidy in humans, however, is often the result of dispermy (BEATTY 1978; COUILLIN *et al.* 1978).

The water temperature at the U.C. Davis hatchery (10 to 11.6° the day that family 267 was fertilized) suggests that thermal shock was probably not the factor that led to production of the triploids. Although it is possible that the dam of family 267 had a genetic predisposition to produce unreduced ova, as discussed by CUELLAR and UYENO (1972), there were no triploids among the 26 progeny of the diploid females in family 267 that we examined chromosomally. Thus, there is no evidence at this point of a genetic tendency toward production of triploids in this family. However, this might still be identified in the next generation if the production of triploids was caused by homozygosity for a recessive allele leading to failure of extrusion of the second polar body in females. Some of the female progeny of the brother-sister mating of fish from family 267 (family 250) might be expected to produce high frequencies of triploid offspring in that case.

The high incidence of triploids in family 267 might have been related to the stage of maturation of the eggs of the dam. Studies in other animals have shown that production of triploids is related to the timing of ovulation and to egg ripeness (YAMAMOTO and INGALLS 1972; MONG *et al.* 1974; NIEBUHR 1974). LINCOLN, AULSTAD and GRAMMELTVEDT (1974) suggested that the stage of egg

maturation could be an important variable in attempts to artificially produce triploids in salmonids.

No evidence for 3n/2n chimerism or mosaicism: We found no evidence of diploid cells in any of the triploid trout on the basis of chromosome number and morphology. This type of variation within individuals is rare, but has been found in several mammalian species (CHU, THULINE and NORBY 1964; NES 1966; DUNN, McENTEE and HANSEL 1970; NIEBUHR 1974) and in chickens (BLOOM and BUSS 1966; ABDEL-HAMEED and SHOFFNER 1971).

However, we did find extensive variation in red blood cell nuclear volume within fish that chromosomally showed no evidence of mosaicism. ALLEN and STANLEY (1978) discussed possible examples of polyploid/diploid mosaicism in fish and proposed that, on the basis of red blood cell nuclear volumes, a number of sterile brook trout they studied were mosaics containing mainly triploid cells but also many cells of other ploidy levels. Our results raise questions about their conclusions and about the accuracy of this method of ploidy determination. Red blood cell size appears to be useful for detecting polyploid individuals, but may be of questionable value for detecting mosaicism within individuals.

Male-determining Y chromosome in trout: Two basic types of "switch mechanisms" for *XY* sex-determining systems are known (WHITE 1973). The "genic balance" mechanism is based on the ratio of *X* (or *Z*) chromosomes to autosomes and is found in Drosophila (BRIDGES 1925) and probably in chickens (ABDEL-HAMEED and SHOFFNER 1971). In forms with a "dominant *Y*" mechanism, the sex is determined by the presence or absence of the odd, *Y* or *W*, sex chromosome. The "dominant *Y*" mechanism is found in mammals (WELSHONS and RUSSELL 1959) and probably in many amphibians (FANKHAUSER and HUMPHREY 1950; BEATTY 1964).

The observation that *XXY* triploid trout are male suggests that the *Y* chromosome is male-determining in trout; these fish might be expected to be intersexes, as in Drosophila and chickens, if a "genic balance" sex-determining mechanism were operating. The switch mechanism apparently varies among other fish species; YAMAMOTO (1963) identified an apparent *XXY* male in the medaka, suggesting that the *Y* chromosome was male-determining in this species, but SWARUP (1957, in BEATTY 1964) found evidence suggesting that *ZWW* triploid sticklebacks were intersexes.

The H-Y antigen has been found to be present on the surface of cells of the heterogametic sex in several groups of animals (WACHTEL, KOO and BOYSE 1975) and has been proposed to be the primary determinant of sex (WACHTEL *et al.* 1975). Although male rainbow trout have a heteromorphic sex chromosome pair (THORGAARD 1977) and the *XXY* triploid results suggest that the *Y* chromosome is male determining in rainbow trout, SHALEV, BERCZI and HAMERTON (1978) found that the H-Y antigen is present on the surface of female, but not male, rainbow trout red blood cells. This apparent contradiction deserves further investigation.

Potential use of triploids in fish culture: We found no evidence that triploid trout are larger than diploids. PURDOM (1972), in flatfish, and VALENTI (1975),

in Tilapia, found that some triploid fish may grow faster than diploids, while SWARUP (1959a) observed that triploid sticklebacks were no larger than diploids. Triploid amphibians are usually no larger than diploids (FANKHAUSER 1945). Triploid rainbow trout appear to have good viability, as has been observed in triploids of other fish species (SWARUP 1959b; PURDOM 1972; VALENTI 1975) and amphibians (FANKHAUSER 1945).

Like triploids in flatfish (PURDOM 1972, 1976) and brook trout (ALLEN and STANLEY 1978), triploid rainbow trout appear to be sterile. The gonads in triploid males were much larger than those in triploid females. It seems likely that the disturbed meiotic pairing may have prevented maturation of the ovaries in triploids; the testes in the triploid males probably developed further because germ cells in male salmonids enter meiosis later than do those in females (ROBERTSON 1953). It is possible that the secondary signs of sexual maturation observed in the male, but not the female, triploids were the result of the greater gonad development in the males.

Trout and salmon undergo large losses in survival, growth and meat quality at the time of sexual maturation. Interest in the potential use of triploid salmonids in fish culture has been based on the assumption that these fish would be sterile and would not be subject to the production losses associated with sexual maturation. We found that triploid trout probably are reproductively sterile, but that triploid males, at least, may show external signs of sexual maturation. Our observations on fish G176, a female triploid, however, did show that some triploids maintain growth and meat quality better than normal fish at the time of sexual maturation. Triploid salmonids, especially females, might potentially be useful in fish culture and management if consistent methods for producing them are developed.

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