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# Studies on Polyploidy in Japanese Treefrogs

By

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(With 9 Text-figures)

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## INTRODUCTION

The existence of natural polyploids in the genus *Hyla* was first reported by WASSERMAN (1970). He described that *Hyla chrysoscelis* (2n=24) and *Hyla versicolor* (2n=48) of the *Hyla versicolor* complex distributed widely in eastern North America are diploid and tetraploid in chromosome number, respectively. According to JOHNSON (1966), these two species are very similar in appearance and only distinguished by the trill rate of the male's call. While *Hyla chrysoscelis* is of fast call-type, *Hyla versicolor* is of slow call-type. They fairly differ in distribution area. While *Hyla versicolor* are distributed in the northern part of the eastern North America, *Hyla chrysoscelis* are in the southern part, although they are sympatric in many districts. RALIN (1968) has confirmed that the two species are ecologically different from each other. *Hyla chrysoscelis* tends to call from trees and bushes in contrast to *Hyla versicolor* which usually calls from the ground. The stomach contents of both species at one locality indicated that *Hyla chrysoscelis* was usually eating arboreal insects, while *Hyla versicolor* was usually eating terrestrial insects.

After diploid-tetraploid species pair of treefrogs was discovered by WASSERMANN (1970), this has been studied by BOGART and WASSERMANN (1972), RALIN (1976, 1977, 1978), MAXSON, PEPPER and MAXSON (1977), RALIN and ROGERS (1979), RALIN and SELANDER (1979), and WILEY (1982) principally in order to elucidate its origin. BOGART and WASSERMANN (1972) postulated that tetraploidy could arise independently in the diploid species through an intermediate triploid stage. While hybrids obtained by RALIN (1976) from matings between female *Hyla versicolor* and male *Hyla chrysoscelis* were triploids, they revealed reduced viability. He explained the poor viability of triploids to be ascribable to triple doses of each gene and consequent genetic imbalance. DANZMANN and BOGART (1982) examined gene dosage effects on MDH isozyme expression in diploid *Hyla chrysoscelis,* tetraploid *Hyla versicolor* and triploid hybrids obtained from reciprocal crossings of the two species.

The present authors considered that genus *Hyla* might be an animal being prone to produce polyploids, because a bisexual tetraploid species exists in the field and, moreover, triploid hybrids can be produced from matings between the diploid and tetraploid species. Then, they made some experiments in order to obtain triploids and tetraploids in the Japanese treefrog, *Hyla arborea japonica* GÜNTHER. The results of these experiments may contribute toward elucidating the origin of the spontaneous tetraploid anuran species in eastern North America as well as those found in South America, Africa and Kirghizia, the Soviet Union.

## MATERIALS AND METHODS

In the present studies, both field-caught and laboratory-stocked treefrogs, *Hyla arborea japonica* GÜNTHER, were used. Three mature females and seven mature males were collected from the suburbs of Hiroshima in 1979, 1980 and 1981. Two wild-type females and one albinic female used as materials were produced by the following procedures.

(1) In 1976, 16 wild-type females and 15 wild-type males were produced by matings between two female colored-albinos (Ym II. C-Alb♀, Nos. 1 and 2) collected in 1975 from Yoshiki, Yamaguchi Prefecture and a male albino (Kk.Alb ♂, No. 3) derived from albinos collected in 1970 from Kake, Hiroshima Prefecture (cf. Nishioka and Ueda, 1977, p. 150, Table 24).

(2) In 1978, from backcrossing of one of the above 16 wild-type females obtained by matings, Ym II.C-Alb♀ × Kk.Alb♂, with a male colored-albino (Ym II.C-Alb) collected in 1975 from Yoshiki, Yamaguchi Prefecture, wild-type (YK.Y, W), red-eyed albinic (YK.Y, Alb) and colored-albinic (YK.Y, C-Alb) treefrogs were produced at the rate of 2 : 1 : 1. Two females of the wild-type treefrogs (YK.Y, W♀, Nos. 1 and 2: $f^cF$ or $fF$) and a colored-albinic female (YK.Y, C-Alb♀, No. 3: $f^cf$) were used in the present studies.

Ovulation was accelerated by injecting a mixture of a pituitary body of *Rana catesbeiana* and 100 units of gonadotropin (Gonatropin, Teikokuzoki Co.) as an injection of 0.50 or 0.25 cc into each female. Fertilization was always made by the routine artificial method.

Triploids were produced by suppressing extrusion of the second polar body by refrigerating eggs for one hour at 2～3°C, 20 minutes after insemination. The chromosomes of tadpoles were observed in the tail-tips by the water-pretreatment squash method (Nishioka, 1972). However, colchicine was not applied to the tadpoles, as it was necessary to rear them continuously after examining their chromosomes. The chromosomes of adults were observed by the blood culture method and the bone marrow method (Volpe and Gebhardt, 1968; Omura, 1967).

Tadpoles were fed on boiled spinach or chard, while metamorphosed treefrogs were fed on crickets. Embryos were kept in glass dishes which were 18 cm in diameter, 6 cm in depth and contained dechlorinated tap-water until they hatched and became feeding tadpoles. After their chromosomes were examined, they were usually reared outdoors in concrete aquaria (90 cm × 60 cm × 20 cm).

The testes of mature treefrogs were fixed in Navashin's fluid, sectioned at 12$\mu$ and stained with Heidenhain's iron hematoxylin.

## OBSERVATION

### *I. Production of triploids from fertilized eggs by refrigeration*

#### 1. Developmental capacity

The eggs of two wild-type females (YK. Y, W♀, Nos. 1 and 2) and a colored-albinic female (YK. Y, C-Alb♀, No. 3) stated above were inseminated with sperm of a male (F 79♂, No. 1) collected from the field in 1979. These eggs were refrigerated at 2～3°C for one hour, 20 minutes after insemination. As presented in Table 1, 295 (72.1%) of 409 eggs obtained from the wild-type females and 76 (37.8%) of 201 eggs from the albinic female cleaved normally. After numerous embryos died of edema, underdevelopment or some other abnormalities, 149 (36.4%) from the wild-type females and 29 (14.4%) from the albinic female became normally feeding tadpoles.

TABLE 1
Production of autotriploids by refrigeration of fertilized eggs (1979)

| Parents | | No. of | No. of cleaved eggs | | No. of feeding tadpoles | |
|---|---|---|---|---|---|---|
| Female | Male | eggs | Normal | Abnormal | Normal | Abnormal |
| YK. Y, W, No. 1 | F 79, No. 1 | 138 | 89 (64.5%) | 26 (18.8%) | 41 (29.7%) | 15 (10.9%) |
| YK. Y, W, No. 2 | | 271 | 206 (76.0%) | 27 (10.0%) | 108 (39.9%) | 51 (18.8%) |
| YK. Y, A, No. 3 | | 201 | 76 (37.8%) | 33 (16.4%) | 29 (14.4%) | 22 (10.9%) |
| Total | | 610 | 371 (60.8%) | 86 (14.1%) | 178 (29.2%) | 88 (14.4%) |

YK, Hybrid between an albinic female from Yamaguchi and an albinic male from Kake
YK. Y, Backcross of a female YK with an albinic male from Yamaguchi
W, Wild-type
A, Colored albino
F, Field-caught

## 2. Chromosome number

Chromosomes were examined in the tail-tips of the 149 tadpoles produced from matings, YK. Y, W♀, Nos. 1 and 2×F 79♂, No. 1, by the water-pretreatment squash method. It was found that 10 were diploids, 86 were triploids, one was a tetraploid and four were 2n-3n mosaics. The remaining 48 were unknown in chromosome number. Chromosomes were also observed in the tail-tips of the 29 feeding tadpoles produced from a mating, YK. Y, C-Alb♀, No. 3×F 79♂, No. 1, by the same method. The results showed that two were diploids, 24 were triploids and the other three were unknown (Table 2).

TABLE 2
Chromosomes of tadpoles produced from refrigerated eggs

| Parents | | Number of tadpoles | | | | |
|---|---|---|---|---|---|---|
| | | | No. of chromosomes | | | |
| Female | Male | Analyzed | 24 (2n) | 36 (3n) | 48 (4n) | 24/36 (2n/3n) |
| YK. Y, W, No. 1 | F 79, No. 1 | 32 | 1 | 30 | 0 | 1 |
| YK. Y, W, No. 2 | | 69 | 9 | 56 | 1 | 3 |
| YK. Y, A, No. 3 | | 26 | 2 | 24 | 0 | 0 |
| Total | | 127 | 12 | 110 | 1 | 4 |

### 3. Metamorphosis and sex ratio

Of 30 feeding triploid tadpoles produced from a mating, YK. Y, W♀, No. 1 × F 79♂, No. 1, 25 metamorphosed normally and 12 attained sexual maturity. Of the latter, three were females and nine were males. Of 56 feeding triploid tadpoles produced from a mating, YK. Y, W♀, No. 2 × F 79♂, No. 1, 49 metamorphosed normally and 47 attained sexual maturity. Twelve of the latter were females and 35 were males. Of 24 feeding triploid tadpoles produced from a mating, YK. Y, C-Alb♀, No. 3 × F 79♂, No. 1, 20 metamorphosed normally and 17 attained sexual maturity. Of the latter, five were females and 12 were males. Of these 110 triploid tadpoles in total, 94 metamorphosed normally and 76 attained sexual maturity. Of the latter, 20 were females and 56 (73.7%) were males.

While nine diploid tadpoles produced from the mating, YK. Y, W♀, No. 2 × F 79♂, No. 1, and two diploid tadpoles produced from the mating, YK. Y, C-Alb ♀, No. 3 × F 79♂, No. 1, completed metamorphosis, six of these frogs attained sexual maturity. Three were females and three were males.

### 4. Body length

Triploids obtained in 1979 by refrigeration of fertilized eggs were compared in body length with diploids obtained in the same matings immediately after metamorphosis as well as at the age of one year when they were sexually matured. As presented in Table 3, 10 of the 11 metamorphosed diploids were 15.0~16.0 mm, 15.25 mm on the average, in body length. The remainder died before the measurement. Twenty-three triploids obtained from a wild-type female (YK. Y, W♀, No. 1), 45 triploids from a wild-type female (YK. Y, W♀, No. 2) and 20 triploids from an albinic female (YK. Y, C-Alb♀, No. 3) by mating with a field-caught male (F 79♂, No. 1) were 15.9 mm, 15.8 mm and 15.7 mm on the average in body length, respectively, immediately after metamorphosis. These 88 triploids in total were 15.0~17.0 mm, 15.8 mm on the average, in body length.

