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Production of Triploids and their Reproductive Capacity in *Rana rugosa*

By

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ABSTRACT

Of the 1209 eggs of *Rana rugosa* refrigerated after insemination with sperm of the same species, 832 (68.8%) cleaved normally, and 338 (28.0%) became normally feeding tadpoles. By counting the chromosomes of these tadpoles, it was found that 254 (82.5%) were triploids (3n=39), seven were diploids, eight were mosaics, and the remaining 39 were uncertain in ploidy. Of the triploid tadpoles, 242 became normally metamorphosed frogs and 11 of them were reared until sexual maturity. No significant differences were observed between these triploids and the control diploids in development and growth rate. All the 211 triploid frogs were males or hermaphrodites which were transforming into males.

Of the 2272 eggs from four field-caught females mated with eight of the 11 triploid males, 360 (15.8%) cleaved normally and 136 (6.0%) became normally feeding tadpoles. Only one of these tadpoles completed metamorphosis, while none of the eggs of the same four females mated with the other three triploid males cleaved normally. The chromosomes were counted in 81 tadpoles produced from four diploid females mated with five triploid males. The results showed that 79 of them were aneuploids between diploid and triploid, and the remaining two were diploids.

INTRODUCTION

In a previous paper (1980), the present author reported on three mature haploid females which were gynogenetically produced from eggs by insemination with UV-irradiated sperm in *Rana rugosa*. One of these females laid eggs spontaneously, while in another female the ovulation occurred by pituitary injection. The eggs obtained from the latter female cleaved normally and became blastulae. However, all of them died at the late blastula stage. The author also reported on mature gynogenetic diploids produced by refrigeration of eggs after insemination with UV-irradiated sperm in the same species (KASHIWAGI, 1993). Of these gynogenetic diploids, two and two of the four females were completely and partially fertile, respectively, while four and two of the six males were completely and partially fertile, respectively.

It appeared interesting to the present author to produce triploids in *Rana rugosa* and to compare them with gynogenetic haploids and diploids in reproductive capacity. Although numerous tripoids have been discovered or produced by

various methods in urodeles and anurans by numerous investigators, only a comparatively small number of studies have been reported hitherto on mature triploids.

In urodeles, mature triploids have been obtained in *Triturus alpestris* by FISCHBERG (1945, 1948), in *Ambystoma mexicanum* by HUMPHREY and FANKHAUSER (1949) and FANKHAUSER and HUMPHREY (1954), in *Cynops pyrrhogaster* by KAWAMURA (1951a) and in *Pleurodeles waltl* by BEETSCHEN (1960). In anurans, on the other hand, they have been produced in *Rana nigromaculata* by KAWAMURA (1941a, b, 1951b), in *R. nigromaculata* and *R. brevipoda* by KAWAMURA, NISHIOKA and OKUMOTO (1983), in *Bombina orientalis* by UEDA (1980) and in *Hyla japonica* by NISHIOKA and UEDA (1983). KAWAMURA (1951a, b) obtained offspring of triploid males mated with diploid females in *C. pyrrhogaster* and *R. nigromaculata,* FANKHAUSER and HUMPHREY (1954) in *A. mexicanum,* GALLIEN and BEETSCHEN (1959) and BEETSCHEN (1960) in *P. waltl,* UEDA (1980) in *B. orientalis,* and NISHIOKA and UEDA (1983) in *H. japonica.*

It is the purpose of the present study to describe the viability, sex and reproductive capacity of triploids in *Rana rugosa.* Some characteristics of the offspring of triploid males mated with diploid females will be also described.

MATERIALS AND METHODS

Male and female *Rana rugosa* SCHLEGEL were collected from the environs of Hiroshima City. Ovulation was accelerated by injecting bullfrog pituitaries into the body cavity of mature females. Triploids were produced by exposing eggs to low temperature of 0~2°C for two hours, 20 minutes after insemination, in order to suppress extrusion of the second polar body (NISHIOKA, 1972).

Embryos and tadpoles were allowed to develop at room temperature in 18 cm petri dishes containing dechlorinated tap-water. When they reached SHUMWAY's stage 25, the tadpoles were transferred to enameled pans, 55×31×22 cm in size, and reared there until TAYLOR and KOLLROS' stage V. At this stage, their chromosome numbers were counted by the squash method after water pretreatment as described by NISHIOKA (1972). Thereafter, the tadpoles were raised outdoors in concrete aquaria, 95×65×20 cm in size. Tadpoles were fed on boiled spinach, while metamorphosed frogs were fed on crickets. The gonads of frogs were fixed in NAVASHIN's fluid, sectioned at 12 μ and stained with HEIDENHAIN's hematoxylin.

OBSERVATION

I. Production of triploids

1. Developmental capacity

The eggs of 10 females (W♀, Nos. 1~10) were inseminated with sperm of 10

males (W♀, Nos. 1~10) and 20 minutes later they were refrigerated at 0~2°C for two hours. It was found that 832 (68.8%) of the 1209 eggs in the experimental series cleaved normally and then 672 (55.6%) eggs hatched normally. In the control series, 757 (88.0%) of the 860 eggs cleaved normally and 740 (86.0%) hatched normally (Table 1). In the experimental series, 338 (28.0%) became normally feeding tadpoles, while in the control series, 603 (70.1%) attained the same stage.

Chromosome numbers were counted in the 308 tadpoles of the experimental series at stage V. The results showed that 254 (82.5%) of them were triploids, seven (2.3%) were diploids, and eight (2.6%) were four kinds of mosaics, including one haploid-tetraploid, four diploid-triploids, one diploid-tetraploid and two tetraploid-pentaploids. The remaining 39 tadpoles were uncertain in chromosome number. In the control series, chromosome numbers were counted in 84 of the 603 tadpoles at stage V. It was found that 82 (97.6%) tadpoles were diploids and the other two were uncertain (Table 1; Fig. 1). Of the 254 triploids, 247 climbed out of water between 56 and 115 days, 73.8 days on the average, while all the 603 normally feeding tadpoles in the control series did so between 61 and 110 days, 75.8 days on the average. In the experimental series, eventually 242 individuals attained completion of metamorphosis. This number corresponds to 20.0% of all the eggs refrigerated after insemination and to 29.1% of the normally

TABLE 1
Developmental capacity of eggs refrigerated after insemination and the ploidy of the tadpoles produced from them

Series	No. of eggs	No. of normally cleaved eggs	No. of normal neurulae	No. of normally hatched tadpoles	No. of normally feeding tadpoles	Ploidy of tadpoles				No. of metamorphosed frogs
						Total	2n	3n	Other	
Control	860	757 (88.0%)	740 (86.0%)	740 (86.0%)	603 (70.1%)	84	82	—	2	599 (69.7%)
Experimental	1209	832 (68.8%)	680 (56.2%)	672 (55.6%)	338 (28.0%)	308	7	254	47	242 (20.0%)

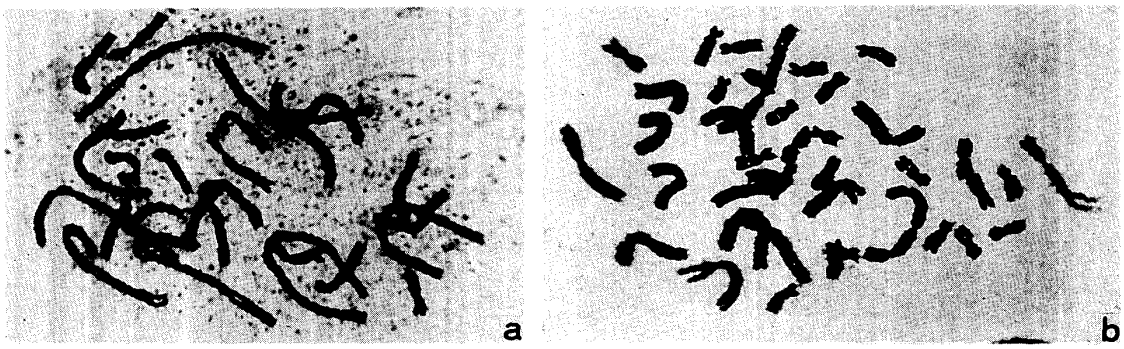

Fig. 1. Metaphase plates in epidermal cells of the tail-tips of a triploid and the control diploid tadpoles. ×1000

a. Control diploid tadpole

b. Triploid tadpole

cleaved ones. In the control series, 599 individuals attained completion of metamorphosis. This number corresponds to 69.7% of the inseminated eggs and to 79.1% of the normally cleaved ones (Table 1).

2. Sex ratio and the structure of gonads

The gonads and kidneys of triploid frogs and the controls were observed at the stage soon after metamorphosis and at the age of one year (Figs. 2 and 3). Table 2 shows the sex ratio of these frogs. While the sex of the control *Rana rugosa* was already differentiated at the stage immediately after metamorphosis, there were several hermaphrodites in the triploids at this stage. Of the 200 juvenile triploids whose sex was examined at the stage soon after metamorphosis, 193 were normal males (♂) and seven were juvenile hermaphrodites, including five of type 1 (⚥1), one of type 2 (⚥2), and one of type 3 (⚥3). The hermaphrodites of type 1 have

TABLE 2
Sex of triploids and the controls

Series	Juvenile frogs						Mature frogs			All frogs examined			
	Total	♀	⚥1	⚥2	⚥3	♂	Total	♀	♂	Total	♀	♂	(%)
Control	488	255	0	0	0	233	21	11	10	509	266	243	(47.7)
Experimental	200	0	5	1	1	193	11	0	11	211	0	211	(100.0)