Three female and three male diploids at the age of one year were 35.5 mm and 31.5 mm on the average in body length, respectively. Three, twelve and five

TABLE 3

Body lengths of diploids and triploids produced from refrigerated eggs in *Hyla arborea japonica*

| Parents | | Ploidy | No. of metamorphosed frogs | Immediately after metamorphosis | | One-year-old mature frogs | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Female | | Male | |
| Female | Male | | | No. of frogs | Body length (Mean) mm | No. of frogs | Body length (Mean) mm | No. of frogs | Body length (Mean) mm |
| YK. Y, W, No. 1 | F 79, No. 1 | 3n | 25 | 23 | 15.5~17.0 (15.9±0.08) | 3 | 36.0~37.0 (36.5±0.20) | 9 | 30.5~34.0 (32.0±0.33) |
| YK. Y, W, No. 2 | F 79, No. 1 | 2n | 9 | 8 | 15.0~16.0 (15.3±0.37) | 3 | 35.0~36.5 (35.5±0.38) | 3 | 29.0~33.5 (31.5±0.52) |
| | | 3n | 49 | 45 | 15.0~17.0 (15.8±0.08) | 12 | 35.5~37.5 (36.4±0.15) | 30 | 30.0~34.0 (32.3±0.19) |
| YK. Y, A, No. 3 | F 79, No. 1 | 2n | 2 | 2 | 15.0, 15.0 | — | | — | |
| | | 3n | 20 | 20 | 15.0~16.5 (15.7±0.10) | 5 | 35.5~37.0 (35.8±0.23) | 11 | 31.5~33.0 (31.8±0.18) |

one-year-old female triploids produced from three matings were 36.5 mm, 36.4 mm and 35.8 mm on the average in body length, respectively. These 20 female triploids in total were 35.5 ~ 37.5 mm, 36.2 mm on the average, in body length. Nine, 30 and 11 one-year-old male triploids produced from three matings were 32.0 mm, 32.3 mm and 31.8 mm on the average in body length, respectively. These 50 male triploids in total were 30.0 ~ 34.0 mm, 32.0 mm on the average, in body length. Thus, there were no remarkable difference in body length between diploid and triploid frogs, although the triploids seemed to be slightly larger than the diploids (Table 3).

### *II. Offspring of triploids*

#### 1. Females used in the experiments of 1980

Two one-year-old female triploids (3n 79♀, Nos. 1 and 2: $f^cf^cF, ffF, FFF, f^cFF$ or $fFF$) produced in 1979 by refrigeration from fertilized eggs between two wild-type females (YK. Y, W♀, Nos. 1 and 2) and a field-caught male (F 79♂, No. 1) were 36.5mm and 37.5 mm in body length. Two other one-year-old female triploids (3n 79♀, Nos. 3 and 4: $f^cf^cF, ffF$ or $f^cfF$) produced in 1976 by refrigeration from eggs between an albinic female (YK. Y, C-Alb♀, No. 3) and the above field-caught male (F 79♂, No. 1) were 37.0 mm and 36.5 mm in body length. By injection of a mixture of bullfrog pituitaries and gonadotropin, these four females (Nos. 1, 2, 3 and 4) laid 164, 244, 832 and 26 eggs, respectively.

Although female triploid No. 4 contained many eggs in her ovaries, most of them were not ovulated. Of the eggs laid by female triploids Nos. 1 ~ 3, 20 ~ 30 were measured. It was found that those of female triploids Nos. 1, 2 and 3 were 1.0 ~ 1.4 mm, 1.30 ± 0.25 mm on the average, 1.1 ~ 1.5 mm, 1.33 ± 0.30 mm on the average, and 1.0 ~ 1.5 mm, 1.32 ± 0.28 mm on the average, in diameter, respectively(Table 8). Three females (F 80♀, Nos. 1, 2 and 3) collected from the field in 1980 laid 636, 563 and 296 eggs, respectively. The diameter of these eggs was not measured.

#### 2. Males used in the experiments of 1980

Two one-year-old male triploids (3n 79♂, Nos. 1 and 2) produced in 1979 by refrigeration from fertilized eggs between a wild-type female (YK. Y, W♀, No. 1) and a field-caught male (F 79♂, No. 1), a one-year-old male triploid (3n 79♂, No. 3) produced in 1979 by refrigeration from a fertilized egg between an albinic female (YK. Y, C-Alb♀, No. 3) and the above field-caught male (F 79♂, No. 1) and three males (F 80♂, Nos. 1 ~ 3) collected from the field in 1980 were used in mating experiments. The left and right testes of the three field-caught males (F 80♂, Nos. 1 ~ 3) were 3.0 mm × 1.8 mm and 3.0 mm × 1.8 mm, 3.0 mm × 1.5 mm and 3.0 mm × 2.0 mm, and 3.5 mm × 2.0 mm and 3.5 mm × 2.0 mm in size, respectively, while their body lengths were not measured.

Three one-year-old male triploids (Nos. 1, 2 and 3) produced from fertilized eggs by refrigeration were 32.5 mm, 33.0 mm and 33.0 mm in body length, respectively. The left and right testes of these three males were 4.0 mm × 2.0 mm and 4.0 mm × 2.0 mm, 3.5 mm × 2.0 mm and 3.5 mm × 2.0 mm, and 4.0 mm × 2.0 mm and 4.0 mm × 2.0 mm in size, respectively (Table 9).

### 3. Developmental capacity

a. Control matings, F 80♀, Nos. 1～3 × F 80♂, Nos. 1～3

In 1980, three field-caught females were mated with three field-caught males. It was found that 89.5～96.5% of eggs in the three control matings, 373 (92.8%) of 402 eggs in total, cleaved normally. While 19 embryos died of abnormalities before the hatching stage, 85.5～90.3%, 354 (88.1%) in total, hatched normally and eventually, 82.0～87.5%, 341 (84.8%) in total, became normally feeding tadpoles (Table 4).

TABLE 4
Reproductive capacity of male and female triploids in *Hyla arborea japonica* (1980)

| Parents: Female | Parents: Male | No. of eggs | No. of normal cleavages | No. of normal neurulae | No. of normally hatched tadpoles | No. of normally feeding tadpoles |
|---|---|---|---|---|---|---|
| F 80, No. 1 | F 80, No. 1 | 144 | 139(96.5%) | 136(94.4%) | 130(90.3%) | 126(87.5%) |
| F 80, No. 2 | F 80, No. 2 | 172 | 154(89.5%) | 151(87.8%) | 147(85.5%) | 141(82.0%) |
| F 80, No. 3 | F 80, No. 3 | 86 | 80(93.0%) | 79(91.9%) | 77(89.5%) | 74(86.0%) |
| F 80, No. 1 | (YK. Y)$_1$F, 3n, No. 1 | 312 | 257(82.4%) | 233(74.7%) | 220(70.5%) | 48(15.4%) |
| F 80, No. 2 | | 156 | 122(78.2%) | 86(55.1%) | 73(46.8%) | 15( 9.6%) |
| F 80, No. 1 | (YK. Y)$_1$F, 3n, No. 2 | 179 | 68(38.0%) | 61(34.1%) | 58(32.4%) | 8( 4.5%) |
| F 80, No. 2 | | 235 | 95(40.4%) | 90(38.3%) | 87(37.0%) | 9( 3.8%) |
| F 80, No. 3 | (YK. Y)$_3$F, 3n, No. 3 | 210 | 198(94.3%) | 183(87.1%) | 123(58.6%) | 18( 8.6%) |
| (YK. Y)$_1$F, 3n, No. 1 | F 80, No. 1 | 164 | 108(65.9%) | 63(38.4%) | 20(12.2%) | 6( 3.7%) |
| (YK. Y)$_2$F, 3n, No. 2 | F 80, No. 2 | 244 | 235(96.3%) | 196(80.3%) | 114(46.7%) | 17( 7.0%) |
| (YK. Y)$_3$F, 3n, No. 3 | F 80, No. 3 | 832 | 806(96.9%) | 628(75.5%) | 474(57.0%) | 136(16.3%) |
| F 80, Nos. 1～3 | F 80, Nos. 1～3 | 402 | 373(92.8%) | 366(91.0%) | 354(88.1%) | 341(84.8%) |
| F 80, Nos. 1～3 | 3n 79, Nos. 1～3 | 1092 | 740(67.8%) | 653(59.8%) | 561(51.4%) | 98( 9.0%) |
| 3n 79, Nos. 1～3 | F 80, Nos. 1～3 | 1240 | 1149(92.7%) | 887(71.5%) | 608(49.0%) | 159(12.8%) |

b. Experimental matings, F 80♀, Nos. 1～3 × 3n 79♂, Nos. 1～3

The above three field-caught females (F 80♀, Nos. 1～3) were mated with two one-year-old male triploids (3n 79♂, Nos. 1 and 2) produced in 1979 by refrigeration of fertilized eggs between a wild-type female (YK. Y, W♀, No. 1) and a field-caught male (F 79♂, No. 1) and a one-year-old male triploid (3n 79♂, No. 3) produced in 1979 by refrigeration from a fertilized egg between an albinic female (YK. Y, C-Alb♀, No. 3) and the above field-caught male (F 79♂, No. 1). The results showed that 379 (81.0%) of 468 eggs inseminated with sperm of male triploid No. 1, 163 (39.4%) of 414 eggs inseminated with sperm of male triploid No. 2 and 198 (94.3%) of 210 eggs inseminated with sperm of male triploid No. 3 cleaved normally. Of these 1092 eggs in total, 740 (67.8%) cleaved normally. Before the neurula stage, 60 from male triploid No. 1, 12 from male triploid No. 2 and 15 from male triploid No. 3 became abnormal and died, while 319 (68.2%), 151 (36.5%) and 183 (87.1%), 653 (59.8%) in total, became normal neurulae, respectively. Thereafter, 26 from male triploid No. 1, six from male triploid No. 2 and 60 from male triploid No. 3 became abnormal before the hatching stage, while 293 (62.6%), 145 (35.0%) and 123 (58.6%), 561 (51.4%) in total, hatched normally, respectively. Many of the hatched tadpoles became edematous or abnormal in gills or some other organs and died without taking food. Eventually, 63 (13.5%) from male triploid No. 1, 17 (4.1%) from male triploid No. 2 and 18 (8.6%) from male triploid No. 3, 98 (9.0%) in total, became normally feeding tadpoles (Table 4).

c. Experimental matings, 3n 79♀, Nos. 1～3×F 80♂, Nos. 1～3

In 1980, three one-year-old female triploids (3n 79♀, Nos. 1～3) produced in 1979 by refrigeration from fertilized eggs between two wild-type females (YK. Y, W♀, Nos. 1 and 2) and a colored-albinic female (YK. Y, C-Alb♀, No. 3) and a field-caught male (F 79♂, No. 1) were mated with the same three males (F 80♂, Nos. 1～3) as used in the control matings. It was found that 65.9～96.9%, 1149 (92.7%) of 1240 eggs in total, cleaved normally. Before the neurula stage, many embryos died of incomplete invagination at the gastrula stage or became abnormal, while 38.4～80.3%, 887 (71.5%) in total, became normal neurulae. At the tail-bud and hatching stages, many embryos died of edema or some other abnormalities, while 12.2～57.0%, 608 (49.0%) in total, hatched normally. Eventually, only 3.7～16.3%, 159 (12.8%) in total, became normally feeding tadpoles, while most of the hatched tadpoles died of edema or emaciation without taking food (Table 4).

4. Chromosome number

a. Control matings, F 80♀, Nos. 1～3×F 80♂, Nos. 1～3

From the three control matings between three field-caught females (F 80♀, Nos. 1～3) and three field-caught males (F 80♂, Nos. 1～3) 126, 141 and 74 normally feeding tadpoles were produced. Chromosomes were examined in the tail-tips of 20, 10 and 10, 40 in total, of these tadpoles by the water-pretreatment squash method. The results indicated that all these tadpoles were diploid (Table 5).

b. Experimental matings, F 80♀, Nos. 1～3×3n 79♂, Nos. 1～3

i) Lethal embryos and tadpoles

Chromosomes were examined in 55 lethal embryos and tadpoles produced from the three matings between the same three field-caught females as those used in the control matings and three male triploids (3n 79♂, Nos. 1～3). It was found that

TABLE 5

Chromosomes of the offspring between diploids and triploids and the controls in *Hyla arborea japonica*

| Parents: Female | Parents: Male | Number of tadpoles: Analyzed | 24 (2n) | 25 | 26 | 27 | 28~30 (2n+) | 31~35 (3n−) | 36 (3n) | 37~42 (3n+) | 43~44 (4n−) | 48 (4n) | 66 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2n, No. 1 | 2n, No. 1 | 20 | 20 | | | | | | | | | | |
| 2n, No. 2 | 2n, No. 2 | 10 | 10 | | | | | | | | | | |
| 2n, No. 3 | 2n, No. 3 | 10 | 10 | | | | | | | | | | |
| 2n, No. 1 | 3n, No. 1 | 41 | 14 | 2 | 9 | 8 | 3 | | 2 | 2 | 1 | | |
| 2n, No. 2 | | 14 | 6 | 1 | 3 | 1 | 1 | 1 | 1 | | | | |
| 2n, No. 1 | 3n, No. 2 | 7 | 2 | | | 2 | 1 | 1 | | 1 | | | |
| 2n, No. 2 | | 9 | | 1 | | 6 | 1 | | | | | | 1 |
| 2n, No. 3 | 3n, No. 3 | 13 | | 1 | 2 | | | 2 | | 7 | 1 | | |
| 3n, No. 1 | 2n, No. 1 | 6 | | | | | | 1 | | | | 5 | |
| 3n, No. 2 | 2n, No. 2 | 16 | 2 | 2 | 3 | | | | 1 | | | 8 | |
| 3n, No. 3 | 2n, No. 3 | 120 | 6 | | 2 | | | 2 | 7 | 1 | | 102 | |

11 of them had each 27, 36, 37, 38, 39, 40, 41, 43, 49, 52 or 66 chromosomes. Two individuals had 28 and three others had 34. Five individuals had 29 and five others had 33. Ten individuals had 30, eight had 31 and six others had 32 chromosomes (Fig. 1) and the remaining five were mosaics or unanalyzable in chromosome number.

ii) Normally feeding tadpoles

Chromosomes were examined in the tail-tips of 98 normally feeding tadpoles produced from the same matings as the above. It was found that 22 were diploids, three were triploids, 42 were hyperdiploids, four were hypotriploids, 10 were hypertriploids, two were hypotetraploids, one was a hyperpentaploid and the remaining 14 were mosaics or unanalyzable in chromosome number (Table 5). More specifically, five of the hyperdiploids had 25 (2n+1), 14 had 26 (2n+2), 17 had 27 (2n+3), five had 28 (2n+4) and the remainder had 29 (2n+5) chromosomes. Of the four hypotriploids, one had 31(3n−5), one had 32 (3n−4), one had 33 (3n−3) and the remainder had 34 (3n−2) chromosomes. Of the 10 hypertriploids, one had 37 (3n+1), three had 39 (3n+3), four had 40 (3n+4), one had 41 (3n+5) and the remainder had 42 (3n+6) chromosomes. Of the two hypotetraploids, one had 43 (4n−5) and the other had 44 (4n−4) chromosomes (Fig. 2).

c. Experimental matings, 3n 79♀, Nos. 1~3×F 80♂, Nos. 1~3

Chromosomes were examined in the tail-tips of 159 normally feeding tadpoles produced from three matings between three female triploids (3n 79♀, Nos. 1~3) and the same three field-caught males as those used in the control matings. It was found that eight tadpoles were diploids, eight were triploids, 115 were tetraploids, seven were hyperdiploids, three were hypotriploids, one was a hypertriploid and the remaining 17 were mosaics or unanalyzable in chromosome number. Of the seven hyperdiploids, two had 25 (2n+1) and five had 26 (2n+2) chromosomes, while the three hypotriploids had 33 (3n−3) chromosomes. The single hypertriploid had 40 (3n+4) chromosomes (Fig. 2).

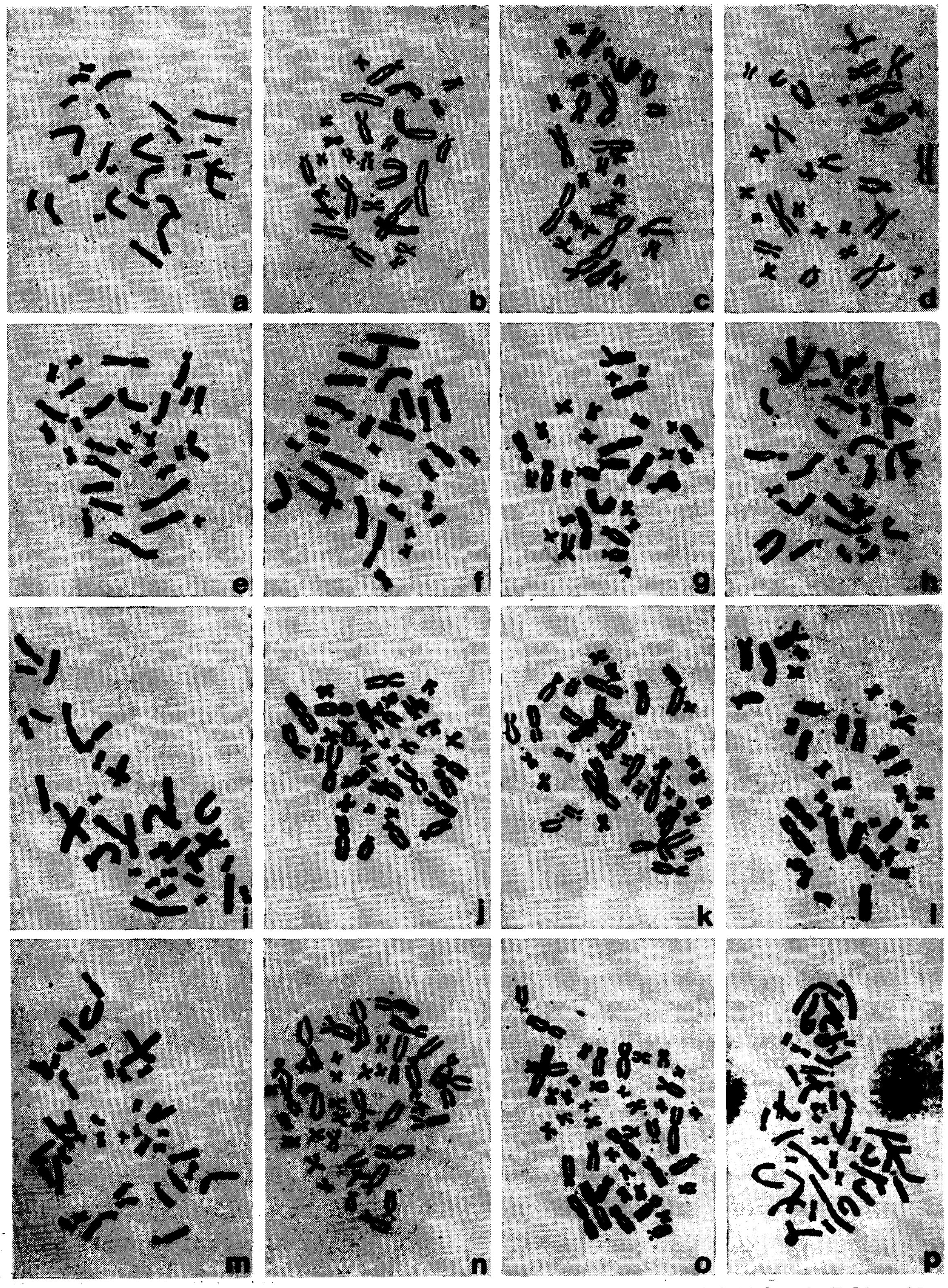


Fig. 1. Chromosomes of lethal embryos in the offspring between diploid female F 80♀, No. 3 and triploid male 3n 79♂, No. 3. ×750

a. 27 (2n+3) b. 28 (2n+4) c. 29 (2n+5) d. 30 (2n+6) e. 31 (3n−5)
f. 32 (3n−4) g. 33 (3n−3) h. 34 (3n−2) i. 36 (3n) j. 37 (3n+1)
k. 38 (3n+2) l. 39 (3n+3) m. 40 (3n+4) n. 41 (3n+5) o. 43 (4n−5)
p. 49 (4n+1)