⚥, Hermaphrodite with gonads transforming from ovaries into testes

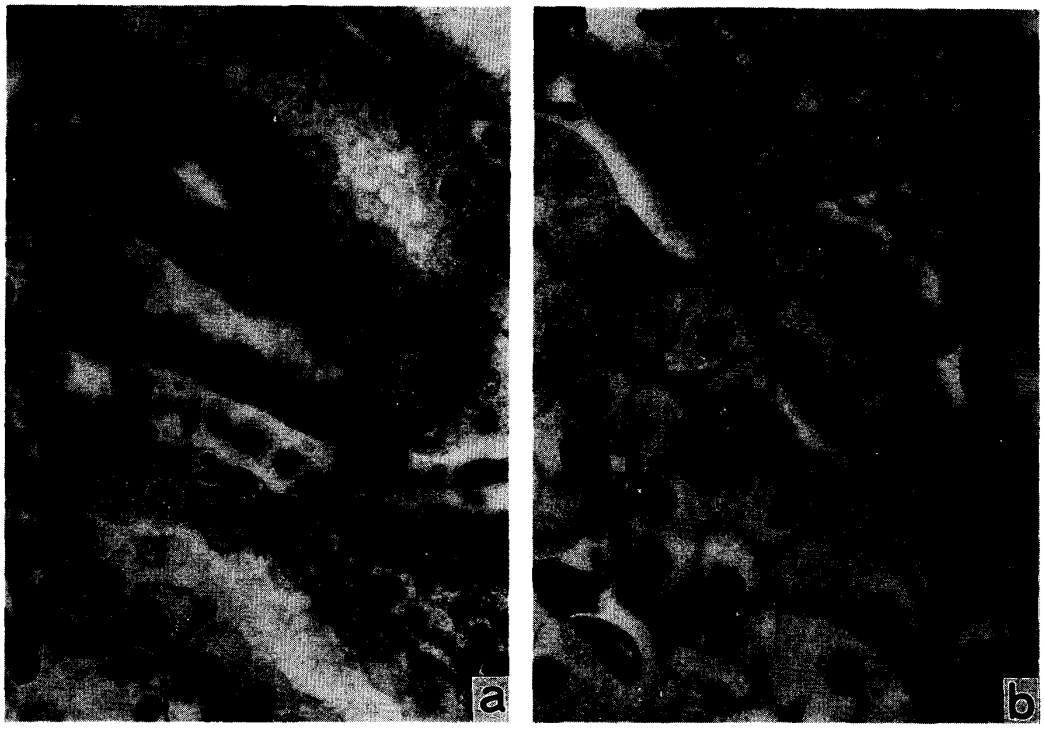

Fig. 2. Cross-sections of the kidneys of a triploid male and the control diploid male frogs. ×840
a. Control diploid frog
b. Triploid frog

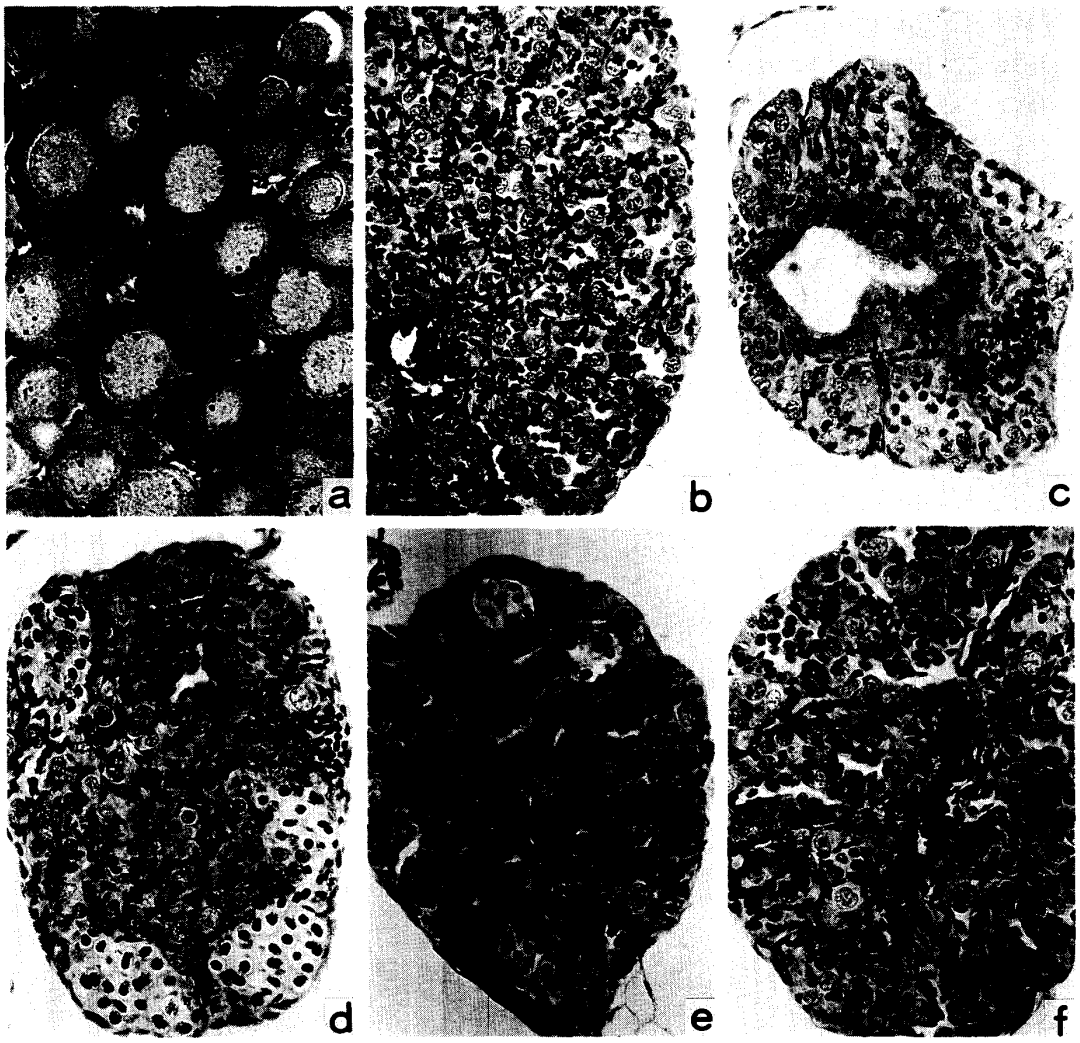

Fig. 3. Cross-sections of the gonads of triploid frogs and the control diploid frogs shortly after completion of metamorphosis. ×170