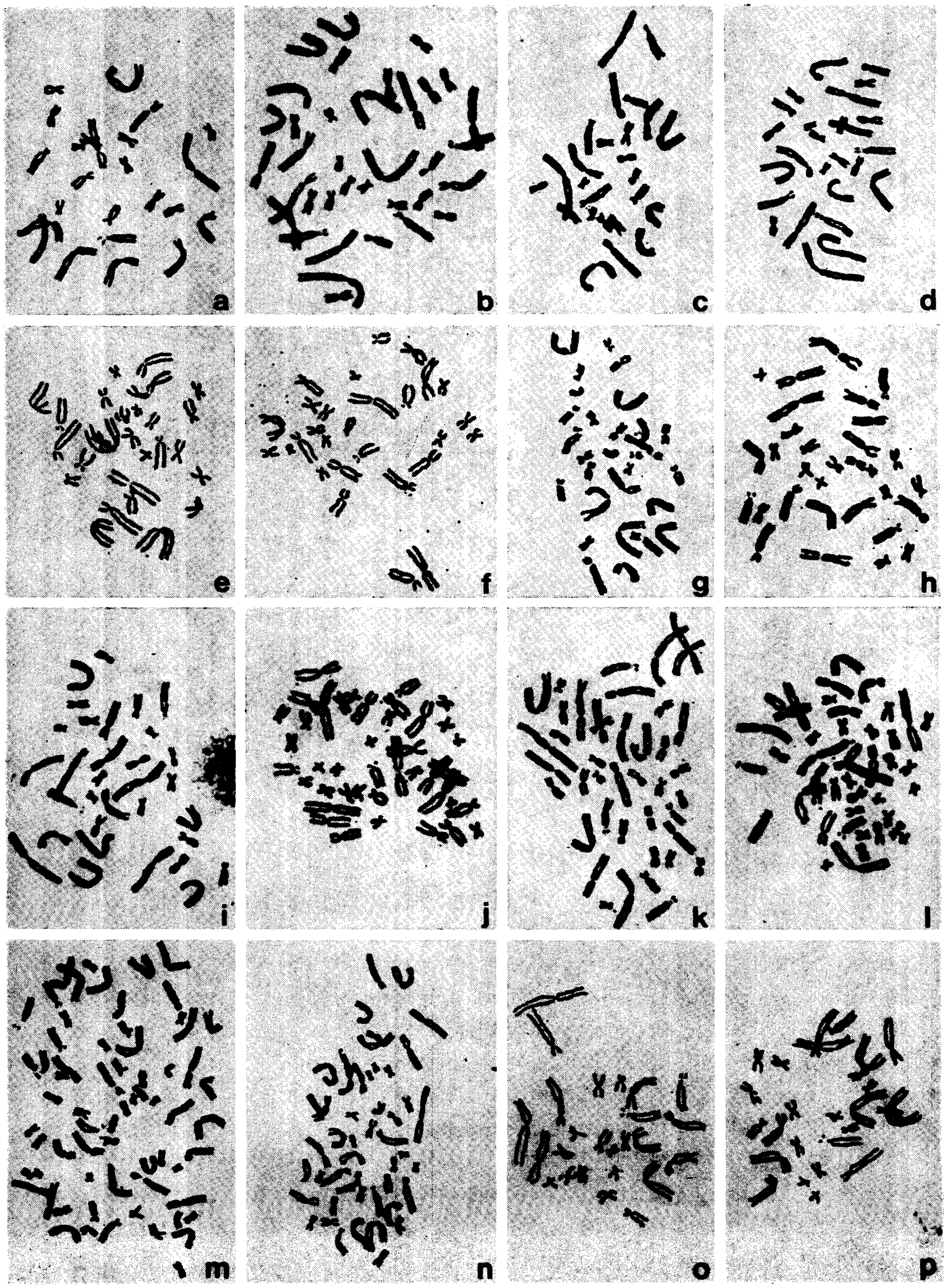



Fig. 2. Chromosomes of normally feeding tadpoles in the offspring between diploids and triploids.

a∼m. Offspring between diploid females and triploid males ×750

| | | | | |
|---|---|---|---|---|
| a. 24 (2n) | b. 36 (3n) | c. 25 (2n+1) | d. 26 (2n+2) | e. 27 (2n+3) |
| f. 28 (2n+4) | g. 29 (2n+5) | h. 31 (3n−5) | i. 32 (3n−4) | j. 41 (3n+5) |
| k. 42 (3n+6) | l. 43 (4n−5) | m. 66 (5n+6) | | |

n∼p. Offspring between a triploid female and a diploid male

| | | |
|---|---|---|
| n. 49 (4n) | o. 25 (2n+1) | p. 26 (2n+2) |

5. Metamorphosis and sex ratio

a. Control matings, F 80♀, Nos. 1～3×F 80♂, Nos. 1～3

Of 126, 141 and 74, 341 in total, normally feeding tadpoles produced from three control matings, 112, 125 and 69, 306 in total, completed metamorphosis, respectively. Of these metamorphosed frogs, sex ratios were examined in 103, 124 and 66, 293 in total, respectively. It was found that 48 were females and 55 (53.4%) were males in control mating No. 1, 61 were females and 63 (50.8%) were males in control mating No. 2 and 34 were females and 32 (48.5%) were males in control mating No. 3. In total, 143 were females and 150 (51.2%) were males (Table 6).

TABLE 6

Sex of the offspring between female triploids and male diploids and the controls in *Hyla arborea japonica*

| Parents | | Ploidy | No. of metamorphosed frogs | Sex of immature frogs | | | Sex of mature frogs | | | Sex of all frogs | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Female | Male | | | No. of frogs | ♀ | ♂ | No. of frogs | ♀ | ♂ | Total | ♀ | ♂ (%) |
| F 80, No. 1 | F 80, No. 1 | 2n | 112 | 57 | 27 | 30 | 46 | 21 | 25 | 103 | 48 | 55(53.4) |
| F 80, No. 2 | F 80, No. 2 | 2n | 125 | 124 | 61 | 63 | — | — | — | 124 | 61 | 63(50.8) |
| F 80, No. 3 | F 80, No. 3 | 2n | 69 | 66 | 34 | 32 | — | — | — | 66 | 34 | 32(48.5) |
| 3n 79, No. 1 | F 80, No. 1 | 4n | 3 | 1 | 0 | 1 | 2 | 2 | 0 | 3 | 2 | 1(33.3) |
| 3n 79, No. 2 | F 80, No. 2 | 4n | 7 | 3 | 2 | 1 | 4 | 3 | 1 | 7 | 5 | 2(28.6) |
| 3n 79, No. 3 | F 80, No. 3 | 2n | 5 | 3 | 2 | 1 | 2 | 1 | 1 | 5 | 3 | 2(40.0) |
| | | 3n | 5 | 1 | 0 | 1 | 3 | 2 | 1 | 4 | 2 | 2(50.0) |
| | | 4n | 79 | 21 | 16 | 5 | 58 | 43 | 15 | 79 | 59 | 20(25.3) |

b. Experimental matings, F 80♀, Nos. 1～3×3n 79♂, Nos. 1～3

Of 22 normally feeding diploid tadpoles produced from five experimental matings between three field-caught females and three triploid males, 13 from F 80♀, No. 1×3n 79♂, No. 1 and three from F 80♀, No. 2×3n 79♂, No. 1, metamorphosed normally. All the three triploids produced from F 80♀, Nos. 1 and 2×3n 79♂, No. 1, also metamorphosed normally. Of 42 hyperdiploids and four hypotriploids, only nine completed metamorphosis. More specifically, two tadpoles having 25 (2n+1) chromosomes and five having 26 (2n+2) chromosomes in F 80♀, No. 1×3n 79♂, No. 1, one tadpole having 26 (2n+2) chromosomes in F 80♀, No. 2×3n 79♂, No. 1 and one tadpole having 25 (2n+1) chromosomes in F 80♀, No. 2×3n 79♂, No. 2 completed metamorphosis. Of nine hypertriploids and three hypotetraploids, one tadpole having 40 (3n+4) chromosomes, one having 41 (3n+5) chromosomes and one having 43 (4n−5) chromosomes in F 80♀, No. 1×3n 79♂, No. 1 and one having 43 chromosomes (4n−5) in F 80♀, No. 1×3n 79♂, No. 2, four tadpoles in total, completed metamorphosis. The sex of these metamorphosed aneuploids could not be determined except one, as they died immediately after metamorphosis. The single exceptional aneuploid was a hyperdiploid having 25 chromosomes and became a sexually mature male.

c. Experimental matings, 3n 79♀, Nos. 1～3×F 80♂, Nos. 1～3

Of the eight normally feeding diploid tadpoles produced from the three experimental matings between three triploid females (3n 79♀, Nos. 1～3) and three field-caught males, two from triploid female No. 2 and five from triploid female No. 3 completed metamorphosis. Of these metamorphosed diploids, two from triploid female No. 2 died immediately after metamorphosis and their sex could not be determined owing to postmortem changes. The sex of the other five diploids from triploid female No. 3 was determined; three were females and two were males.

Of eight normally feeding triploid tadpoles produced from the same three experimental matings, one from triploid female No. 2 and five from triploid female No. 3, six in total, completed metamorphosis. When the sex of four of these triploids was examined, two were females and two were males. The sex of the remaining two was not determined owing to postmortem changes.

Of 115 normally feeding tetraploid tadpoles produced from the same three experimental matings, three from triploid female No. 1, seven from triploid female No. 2 and 79 from triploid female No. 3, 89 in total, completed metamorphosis. It was found that 66 of them were females and 23 (25.8%) were males. Thereafter, 48 females and 16 (25.0%) males of these tetraploids attained sexual maturity.

Of 11 normally feeding aneuploid tadpoles which were between diploid and tetraploid in chromosome number, two hyperdiploids produced from triploid female No. 2, having each 25 (2n+1) and 26 (2n+2) chromosomes, and one hyperdiploid and one hypertriploid produced from triploid female No. 3, having 26 (2n+2) and 40 (3n+4) chromosomes, respectively, four tadpoles in total, completed metamorphosis. Of these four aneuploid frogs, only one having 25 (2n+1) chromosomes attained sexual maturity. This hyperdiploid was a female (Table 6).

6. Body length

a. Control matings, F 80♀, Nos. 1～3×F 80♂, Nos. 1～3

Twenty frogs produced from each of the three control matings were measured immediately after metamorphosis. Twenty, 20 and 20 frogs were 15.8 mm, 15.6 mm and 15.7 mm in mean body length, respectively. In total, they were 15.0～17.0 mm and 15.7 mm on the average. The body lengths of mature frogs were measured in those produced from a mating, F 80♀, No. 1×F 80♂, No. 1. It was found that 20 mature females were 34.0～37.0 mm, 35.8 mm on the average, in body length, while 20 mature males were 30.5～33.5 mm, 32.1 mm on the average (Table 7).

b. Experimental matings, 3n 79♀, Nos. 1～3×F 80♂, Nos. 1～3

i) Tetraploids

The body lengths of tetraploids produced from the three matings between three female triploids (3n 79♀, Nos. 1～3) and three field-caught males were measured immediately after metamorphosis as well as at the sexually mature stage. Three,

TABLE 7

Body lengths of the offspring between female triploids and male diploids and the controls in *Hyla arborea japonica*

| Parents | | Ploidy | No. of metamorphosed frogs | Immediately after metamorphosis | | One-year-old mature frogs | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Female | | Male | |
| Female | Male | | | No. of frogs | Body length (Mean) mm | No. of frogs | Body length (Mean) mm | No. of frogs | Body length (Mean) mm |
| F 80, No. 1 | F 80, No. 1 | 2n | 112 | 20 | 15.0~16.5 (15.8±0.10) | 20 | 34.0~37.0 (35.8±0.15) | 20 | 30.5~33.5 (32.1±0.18) |
| F 80, No. 2 | F 80, No. 2 | 2n | 125 | 20 | 15.0~16.5 (15.6±0.13) | — | | — | |
| F 80, No. 3 | F 80, No. 3 | 2n | 69 | 20 | 15.0~17.0 (15.7±0.13) | — | | — | |
| 3n 79, No. 1 | F 80, No. 1 | 4n | 3 | 3 | 16.0~17.0 (16.6±0.14) | 2 | 35.5, 36.0 (35.8±0.18) | — | |
| 3n 79, No. 2 | F 80, No. 2 | 4n | 7 | 7 | 16.0~17.0 (16.6±0.19) | 3 | 35.0~36.5 (35.7±0.36) | 1 | 31.0 |
| 3n 79, No. 3 | F 80, No. 3 | 2n | 5 | 5 | 15.0~16.5 (15.7±0.20) | 1 | 32.0 | 1 | 28.5 |
| | | 3n | 5 | 5 | 15.5~16.5 (16.0±0.14) | 2 | 35.5, 36.0 (35.8±0.18) | 1 | 32.0 |
| | | 4n | 79 | 51 | 15.0~17.5 (16.7±0.08) | 31 | 36.0~38.0 (37.0±0.12) | 12 | 31.5~35.5 (33.4±0.38) |

seven and 51 tetraploids in the three experimental matings were 16.6 mm, 16.6 mm and 16.7 mm on the average, respectively, 15.0~17.5 mm and 16.6 mm on the average in total, in body length immediately after metamorphosis. The tetraploids produced from female triploids Nos. 1 and 2 were reared indoors in small plastic aquaria. Thus, two and three mature female tetraploids from female triploids Nos. 1 and 2, respectively, were insufficient in growth. They were 35.8 mm and 35.7 mm on the average, respectively. In contrast, the female tetraploids produced from female triploid No. 3 grew satisfactorily, as they were reared outdoors in large concrete aquaria. The results of measurement showed that 31 mature female tetraploids were 36.0~38.0 mm, 37.0 mm on the average, in body length.

Mature male tetraploids were somewhat smaller than mature female tetraploids. One male from female triploid 3n 79♀, No. 2 was 31.0 mm and 12 males from 3n 79♀, No. 3 were 31.5~35.5 mm, 33.4 mm on the average, in body length.

ii) Diploids and triploids

Five diploids produced from a mating, 3n 79♀, No. 3×F 80♂, No. 3, were 15.7 mm in mean body length immediately after metamorphosis. A mature female was 32.0 mm in body length, while a mature male was undergrown, being 28.5 mm in body length.

Five triploids produced from the same mating were 16.0 mm in mean body length immediately after metamorphosis. Two mature females were 35.5 mm and 36.0 mm and a mature male was 32.0 mm in body length.

### III. *First-generation offspring of tetraploids*

In the breeding season of 1981, first-generation offspring were produced from matings among seven one-year-old female tetraploids (4n 80♀, Nos. 1~7) and three one-year-old male tetraploids (4n 80♂, Nos. 1~3) obtained in 1980 from 3n 79♀, No. 3×F 80♂, No. 3, and three one-year-old male diploids (2n 80♂, Nos. 1~3) obtained from F 80♀, No. 1×F 80♂, No. 1 (Tables 8 and 9).

TABLE 8

Eggs of mature female diploids, triploids and tetraploids in *Hyla arborea japonica*

| Parents | | Individual no. | Ploidy | Age years | Body length mm | No. of eggs | Diameter of 20 or 30 eggs (Mean) mm |
|---|---|---|---|---|---|---|---|
| Female | Male | | | | | | |
| F 80, No. 1 | F 80, No. 1 | 2n 80, No. 1 | 2n | 1 | 35.5 | 866 | 1.2~1.3 (1.21±0.02) |
| | | 2n 80, No. 2 | 2n | 1 | 36.5 | 925 | 1.2~1.3 (1.20±0.02) |
| | | 2n 80, No. 3 | 2n | 1 | 36.0 | 673 | 1.1~1.2 (1.20±0.03) |
| | | 2n 80, No. 4 | 2n | 2 | 36.5 | 1052 | 1.2~1.3 (1.22±0.03) |
| | | 2n 80, No. 5 | 2n | 2 | 37.0 | 923 | 1.2~1.3 (1.21±0.02) |
| | | 2n 80, No. 6 | 2n | 2 | 37.0 | 788 | 1.2~1.3 (1.21±0.02) |
| YK. Y, W, No. 1 | F 79, No. 1 | 3n 79, No. 1 | 3n | 1 | 36.5 | 164 | 1.0~1.4 (1.30±0.25) |
| | | 3n 79, No. 2 | 3n | 1 | 37.5 | 244 | 1.1~1.5 (1.33±0.30) |
| YK. Y, A, No. 3 | F 79, No. 1 | 3n 79, No. 3 | 3n | 1 | 37.0 | 832 | 1.0~1.5 (1.32±0.28) |
| | | 3n 79, No. 4 | 3n | 1 | 36.5 | 26 | |
| 3n 79, No. 3 | F 80, No. 3 | 4n 80, No. 1 | 4n | 1 | 37.0 | 804 | 1.2~1.4 (1.32±0.20) |
| | | 4n 80, No. 2 | 4n | 1 | 36.5 | 792 | 1.2~1.4 (1.35±0.15) |
| | | 4n 80, No. 3 | 4n | 1 | 38.0 | 681 | 1.2~1.5 (1.34±0.14) |
| | | 4n 80, No. 4 | 4n | 1 | 37.5 | 793 | 1.2~1.4 (1.35±0.11) |
| | | 4n 80, No. 5 | 4n | 1 | 37.0 | 1066 | 1.2~1.4 (1.33±0.11) |
| | | 4n 80, No. 6 | 4n | 1 | 36.0 | 936 | 1.2~1.4 (1.34±0.18) |
| | | 4n 80, No. 7 | 4n | 1 | 37.5 | 530 | 1.2~1.4 (1.35±0.10) |
| 4n 80, No. 6 | 4n 80, No. 3 | 4n 81, No. 1 | 4n | 1 | 38.5 | 521 | 1.2~1.5 (1.34±0.20) |
| | | 4n 81, No. 2 | 4n | 1 | 37.0 | 1154 | 1.2~1.4 (1.34±0.14) |
| | | 4n 81, No. 3 | 4n | 1 | 37.5 | 726 | 1.3~1.4 (1.36±0.09) |
| | | 4n 81, No. 4 | 4n | 1 | 36.5 | 785 | |
| | | 4n 81, No. 5 | 4n | 1 | 36.0 | 216 | |
| | | 4n 81, No. 6 | 4n | 1 | 37.0 | 62 | |

1. Females used in the experiments of 1981

a. Control diploids

Three one-year-old female diploids (2n 80♀, Nos. 1~3) were 35.5 mm, 36.5 mm and 36.0 mm, 36.0 mm on the average, in body length. These females laid 866, 925 and 673 eggs, 821.3 eggs on the average, respectively, after injection of a mixture of bullfrog pituitaries and gonadotropin. When 20~30 of the eggs laid by each female were measured, they were 1.21 mm, 1.20 mm or 1.20 mm in mean diameter. The eggs of the three females were 1.1~1.3 mm, 1.20 mm on the average, in diameter (Table 8).

b. Tetraploids

Seven one-year-old female tetraploids (4n 80♀, Nos. 1~7) were 36.0~38.0 mm, 37.1 mm on the average, in body length. After injection of a mixture of bullfrog pituitaries and gonadotropin, all of them laid 530~1066 eggs, 800.3 eggs on the average. The eggs laid by each female were 1.32~1.35 mm in mean diameter, when 20~30 of the eggs were measured. Those laid by the seven females were 1.2~1.5 mm, 1.34 mm on the average, in diameter (Table 8).

2. Males used in the experiments of 1981

a. Control diploids

Three one-year-old male diploids (2n 80♂, Nos. 1~3) were 31.5 mm, 31.0 mm and 32.0 mm, 31.5 mm on the average, in body length. Their testes were 3.0 mm or 3.5 mm in length and 2.0 mm in width (Table 9).

b. Tetraploids

Three one-year-old male tetraploids (4n 80♂, Nos. 1~3) were 33.5 mm, 32.0 mm and 34.0 mm, 33.2 mm on the average, in body length. Their left testes were 4.0~4.5 mm in length and 2.0 mm in width (Table 9).

3. Developmental capacity

a. Experimental matings, 4n 80♀, Nos. 1~7 × 4n 80♂, Nos. 1~3

In 1981, seven matings were made between seven female tetraploids (4n 80♀, Nos. 1~7) and three male tetraploids (4n 80♂, Nos. 1~3). It was found that 92.6~98.6% of 298~659 eggs in the seven matings, 3291 (96.5%) of 3412 eggs in total, cleaved normally. After 431 normally cleaved eggs died of incomplete invagination at the gastrula stage, 65.0~92.9%, 2860 (83.8%) eggs in total, became normal neurulae. At the tail-bud and hatching stages, about half of the embryos died of edema or underdevelopment, and 21.8~54.2%, 1422 (41.7%) in total, hatched normally. Eventually , 12.5~45.3%, 1131 (33.1%) in total, became normally feeding tadpoles, while 291 of the normally hatched tadpoles died of edema or underdevelopment without taking food (Table 10).

TABLE 9

Testes of mature male diploids, triploids, tetraploids and pentaploids in *Hyla arborea japonica*

| Parents | | Individual | Ploidy | Age | Body length | Size of testes | |
|---|---|---|---|---|---|---|---|
| Female | Male | no. | | years | mm | Left mm | Right mm |
| | Field caught | F. 80, No. 1 | 2n | — | — | 3.0×1.8 | 3.0×1.8 |
| | | F. 80, No. 2 | 2n | — | — | 3.0×1.5 | 3.0×2.0 |
| | | F. 80, No. 3 | 2n | — | — | 3.5×2.0 | 3.5×2.0 |
| F 80, No. 1 | F 80, No. 1 | 2n 80, No. 1 | 2n | 1 | 31.5 | 3.5×2.0 | 3.5×2.0 |
| | | 2n 80, No. 2 | 2n | 1 | 31.0 | 3.5×2.0 | 3.5×2.0 |
| | | 2n 80, No. 3 | 2n | 1 | 32.0 | 3.0×2.0 | 3.0×2.0 |
| | | 2n 80, No. 4 | 2n | 2 | 32.5 | 4.0×2.0 | 3.5×2.0 |
| | | 2n 80, No. 5 | 2n | 2 | 33.0 | 3.5×2.0 | 3.5×2.0 |
| YK. Y, W, No. 1 | F 79, No. 1 | 3n 79, No. 1 | 3n | 1 | 32.5 | 4.0×2.0 | 4.0×2.0 |
| | | 3n 79, No. 2 | 3n | 1 | 33.0 | 3.5×2.0 | 3.5×2.0 |
| YK. Y, A, No. 3 | F 79, No. 1 | 3n 79, No. 3 | 3n | 1 | 33.0 | 4.0×2.0 | 4.0×2.0 |
| 3n 79, No. 3 | F 80, No. 3 | 4n 80, No. 1 | 4n | 1 | 33.5 | 4.0×2.0 | |
| | | 4n 80, No. 2 | 4n | 1 | 32.0 | 4.5×2.0 | |
| | | 4n 80, No. 3 | 4n | 1 | 34.0 | 4.0×2.0 | |
| 4n 80, No. 3 | 4n 80, No. 2 | 4n 81, No. 1 | 4n | 1 | 33.0 | 4.5×2.0 | 4.5×2.0 |
| | | 4n 81, No. 2 | 4n | 1 | 32.5 | 4.0×2.5 | 4.0×2.0 |
| | | 4n 81, No. 3 | 4n | 1 | 34.5 | 4.0×2.0 | 4.0×2.0 |
| 4n, Nos. 1∼7 | 2n, Nos. 1∼3 | 5n 81, No. 1 | 5n | 1 | 32.0 | 4.0×2.0 | 4.0×2.0 |
| | | 5n 81, No. 2 | 5n | 1 | 32.5 | 4.0×2.0 | 4.0×2.0 |
| | | 5n 81, No. 3 | 5n | 1 | 33.5 | 4.5×2.0 | 4.5×2.0 |