a. Normal ovary (♀N) of the control diploid female frog
b. Normal testis (♂N) of the control diploid male frog
c. Hermaphroditic gonad (⚥1) of a triploid frog
d. Hermaphroditic gonad (⚥2) of a triploid frog
e. Hermaphroditic gonad (⚥3) of a triploid frog
f. Normal testis (♂N) of a triploid male frog

gonads showing multiplication of rete cells in the medullary portions and abundant oogonia and young oocytes in the cortical portions. The hermaphrodites of type 2 have gonads showing testicular structure in the medullary portions, while the wide areas are still of the ovarian structure in the outer cortical portions. The hermaphrodites of type 3 have testes as a whole, in spite of the presence of some small groups of oocytes. Normal males, on the other hand, have typical testes, although some have a few testis-ova (KAWAMURA and NISHIOKA, 1972). In the control series, there were 255 females and 233 males (Fig. 3a~f). In the experimental and control series, 11 triploid and 21 diploid frogs were further reared, respectively. At the age of one year, all the triploids were males, while 11 of the

21 control diploids were females and 10 were males. When the hermaphrodites were counted as males, all the 211 juvenile and adult triploid frogs were males. In contrast, there were 266 females and 243 (47.7%) males in the 509 juvenile and adult control frogs (Table 2).

II. Reproductive capacity

1. Testes of triploid males

Eleven one-year-old triploid males (3n♂, Nos. 1~11) produced by refrigerating eggs after insemination attained sexual maturity in the next year and together with a field-caught male (W♂, No. 5) were mated with four field-caught females (W♀, Nos. 1~4) to produce offspring (Fig. 4). The left and right testes of each of the 11 triploids were used for histological observation and insemination, respectively. The field-caught male was 35.2 mm in body length. The testes measured 3.1 mm and 3.2 mm in length and 2.1 and 2.2 mm in width (Table 3). The eleven male triploids (Nos. 1~11) were 31.0~41.2 mm, 36.3 mm on the average, in body length. Testes were 2.2~4.1 mm, 3.2 mm on the average, in length, and 1.3~2.6 mm, 2.2 mm on the average, in width.

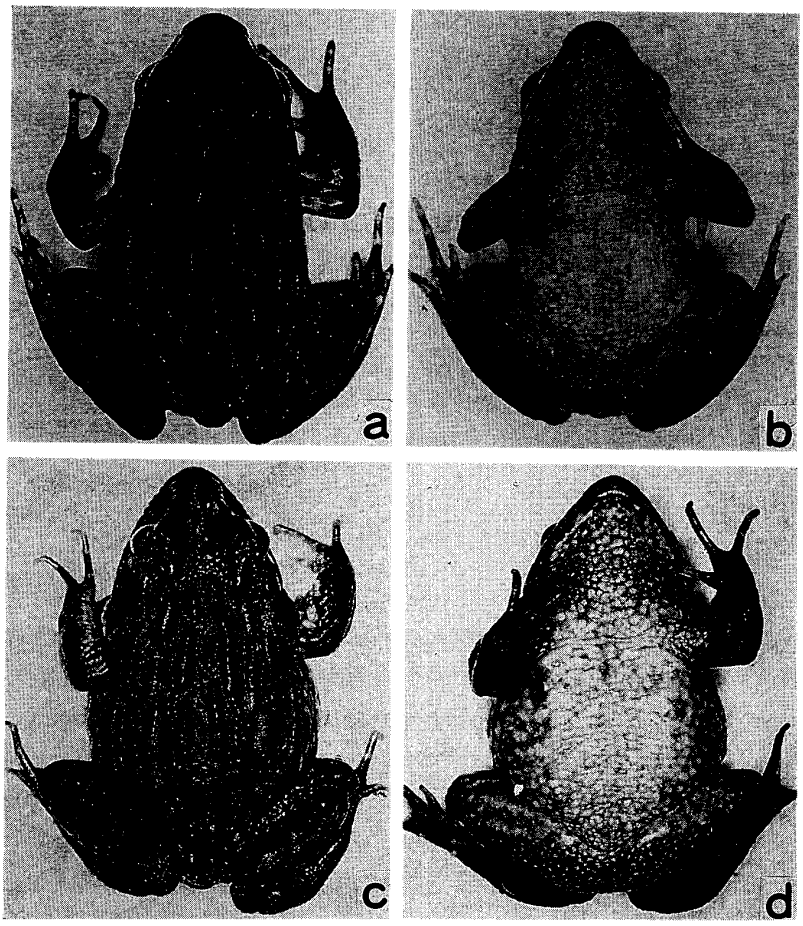

Fig. 4. A triploid male and the control diploid male frogs at the age of one year. ×1.3
a, b. Control diploid male, W, No. 5
c, d. Triploid male, 3n, No. 4

TABLE 3
Size of the testes of mature triploid males

Series	Individual no.	Body length (mm)	Size of testes	
			Right (mm)	Left (mm)
Control	W, No. 5	35.2	3.1×2.2	3.2×2.1
Experimental	3n, No. 1	35.9	4.0×2.0	4.1×2.1
	3n, No. 2	37.0	3.2×2.4	3.0×2.4
	3n, No. 3	39.5	3.0×2.5	3.1×2.4
	3n, No. 4	35.0	3.5×2.5	3.3×2.6
	3n, No. 5	34.0	3.0×2.5	3.2×2.5
	3n, No. 6	41.2	3.8×2.2	3.6×2.4
	3n, No. 7	37.7	3.4×2.3	3.4×2.5
	3n, No. 8	38.0	3.9×2.4	3.9×2.0
	3n, No. 9	35.0	2.2×1.5	2.3×1.5
	3n, No. 10	35.0	2.8×2.0	2.9×2.1
	3n, No. 11	31.0	2.5×1.3	2.7×1.3

The testes of the field-caught male were quite normal in inner structure. The cavities of seminiferous tubules were almost filled with bundles of normal spermatozoa. There were abundant first and second spermatocytes along the inner walls of the tubules (Fig. 5a). All the testes of 11 one-year-old triploid males were characteristic of triploid testes and very similar to each other in inner structure (Fig. 5b~e). Seminiferous tubules contained a small number of abnormal spermatozoa, most of which were distinctly thicker and obtuser than the normal spermatozoa of diploid males. The cavities of the seminiferous tubules were surrounded with abundant first spermatocytes which were evidently larger than those of diploid males. While there were comparatively numerous figures of first meiotic divisions, the second spermatocytes and spermatids were scarcely found. One (No. 4) of the 11 one-year-old triploid males contained a comparatively numerous spermatozoa in the seminiferous tubules, although nearly all of them were abnormal in size and shape (Fig. 5b).

2. Developmental capacity of offspring

a. Control series, W♀, Nos. 1~4×W♂, No. 5

Four control matings were made between four field-caught females, W♀, Nos. 1~4, and a field-caught male, W♂, No. 5 (Table 4). The results showed that 58.1~72.7% of the eggs, 207 (67.4%) of the 307 eggs in total, cleaved normally. While 13 embryos died of abnormalities before the hatching stage, 53.8~64.9%, 188 (61.2%) in total, became normally feeding tadpoles. Eventually, 45.2~62.3%, 171 (55.7%) in total, became normally metamorphosed frogs (Table 4).