TABLE 10

Reproductive capacity of tetraploids in *Hyla arborea japonica* (1981)

| Parents | | No. of eggs | No. of normal cleavages | No. of normal neurulae | No. of normally hatched tadpoles | No. of normally feeding tadpoles |
|---|---|---|---|---|---|---|
| Female | Male | | | | | |
| 4n 80, No. 1 | 4n 80, No. 1 | 659 | 650(98.6%) | 612(92.9%) | 327(49.6%) | 270(41.0%) |
| | 2n 80, No. 1 | 145 | 140(96.6%) | 123(84.8%) | 96(66.2%) | 87(60.0%) |
| 4n 80, No. 2 | 4n 80, No. 1 | 349 | 323(92.6%) | 282(80.8%) | 108(30.9%) | 82(23.5%) |
| | 2n 80, No. 1 | 343 | 312(91.0%) | 254(74.1%) | 132(38.5%) | 102(29.7%) |
| 4n 80, No. 3 | 4n 80, No. 2 | 384 | 372(96.9%) | 319(83.1%) | 208(54.2%) | 173(45.1%) |
| | 2n 80, No. 2 | 161 | 160(99.4%) | 130(80.7%) | 104(64.6%) | 88(54.7%) |
| 4n 80, No. 4 | 4n 80, No. 2 | 298 | 279(93.6%) | 224(75.2%) | 107(35.9%) | 59(19.8%) |
| | 2n 80, No. 2 | 231 | 218(94.4%) | 174(75.3%) | 137(59.3%) | 103(44.6%) |
| 4n 80, No. 5 | 4n 80, No. 3 | 657 | 633(96.3%) | 570(86.8%) | 234(35.6%) | 199(30.3%) |
| | 2n 80, No. 3 | 409 | 388(94.9%) | 238(58.2%) | 188(46.0%) | 144(35.2%) |
| 4n 80, No. 6 | 4n 80, No. 3 | 656 | 639(97.4%) | 587(89.5%) | 349(53.2%) | 297(45.3%) |
| | 2n 80, No. 3 | 277 | 264(95.3%) | 203(73.3%) | 175(63.2%) | 154(55.6%) |
| 4n 80, No. 7 | 4n 80, No. 3 | 409 | 395(96.6%) | 266(65.0%) | 89(21.8%) | 51(12.5%) |
| | 2n 80, No. 3 | 121 | 96(79.3%) | 80(66.1%) | 54(44.6%) | 40(33.1%) |
| 4n, Nos. 1∼7 | 4n, Nos. 1∼3 | 3412 | 3291(96.5%) | 2860(83.8%) | 1422(41.7%) | 1131(33.1%) |
| | 2n, Nos. 1∼3 | 1687 | 1578(93.5%) | 1202(71.3%) | 886(52.5%) | 718(42.6%) |

b. Experimental matings, 4n 80♀, Nos. 1∼7×2n 80♂, Nos. 1∼3

Seven matings were made between the above seven female tetraploids and three male diploids (2n 80♂, Nos. 1∼3). The results showed that 79.3∼99.4% of

121 ~ 409 eggs in the seven matings, 1578 (93.5%) of 1687 eggs in total, cleaved normally. After 376 eggs died of incomplete or retarded invagination at the gastrula stage, 58.2 ~ 84.8%, 1202 (71.3%) in total, became normal neurulae. At the tail-bud and hatching stages, 316 embryos died of edema or some other abnormalities, and 38.5 ~ 66.2%, 886 (52.5%) in total, hatched normally. Thereafter, 168 tadpoles died of edema or underdevelopment without taking food, and eventually, 29.7 ~ 60.0%, 718 (42.6%) in total, became normally feeding tadpoles (Table 10).

## 4. Chromosome number

a. Experimental matings, 4n 80♀, Nos. 1 ~ 7 × 4n 80♂, Nos. 1 ~ 3

i) Tetraploids

In two matings between two female tetraploids (4n 80♀, Nos. 1 and 2) and a male tetraploid (4n 80♂, No. 1), 72 of 270 and 56 of 82 feeding tadpoles were examined in chromosome number. Of these analyzed tadpoles, 62 (86.1%) and 52 (92.9%) were tetraploids. In three other matings between three female tetraploids (4n 80♀, Nos. 3 ~ 5) and a male tetraploid (4n 80♂, No. 2), 119 of 173, 26 of 59 and 141 of 199 feeding tadpoles were examined in chromosome number. Of these analyzed tadpoles, 102 (85.7%), 23 (88.5%) and 106 (75.2%) were tetraploids. In the remaining two matings between two female tetraploids (4n 80♀, Nos. 6 and 7) and a male tetraploid (4n 80♂, No. 3), 216 of 297 and 32 of 51 feeding tadpoles were examined in chromosome number. Of these analyzed tadpoles, 193 (89.4%) and 25 (78.1%) were tetraploids (Table 11).

TABLE 11

Chromosome number of the offspring between female tetraploids and male tetraploids or diploids

| Parents | | Number of tadpoles | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ana-lyzed | Number of chromosomes | | | | | | | | | Mosaics |
| Female | Male | | 31~35 (3n−) | 36 (3n) | 37~42 (3n+) | 43~47 (4n−) | 48 (4n) | 49~54 (4n+) | 60 (5n) | 61~66 (5n+) | 72 (6n) | |
| 4n 80, No. 1 | 4n 80, No. 1 | 72 | | | | 2 | 62 | | | | 1 | 7 |
| | 2n 80, No. 1 | 73 | 1 | 49 | 1 | | | | 21 | | | 1 |
| 4n 80, No. 2 | 4n 80, No. 1 | 56 | | | | 2 | 52 | | | | 2 | |
| | 2n 80, No. 1 | 52 | | 47 | 1 | | | | 2 | | | 2 |
| 4n 80, No. 3 | 4n 80, No. 2 | 119 | | | | 4 | 102 | | | | 11 | 2 |
| | 2n 80, No. 2 | 27 | 1 | 21 | | | | | 5 | | | |
| 4n 80, No. 4 | 4n 80, No. 2 | 26 | | | | | 23 | | | | 3 | |
| | 2n 80, No. 2 | 30 | | 20 | 3 | | | | 7 | | | |
| 4n 80, No. 5 | 4n 80, No. 2 | 141 | | | | 4 | 106 | 1 | | 1 | 18 | 11 |
| | 2n 80, No. 2 | 49 | | 35 | 2 | | | | 12 | | | |
| 4n 80, No. 6 | 4n 80, No. 3 | 216 | | | | 9 | 193 | 3 | | 1 | 9 | 1 |
| | 2n 80, No. 3 | 97 | | 88 | 3 | | | | 4 | | | 2 |
| 4n 80, No. 7 | 4n 80, No. 3 | 32 | | | | 2 | 25 | | | | 3 | 2 |
| | 2n 80, No. 3 | 20 | | 11 | | | | | 8 | | | 1 |
| 4n, Nos. 1 ~ 7 | 4n, Nos. 1 ~ 3 | 662 | | | | 23 | 563 | 4 | | 2 | 47 | 23 |
| | 2n, Nos. 1 ~ 3 | 348 | 2 | 271 | 10 | | | | 59 | | | 6 |

In total, 662 of 1130 normally feeding tadpoles obtained from the seven matings between seven female tetraploids and three male tetraploids were analyzed in chromosome number. It was found that 563 (85.0%) of them were tetraploids.

ii) Hexaploids

Of the 662 analyzed tadpoles stated above, 47 (7.1%) were hexaploids. It was found that 1.4%, 3.6%, 9.2%, 11.5%, 12.8%, 4.2% and 9.4%, 7.1% on the average, of the respective number of analyzed tadpoles were hexaploids in seven matings Nos. 1～7 between seven female tetraploids and three male tetraploids, respectively (Table 11).

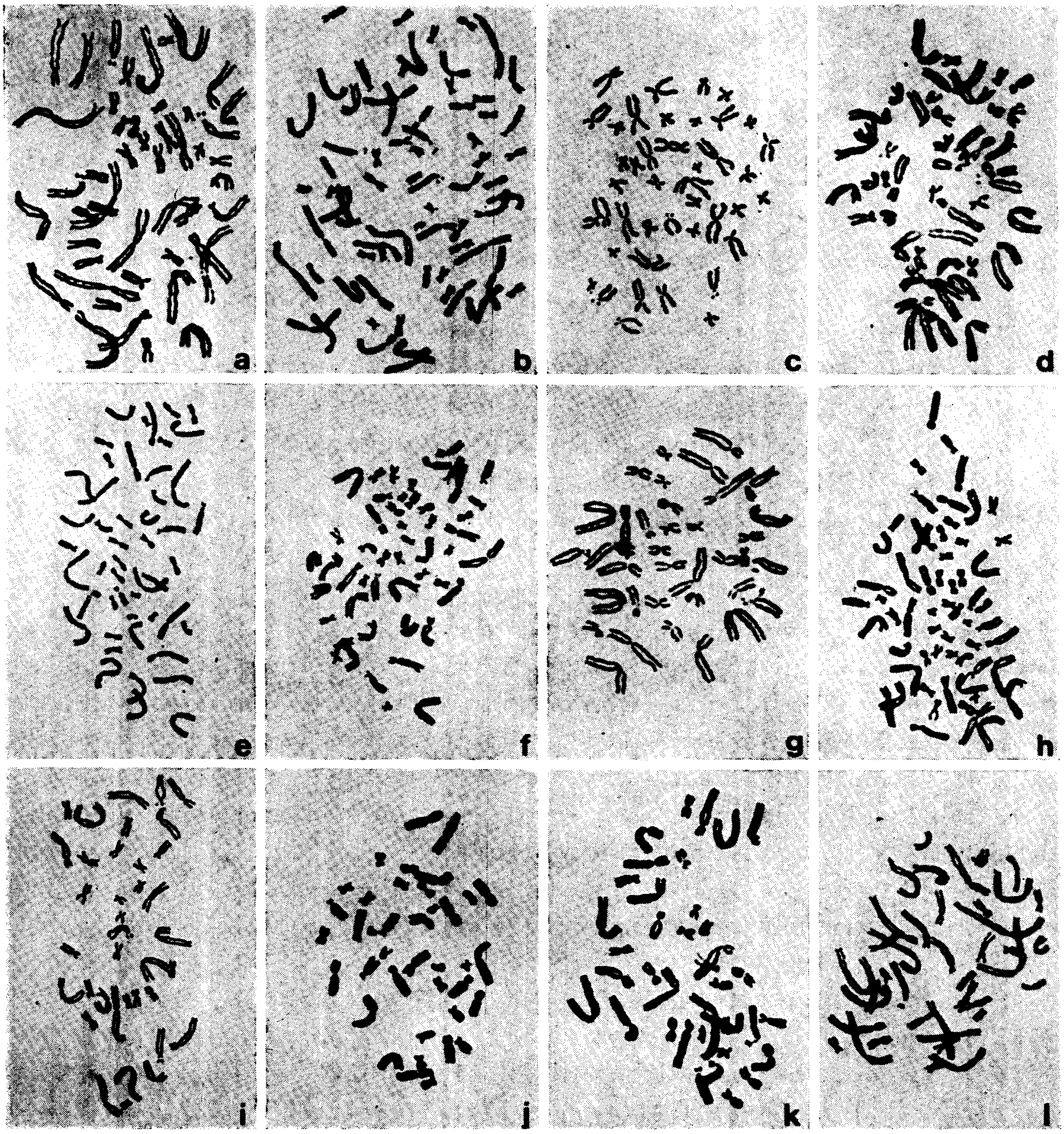


Fig. 3. Chromosomes of normally feeding tadpoles in the offspring between tetraploid females and tetraploid or diploid males. ×750