b. Experimental series, W♀, Nos. 1~4×3n♂, Nos. 1~11

The same four field-caught females as used in the control series were mated with

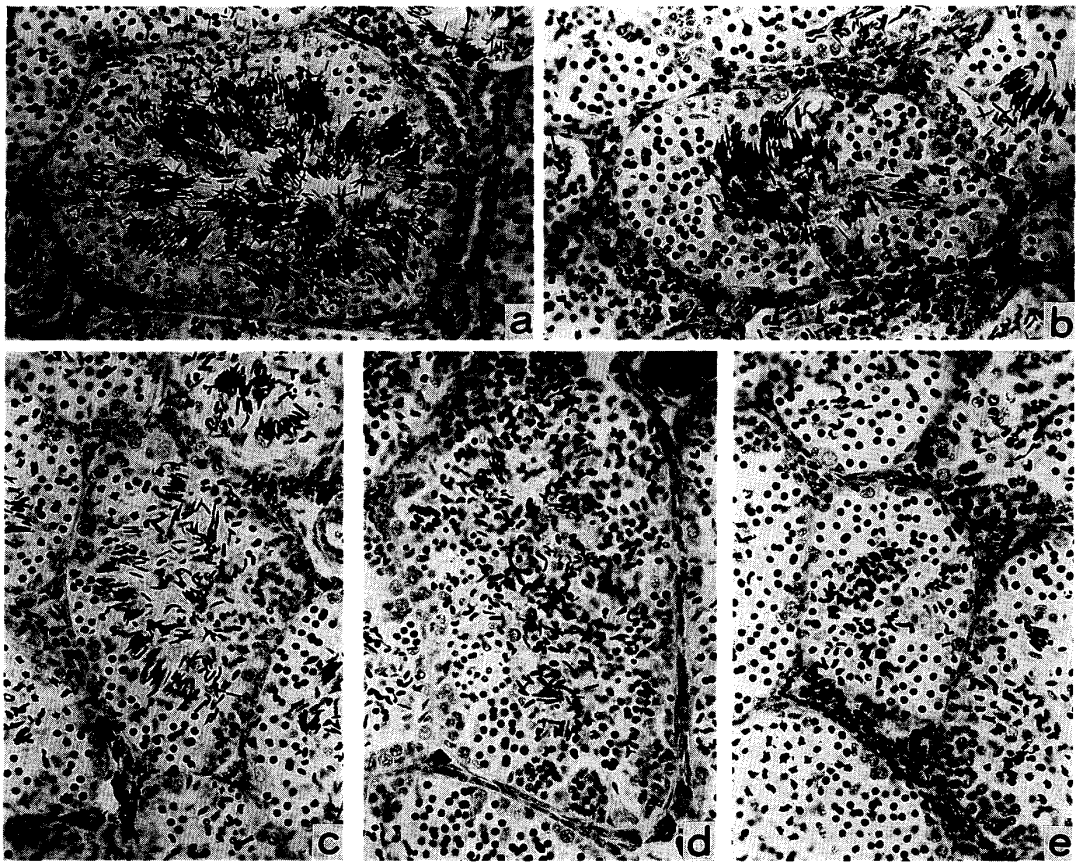

Fig. 5. Cross-sections of the testes of mature triploid male and the control diploid male frogs at the age of one year. ×170

a. Testis of type 1 of the control diploid male, W, No. 5
b. Testis of type 2 of a triploid male, 3n, No. 4
c. Testis of type 3 of a triploid male, 3n, No. 9
d. Testis of type 4 of a triploid male, 3n, No. 2
e. Testis of type 5 of a triploid male, 3n, No. 6

11 one-year-old triploid males, 3n♂, Nos. 1~11 (Table 4). The results showed that none of the eggs of the four females cleaved normally by insemination with sperm of the three triploid males, Nos. 3, 6 and 11. On the other hand, three (1.7%) of the 177 eggs of females W, Nos. 1 and 2, three (1.4%) of the 219 eggs of females W, Nos. 1 and 2, 292 (64.2%) of the 455 eggs of females W, Nos. 1 and 2, 10 (2.4%) of the 412 eggs of females W, Nos. 1 and 2, four (1.9%) of the 211 eggs of females W, Nos. 3 and 4, six (1.9%) of the 309 eggs of females W, Nos. 3 and 4, 28 (12.7%) of the 221 eggs of females W, Nos. 3 and 4 and 14 (5.2%) of the 268 eggs of females W, Nos. 3 and 4 cleaved normally by insemination with sperm of triploid males, Nos. 1, 2, 4, 5, 7, 8, 9 and 10, respectively. A total of 360 (15.8%) of the 2272 eggs obtained from four females cleaved normally. Of the normally cleaved eggs, three from triploid male No. 1, two from triploid male No. 2, five from triploid male No. 4, one from triploid male No. 5, three from triploid male No. 7, four from triploid male No. 8, three from triploid male No. 9 and one from triploid male No. 10 became abnormal and died before the tail-bud stage, and one

TABLE 4
Reproductive capacity of triploid males

Parents		No. of eggs	No. of normally cleaved eggs	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally feeding tadpoles	No. of metamorphosed frogs
Female	Male						
W, No. 1	W, No. 5	77	56 (72.7%)	56 (72.7%)	50 (64.9%)	50 (64.9%)	48 (62.3%)
W, No. 2		75	54 (72.0%)	51 (68.0%)	51 (68.0%)	48 (64.0%)	46 (61.3%)
W, No. 3		62	43 (69.4%)	43 (69.4%)	43 (69.4%)	40 (64.5%)	35 (56.5%)
W, No. 4		93	54 (58.1%)	54 (58.1%)	50 (53.8%)	50 (53.8%)	42 (45.2%)
W, No. 1	3n, No. 1	77	0	—	—	—	—
W, No. 2		100	3 (3.0%)	0	—	—	—
W, No. 1	3n, No. 2	122	1 (0.8%)	1 (0.8%)	0	—	—
W, No. 2		97	2 (2.1%)	0	—	—	—
W, No. 1	3n, No. 3	167	0	—	—	—	—
W, No. 2		152	0	—	—	—	—
W, No. 1	3n, No. 4	228	152 (66.7%)	150 (65.8%)	117 (51.3%)	54 (23.7%)	1 (0.4%)
W, No. 2		227	140 (61.7%)	137 (60.4%)	121 (53.3%)	57 (25.1%)	0
W, No. 1	3n, No. 5	242	3 (1.2%)	3 (1.2%)	3 (1.2%)	3 (1.2%)	0
W, No. 2		170	7 (4.1%)	6 (3.5%)	6 (3.5%)	1 (0.6%)	0
W, No. 3	3n, No. 6	98	0	—	—	—	—
W, No. 4		88	0	—	—	—	—
W, No. 3	3n, No. 7	139	3 (2.2%)	0	—	—	—
W, No. 4		72	1 (1.4%)	1 (1.4%)	1 (1.4%)	0	—
W, No. 3	3n, No. 8	161	0	—	—	—	—
W, No. 4		148	6 (4.1%)	2 (1.4%)	2 (1.4%)	2 (1.4%)	0
W, No. 3	3n, No. 9	117	15 (12.8%)	12 (10.3%)	12 (10.3%)	7 (6.0%)	0
W, No. 4		104	13 (12.5%)	13 (12.5%)	12 (11.5%)	9 (8.7%)	0
W, No. 3	3n, No. 10	118	2 (1.7%)	2 (1.7%)	2 (1.7%)	0	—
W, No. 4		150	12 (8.0%)	11 (7.3%)	11 (7.3%)	3 (2.0%)	0
W, No. 3	3n, No. 11	118	0	—	—	—	—
W, No. 4		161	0	—	—	—	—
W, Nos. 1～4	W, No. 5	307	207 (67.4%)	204 (66.4%)	194 (63.2%)	188 (61.2%)	171 (55.7%)
W, Nos. 1～4	3n, Nos. 1～11	3056	360 (11.8%)	338 (11.1%)	287 (9.4%)	136 (4.5%)	1 (0.03%)

from triploid male No. 2, 49 from triploid male No. 4 and one from triploid male No. 9 became abnormal before the hatching stage. Eventually, 238 (52.3%) embryos from triploid male No. 4, nine (2.2%) embryos from triploid male No. 5, one (0.5%) embryo from triploid male No. 7, two (0.6%) embryos from triploid male No. 8, 24 (10.9%) embryos from triploid male No. 9 and 13 (4.9%) embryos from triploid male No. 10 hatched normally. Of these tadpoles, 127 from triploid male No. 4, five from triploid male No. 5, one from triploid male No. 7, eight from triploid male No. 9 and 10 from triploid male No. 10 became edematous or revealed various abnormalities and died without taking food, while 111 (24.4%) from triploid male No. 4, four (1.0%) from triploid male No. 5, two (0.6%) from triploid male No. 8, 16 (7.2%) from triploid male No. 9 and three (1.1%) from triploid male No. 10 became normally feeding tadpoles. However, the great majority of these tadpoles were ill-developed and gradually died before metamorphosis, and eventually, a single tadpole obtained from triploid male No. 4 attained completion of metamorphosis (Table 4).

3. Chromosome numbers of offspring

When chromosomes were counted in 28 normal tadpoles produced from four control matings between four field-caught females (W♀, Nos. 1~4) and a field-caught male (W♂, No. 5), it was found that 26 were diploids (Table 5).

In the experimental series, chromosomes of 81 tadpoles produced from seven matings between four females, W♀, Nos. 1~4, and five triploid males, 3n♂, Nos. 4, 5, 8, 9 and 10, were counted. All the tadpoles were somewhat abnormal in external features. The results of chromosome counts showed that two were diploids, 61 were hyperdiploids and the remaining 18 were hypotriploids. Of the 61 hyperdiploids, one was 27 (2n+1), one was 28 (2n+2), 11 were 29 (2n+3), 12 were 30 (2n+4), 23 were 31 (2n+5) and 13 were 32 (2n+6) in chromosome number. Of the 18 hypotriploids, nine were 33 (3n-6), three were 34 (3n−5),

TABLE 5

Number of offspring having different chromosome numbers in the experimental series, 2n♀×3n♂, and the control

Series	Parents		Number of chromosomes							
	Female	Male	Total	26 (2n)	27	28	29	30~32 (2n+)	33~38 (3n−)	39 (3n)
Control	W, Nos. 1~4	W, No. 5	26	26						
Experimental	W, No. 1	3n, No. 4	32	1			5	19	7	
	W, No. 2	3n, No. 4	30	1		1	5	18	5	
	W, No. 1	3n, No. 5	3					2	1	
	W, No. 4	3n, No. 8	2		1				1	
	W, No. 3	3n, No. 9	5					2	3	
	W, No. 4	3n, No. 9	7				1	6		
	W, No. 4	3n, No. 10	2					1	1	

two were 35 (3n−4), one was 36 (3n−3) and three were 38 (3n−1) in chromosome number (Table 5).

DISCUSSION

Since FANKHAUSER (1940) observed the structures of the gonads in four triploid *Notophthalmus viridescens* which were reared until metamorphosis, the sex differentiation of triploid amphibians has been reported by many investigators, including BÖÖK (1940) in *Triturus vulgaris*, GRIFFITHS (1941) in *Notophthalmus viridescens*, FISCHBERG (1945, 1948) in *Triturus alpestris*, HUMPHREY and FANKHAUSER (1946), and FANKHAUSER and HUMPHREY (1954) in *Ambystoma mexicanum*, KAWAMURA (1940, 1941a, b, 1949, 1951a) in *Cynops pyrrhogaster*, *Rana nigromaculata* and *Rana japonica*, KAWAMURA and SANADA (1943) in *Cynops pyrrhogaster*, HUMPHREY, BRIGGS and FANKHAUSER (1950) in *Rana pipiens*, KAWAMURA and TOKUNAGA (1952) in *R. japonica*, NISHIOKA (1971), and KAWAMURA, NISHIOKA and OKUMOTO (1983) in *R. nigromaculata* and *R. brevipoda*, SATO (1952) in *Rana limnocharis*, MUTO (1952) in *Bufo japonicus*, UEDA (1980) in *Bombina orientalis*, and NISHIOKA and UEDA (1983) in *Hyla japonica*.