a～f. Offspring between tetraploid females and tetraploid males

a. 48 (4n) b. 72 (6n) c. 42 (3n+6) d. 45 (4n−3) e. 49 (4n+1) f. 50 (4n+2)

g～l. Offspring between tetraploid females and diploid males

g. 36 (3n) h. 60 (5n) i. 37 (3n+1) j. 38 (3n+2) k. 39 (3n+3) l. 40 (3n+4)

iii) Aneuploids

Of the 662 analyzed tadpoles, 29 (4.4%) were aneuploids. Of the latter, 23 (3.5%) were hypotetraploids having 43～47 chromosomes and four others (0.6%) were hypertetraploids having 49～54 chromosomes. The other two aneuploids were hyperpentaploids having 66 (5n+6) chromosomes. More specifically, these aneuploids were made up as follows.

The 23 hypotetraploids included five having 44 (4n−4), five having 45 (4n−3), eight having 46 (4n−2) and five having 47 (4n−1) chromosomes. Of the five hypotetraploids having 44 chromosomes, three were obtained from tetraploid females Nos. 2, 3 and 5 and the other two were from tetraploid female No. 6. Of the five hypotetraploids having 45 chromosomes, three were obtained from tetraploid female No. 6 and the other two were from tetraploid females Nos. 3 and 7. Of the eight hypotetraploids having 46 (4n−2) chromosomes, two were obtained from tetraploid females Nos. 1 and 7, and six were two each from tetraploid females Nos. 3, 5 and 6. Of the five hypotetraploids having 47 (4n−1) chromosomes, three were obtained from tetraploid females Nos. 1, 2 and 5, and two were from tetraploid female No. 6.

The four hypertetraploids included two having 49 (4n+1) chromosomes and two having 50 (4n+2) chromosomes. Of the latter two, one was obtained from tetraploid female No. 5, and the other was from tetraploid female No. 6.

The two hyperpentaploids were obtained from tetraploid females Nos. 5 and 6.

iv) Mosaics

Of the 662 analyzed tadpoles stated above, 23 (3.5%) were chromosome mosaics. They included three 2n-4n, seven 3n-4n, five 2n-6n, one 3n-6n and seven 4n-6n mosaics. The three 2n-4n mosaics were obtained from tetraploid females Nos. 1, 3 and 5. Of the seven 3n-4n mosaics, six were obtained by threes from tetraploid females Nos. 1 and 5, and the remainder was from tetraploid female No. 3. Of the five 2n-6n mosaics, two were obtained from tetraploid female No. 5, one was from No. 6 and two were from No. 7. The single 3n-6n mosaic was obtained from tetraploid female No. 5. Of the seven 4n-6n mosaics, three were obtained from tetraploid female No. 1 and four were from No. 5.

b. Experimental matings, 4n 80♀, Nos. 1～7×2n 80♂, Nos. 1～3

i) Triploids

Of 718 normally feeding tadpoles produced from seven matings between seven female tetraploids (4n 80♀, Nos. 1～7) and three male diploids (2n 80♂, Nos. 1～3), the chromosomes of 348 were analyzed in the tail-tips by the water-pretreatment squash method. It was found that 49 (67.1%) of 73, 47 (90.4%) of 52, 21 (77.8%) of 27, 20 (66.7%) of 30, 35 (71.4%) of 49, 88 (90.7%) of 97 and 11 (55.0%) of 20 analyzed tadpoles, 271 (77.9%) of 348 in total, were triploids in the seven matings, respectively (Table 11).

ii) Pentaploids

Of the 348 analyzed tadpoles stated above, 59 (17.0%) were pentaploids. In matings Nos. 1∼7, 21 (28.8%), 2 (3.8%), 5 (18.5%), 7 (23.3%), 12 (24.5%), 4 (4.1%) and 8 (40.0%) were pentaploids, respectively.

iii) Aneuploids

Of the 348 analyzed tadpoles, 12 were aneuploids including two (0.6%) hypotriploids and 10 (2.9%) hypertriploids. The two hypotriploids had 35 (3n−1) chromosomes and had been produced from tetraploid females Nos. 1 and 3. Of the ten hypertriploids, five had 37 (3n+1), two had 38 (3n+2), two had 39 (3n+3) and the remainder had 40 (3n+4) chromosomes.

iv) Mosaics

Six (1.7%) of the 348 analyzed tadpoles were chromosome mosaics. These mosaics included three 3n-5n, one hypertriploid-7n, one 5n-10n and one 3n-*x*n mosaics. Two of the three 3n-5n mosaics were obtained from tetraploid female No. 2, while the other was from No. 7. The hypertriploid-7n mosaic was a mixture of 42 and 84 in chromosome number and obtained from tetraploid female No. 1. The 5n-10n and 3n-*x*n mosaics were obtained from tetraploid female No. 6. The *x*n shows that the chromosomes were too numerous to be exactly counted.

## 5. Metamorphosis and sex ratio

a. Experimental matings, 4n 80♀, Nos. 1∼7 × 4n 80♂, Nos. 1∼3

Of 563 tetraploid tadpoles produced from seven matings between seven female and three male tetraploids, 351 metamorphosed normally. The sex of 181 normally metamorphosed frogs which died or were killed within three months after metamorphosis was examined. The results indicated that 90 were females and 91 (50.3%) were males. Of the remaining tetraploid frogs, 143 attained sexual maturity at the age of one year. Of these mature tetraploids, 78 were females and 65 (45.5%) were males. In total, 168 of 324 immature and mature

TABLE 12

Sex of triploid, tetraploid and pentaploid offspring between female tetraploids and male diploids or tetraploids

| Parents | | Ploidy | No. of metamorphosed frogs | Sex of immature frogs | | | Sex of mature frogs | | | Sex of all frogs | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Female | Male | | | No. of frogs | ♀ | ♂ | No. of frogs | ♀ | ♂ | Total | ♀ | ♂ (%) |
| 4n 80, No. 1 | 2n 80, No. 1 | 3n | 23 | 23 | 10 | 13 | — | — | — | 23 | 10 | 13(56.5) |
| | 4n 80, No. 1 | 4n | 53 | 16 | 7 | 9 | 35 | 19 | 16 | 51 | 26 | 25(49.0) |
| 4n 80, No. 2 | 2n 80, No. 1 | 3n | 28 | 9 | 5 | 4 | 19 | 8 | 11 | 28 | 13 | 15(53.6) |
| | 4n 80, No. 1 | 4n | 23 | 23 | 13 | 10 | — | — | — | 23 | 13 | 10(43.5) |
| 4n 80, No. 3 | 2n 80, No. 2 | 3n | 15 | 15 | 7 | 8 | — | — | — | 15 | 7 | 8(53.3) |
| | 4n 80, No. 2 | 4n | 50 | 12 | 6 | 6 | 38 | 20 | 18 | 50 | 26 | 24(48.0) |
| 4n 80, No. 4 | 2n 80, No. 2 | 3n | 16 | 16 | 7 | 9 | — | — | — | 16 | 7 | 9(56.3) |
| | 4n 80, No. 2 | 4n | 15 | 15 | 6 | 9 | — | — | — | 15 | 6 | 9(60.0) |
| 4n 80, No. 5 | 2n 80, No. 3 | 3n | 34 | 6 | 4 | 2 | 26 | 10 | 16 | 32 | 14 | 18(56.3) |
| | 4n 80, No. 3 | 4n | 76 | 46 | 22 | 24 | 26 | 16 | 10 | 72 | 38 | 34(47.2) |
| 4n 80, No. 6 | 2n 80, No. 3 | 3n | 51 | 12 | 6 | 6 | 35 | 17 | 18 | 47 | 23 | 24(51.1) |
| | 4n 80, No. 3 | 4n | 118 | 53 | 27 | 26 | 44 | 23 | 21 | 97 | 50 | 47(48.5) |
| 4n 80, No. 7 | 2n 80, No. 3 | 3n | 11 | 11 | 6 | 5 | — | — | — | 11 | 6 | 5(45.5) |
| | 4n 80, No. 3 | 4n | 16 | 16 | 9 | 7 | — | — | — | 16 | 9 | 7(43.8) |
| 4n, Nos. 1∼7 | 2n, Nos. 1∼3 | 3n | 178 | 92 | 45 | 47 | 80 | 35 | 45 | 172 | 80 | 92(53.5) |
| | | 5n | 40 | 27 | 13 | 14 | 10 | 4 | 6 | 37 | 17 | 20(54.1) |
| 4n, Nos. 1∼7 | 4n, Nos. 1∼3 | 4n | 351 | 181 | 90 | 91 | 143 | 78 | 65 | 324 | 168 | 156(48.1) |

tetraploids were females and 156 (48.1%) were males. The sex of the remaining 27 of the metamorphosed tetraploids was unknown (Table 12).

Of 47 hexaploid tadpoles produced from the above seven matings, only three could complete metamorphosis. These metamorphosed hexaploids were undergrown and remarkably smaller than tetraploids and triploids. They died without taking food. The sex of them was not identified owing to postmortem changes.

All 29 aneuploid tadpoles produced from the above seven matings could not metamorphose. Twenty-three mosaics were not reared after the chromosomes were examined.

b. Experimental matings, 4n 80♀, Nos. 1~7×2n 80♂, Nos. 1~3

Of 271 triploid tadpoles produced from the above seven matings between seven female tetraploids and three male diploids, 178 metamorphosed normally. Of 92 normally metamorphosed triploids which died or were killed within three months after metamorphosis, 45 were females and 47 (51.1%) were males. Of 80 one-year-old mature triploids, 35 were females and 45 (56.3%) were males. In total, 80 of 172 immature and mature triploids were females and the other 92 (53.5%) were males (Table 12). The sex of the remaining six metamorphosed triploids was unknown.

Of 59 pentaploids produced from the above seven matings, 40 metamorphosed normally and 27 of them died within three months after metamorphosis. Thirteen of them were females and 14 (51.9%) were males. Of 10 one-year-old mature pentaploids, four were females and six (60.0%) were males. In total, 17 of 37 immature and mature pentaploids were females and 20 (54.1%) were males (Table 12).