Triploids produced by cold treatment of fertilized eggs in *R. japonica* were all males (KAWAMURA and TOKUNAGA, 1952). In contrast, almost all triploid individuals of *B. japonicus* raised from cold-treated or heat-shocked eggs were females immediately after metamorphosis (MUTO, 1952). NISHIOKA and UEDA (1983) found that mature triploid frogs of *H. japonica* likewise included an excessive number of males, that is, 50 males of the 70 triploids. Nearly an equal number of males and females was reported in triploids of *R. nigromaculata* and *R. brevipoda* (KAWAMURA and NISHIOKA, 1967). A similar observation was made on mature triploid frogs of *B. orientalis* by UEDA (1980).

KAWAMURA (1941a, b) obtained 43 *Rana nigromaculata* triploid frogs by cold treatment of fertilized eggs and reported that only five of these frogs were females, while the other 38 were males. KAWAMURA pointed out that the preponderance of triploid males is due to overripeness of the eggs of one mother frog used for his experiments, as this female was collected about 50 days after the height of the breeding season and fertilization was made at 20°C. In fact, the diploid offspring of the same mother frog likewise were largely males. Triploid frogs developed from the eggs of three other mother frogs however, included both sexes in equal numbers. In the present study, all the 211 triploid frogs of *Rana rugosa* were males or hermaphrodites destined to transform into males, in contrast to the diploid controls which consisted of 266 females and 243 males. This result seems to exclude the overripeness of the eggs used to produce the triploids. NISHIOKA, MIURA and SAITOH (1993) suggested the male heterogamety of *R. rugosa* distributed in the Hiroshima district, as half of the males raised from tadpoles which had been injected with testosterone propionate produced only females by mating with normal females, while the other males produced both females and males in nearly an equal number. The male heterogamety was supported by two observa-

tions, (1) females were much more frequent than males among gynogenetically produced diploids, in contrast to the control frogs in which the sex ratio was approximately 1:1 and (2) gynogenetic diploid males produced exclusively female offspring when mated with normal females (KASHIWAGI, 1993). The triploidy obtained from refrigerated eggs is believed to result from retention of the second polar body. Accordingly, it is highly probable that in *R. rugosa*, triploid females are XXX and males are XXY in sex chromosome constitution. HUMPHREY, BRIGGS and FANKHAUSER (1950) noted that in *Rana pipiens* triploids at metamorphosis usually appeared to be males, owing to the sex reversal of genetic females. The triploids of *R. rugosa* resembled those of *R. pipiens* which were all males. According to KASHIWAGI (1980), all the gynogenetic haploids of *R. rugosa* were genetic females having X chromosomes and sex reversal often occurred in these females. Thus, it seems evident that the triploid males in *R. rugosa* contained sex reversed genetic females.

Concerning the reproductive capacity of triploids in amphibians, mature triploid males and females were not completely sterile in the axolotl and *C. pyrrhogaster*. The overwhelming majority of the offspring between diploids and triploids were intermediate between the diploid and triploid in chromosome number (HUMPHREY and FANKHAUSER, 1946, 1949; KAWAMURA, 1951a; FANKHAUSER and HUMPHREY, 1954). Fischberg (1945, 1948) reported that in mature *T. alpestris* female triploids were sterile, and the triploid males did not emit normal spermatophores. Numerous offspring were obtained from triploid males of *Pleurodeles waltl* by mating with diploid females, although they were non-viable because of their aneuploidy in chromosome number (GALLIEN and BEETSCHEN, 1959; BEETSCHEN, 1960).

In anurans, no offspring were produced from triploid females of *R. nigromaculata* by mating with diploid males (KAWAMURA, 1941a, b), while triploid males mated with diploid females produced embryos and tadpoles which were aneuploids and lethal (KAWAMURA, 1951b). In *R. nigromaculata* and *R. brevipoda*, NISHIOKA (1971) noted that triploid females were completely sterile, although a few eggs of diploid females fertilized with sperm of triploid males could develop until the stage before the hatching stage. However, KAWAMURA, NISHIOKA and OKUMOTO (1983) reported later that triploid females of these two species produced a number of diploids, triploids, tetraploids and aneuploids at the completion of metamorphosis by mating with diploid males. NISHIOKA and UEDA (1983) obtained the offspring of triploid males and females in *Hyla japonica*. The matings between triploid females and diploid males produced offspring which were diploids, triploids, tetraploids and aneuploids. A considerable number of these offspring completed metamorphosis. Of four metamorphosed aneuploid frogs, one having 25 (2n+1) chromosomes attained sexual maturity. The matings between diploid females and triploid males produced mostly aneuploid frogs between the diploid and triploid, and no tetraploid frogs were produced. Nine of 46 aneuploid tadpoles completed metamorphosis and a single aneuploid having 25 chromosomes finally attained sexual maturity. UEDA (1980) reported that eggs of diploid females of *B. orientalis* inseminated with sperm of triploid males developed into a few abnormal

tadpoles which were hyperdiploids and died before attaining the metamorphosis.

Of the 2272 eggs from four field-caught females of *R. rugosa* mated with eight of the 11 triploid males obtained in the present study, 360 cleaved normally and 136 became normally feeding tadpoles. However, only one of them could complete metamorphosis. From matings of the same four females with the other three triploid males, no normally cleaved eggs were obtained. Of 81 tadpoles produced from four diploid females mated with five of the above eight triploid males, 79 were aneuploids which were between diploids and triploids and died before metamorphosis. The remaining two were diploids and one of them completed metamorphosis.

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