Two hypotriploids and 10 hypertriploids produced from the seven matings, could not complete metamorphosis. Six mosaics were not reared after their chromosomes were examined.

## 6. Body length

a. Tetraploids

Twelve, 15, 10 and 15 mature female tetraploids produced from four matings, 4n 80♀, No. 1×4n 80♂, No. 1, 4n 80♀, No. 3×4n 80♂, No. 2, 4n 80♀, No. 5×4n 80♂, No. 3 and 4n 80♀, No. 6×4n 80♂, No. 3, respectively, were measured at the age of 1.5 years. It was found that they were 36.8 mm, 36.7 mm, 36.2 mm and 37.1 mm in mean body length, respectively. A total of 52 female tetraploids were 33.0~38.5 mm, 36.7 mm on the average, in body length.

Thirteen, 15, 6 and 15 mature male tetraploids produced from the same four matings as the above were measured at the age of 1.5 years. They were 33.0 mm, 33.3 mm, 33.9 mm and 34.2 mm in mean body length, respectively, and 30.0~35.0 mm, 33.6 mm on the average, in a total of 49 male tetraploids (Table 13).

b. Triploids

Six, seven and 15 mature female triploids produced from three matings, 4n

TABLE 13

Body lengths of mature triploid, tetraploid and pentaploid offspring between female tetraploids and male diploids or tetraploids

| Parents | | Ploidy | Female | | Male | |
|---|---|---|---|---|---|---|
| Female | Male | | No. of frogs | Body length (Mean) mm | No. of frogs | Body length (Mean) mm |
| 4n 80, No. 1 | 4n 80, No. 1 | 4n | 12 | 35.0~37.5 (36.8±0.20) | 13 | 30.0~34.5 (33.0±0.31) |
| 4n 80, No. 2 | 2n 80, No. 1 | 3n | 6 | 35.5~37.0 (36.5±0.25) | 7 | 30.5~34.0 (32.0±0.30) |
| 4n 80, No. 3 | 4n 80, No. 2 | 4n | 15 | 35.0~38.0 (36.7±0.31) | 15 | 31.5~35.0 (33.3±0.38) |
| 4n 80, No. 5 | 2n 80, No. 3 | 3n | 7 | 32.5~37.0 (36.0±0.37) | 12 | 29.5~33.0 (31.5±0.40) |
| | 4n 80, No. 3 | 4n | 10 | 33.0~37.5 (36.2±0.38) | 6 | 30.5~34.0 (33.9±0.25) |
| 4n 80, No. 6 | 2n 80, No. 3 | 3n | 15 | 35.0~37.5 (36.8±0.31) | 18 | 31.0~35.0 (33.5±0.22) |
| | 4n 80, No. 3 | 4n | 15 | 35.5~38.5 (37.1±0.27) | 15 | 32.0~35.0 (34.2±0.20) |
| 4n, Nos. 1~7 | 2n, Nos. 1~3 | 5n | 4 | 32.0~35.5 (34.2±0.80) | 6 | 28.5~33.5 (32.0±0.71) |

80♀, No. 2×2n 80♂, No. 1, 4n 80♀, No. 5×2n 80♂, No. 3 and 4n 80♀, No. 6×2n 80♂, No. 3, respectively, were measured at the age of 1.5 years. The results showed that they were 36.5 mm, 36.0 mm and 36.8 mm in mean body length, respectively, and 32.5~37.5 mm, 36.4 mm on the average, in a total of 28 mature female triploids.

Seven, 12 and 18 mature male triploids produced from the same three matings as the above were 32.0 mm, 31.5 mm and 33.5 mm in mean body length, respectively, and 29.5~35.0 mm, 32.3 mm on the average, in a total of 37 mature male triploids (Table 13).

c. Pentaploids

Four mature female pentaploids produced from among the above seven matings between seven female tetraploids and three male diploids were 32.0~35.5 mm, 34.2 mm on the average, in body length at the age of 1.5 years. Six mature male pentaploids produced from the same seven matings were 28.5~33.5 mm, 32.0 mm on the average, in body length.

### *IV. Second-generation offspring of tetraploids*

In the breeding season of 1982, second-generation offspring of tetraploids were produced from matings among two one-year-old female tetraploids (4n 81♀, Nos. 1 and 2) of the first-generation offspring obtained in 1981 from a mating, 4n 80♀, No. 6×4n 80♂, No. 3 and three one-year-old male tetraploids (4n 81♂, Nos. 1, 2 and 3) of the first-generation offspring obtained in 1981 from a mating, 4n 80♀, No. 3×4n 80♂, No. 2, and among three 2-year-old female diploids (2n

80♀, Nos. 4～6) and two 2-year-old male diploids (2n 80♂, Nos. 4 and 5) obtained in 1980 from a control matings, F 80♀, No. 1 × F 80♂, No. 1.

1. Females used in the experiments of 1982

a. Control diploids

Three 2-year-old female diploids (2n 80♀, Nos. 4～6) used in the mating experiments of 1982 were 36.5 mm, 37.0 mm and 37.0 mm, 36.8 mm on the average, in body length. By injection of a mixture of bullfrog pituitaries and gonadotropin, these three females laid 1052, 923 and 788 eggs, 921 eggs on the average. When 20 or 30 eggs of each female were measured, they were 1.22 mm, 1.21 mm and 1.21 mm, 1.21 mm on the average, in diameter (Table 8).

b. Tetraploids

Two one-year-old female tetraploids (4n 81♀, Nos. 1 and 2) used in the mating experiments of 1982 were selected from among six one-year-old female tetraploids (4n 81♀, Nos. 1～6) obtained in 1981 from a mating, 4n 80♀, No. 6 × 4n 80♂, No. 3 (Table 8). These six female tetraploids were 38.5 mm, 37.0 mm, 37.5 mm, 36.5 mm, 36.0 mm and 37.0 mm, 37.1 mm on the average, in body length. After injection of a mixture of bullfrog pituitaries and gonadotropin, ovulation occurred normally in four of the six female tetraploids, while it partly occurred in the other two. These six females laid 521, 1154, 726, 785, 216 and 62 eggs, respectively. When 20 or 30 eggs of each of three female tetraploids (4n 80♀, Nos. 1～3) were measured, they were 1.34 mm, 1.34 mm or 1.36 mm in mean diameter. The eggs of these three females were 1.35 mm on the average.

2. Males used in the experiments of 1982

a. Control diploids

Two 2-year-old male diploids (2n 80♂, Nos. 4 and 5) used in the experiments of 1982 were 32.5 mm and 33.0 mm in body length. The left testes of these males were 4.0 mm or 3.5 mm in length and 2.0 mm in width, while the right testes were 3.5 mm in length and 2.0 mm in width.

b. Tetraploids

Three one-year-old male tetraploids (4n 81♂, Nos. 1～3) obtained in 1981 from 4n 80♀, No. 3 × 4n 80♂, No. 2 were 33.0 mm 32.5 mm and 34.5 mm, 33.3 mm on the average, in body length. The left testes of these males were 4.0 or 4.5 mm in length and 2.0 or 2.5 mm in width, while the right testes were 4.0 or 4.5 mm in length and 2.0 mm in width (Table 9).

3. Developmental capacity

a. Control matings, 2n 80♀, Nos. 4～6 × 2n 80♂, Nos. 4 and 5

Three control matings were made between three 2-year-old female diploids (2n 80♀, Nos. 4～6) and two 2-year-old male diploids (2n 80♂, Nos. 4 and 5). It was found that 88.4～91.7% of eggs, 381 (89.6%) of 425 eggs in total, cleaved normally. After eight of the normally cleaved eggs became abnormal neurulae owing to incomplete invagination and nine others died of edema or some other abnormalities at the hatching stage, 83.1～88.0%, 364 (85.6%) in total, hatched normally. While seven died of edema without taking food, 81.0～87.0%, 357 (84.0%) in total, became normally feeding tadpoles (Table 14).

TABLE 14
Developmental capacity of the offspring produced in 1982 from male or female first-generation tetraploids

| Parents: Female | Parents: Male | No. of eggs | No. of normal cleavages | No. of normal neurulae | No. of normally hatched tadpoles | No. of normally feeding tadpoles |
|---|---|---|---|---|---|---|
| 2n 80, No. 4 | 2n 80, No. 4 | 108 | 99(91.7%) | 99(91.7%) | 95(88.0%) | 94(87.0%) |
| | 4n 81, No. 1 | 156 | 139(89.1%) | 137(87.8%) | 127(81.4%) | 102(65.4%) |
| | 4n 81, No. 2 | 193 | 180(93.3%) | 176(91.2%) | 169(87.6%) | 132(68.4%) |
| | 4n 81, No. 3 | 159 | 156(98.1%) | 145(91.2%) | 114(71.7%) | 87(54.7%) |
| 2n 80, No. 5 | 2n 80, No. 4 | 189 | 167(88.4%) | 160(84.7%) | 157(83.1%) | 153(81.0%) |
| | 4n 81, No. 1 | 116 | 93(80.2%) | 91(78.4%) | 85(73.3%) | 69(59.5%) |
| | 4n 81, No. 2 | 81 | 61(75.3%) | 59(72.8%) | 54(66.7%) | 42(51.9%) |
| 2n 80, No. 6 | 2n 80, No. 5 | 128 | 115(89.8%) | 114(89.1%) | 112(87.5%) | 110(85.9%) |
| | 4n 81, No. 3 | 175 | 156(89.1%) | 148(84.6%) | 123(70.3%) | 103(58.9%) |
| 4n 81, No. 1 | 2n 80, No. 4 | 219 | 213(97.3%) | 184(84.0%) | 161(73.5%) | 129(58.9%) |
| | 4n 81, No. 1 | 95 | 93(97.9%) | 85(89.5%) | 78(82.1%) | 59(62.1%) |
| | 4n 81, No. 2 | 126 | 119(94.4%) | 111(88.1%) | 107(84.9%) | 88(69.8%) |
| 4n 81, No. 2 | 2n 80, No. 5 | 486 | 482(99.2%) | 437(89.9%) | 335(68.9%) | 311(64.0%) |
| | 4n 81, No. 1 | 255 | 253(99.2%) | 232(91.0%) | 138(54.1%) | 113(44.3%) |
| | 4n 81, No. 2 | 161 | 160(99.4%) | 146(90.7%) | 94(58.4%) | 91(56.5%) |
| | 4n 81, No. 3 | 209 | 204(97.6%) | 164(78.5%) | 110(52.6%) | 101(48.3%) |
| 2n, Nos. 4～6 | 2n, Nos. 4, 5 | 425 | 381(89.6%) | 373(87.8%) | 364(85.6%) | 357(84.0%) |
| | 4n, Nos. 1～3 | 880 | 785(89.2%) | 756(85.9%) | 672(76.4%) | 535(60.8%) |
| 4n, Nos. 1, 2 | 2n, Nos. 4, 5 | 705 | 695(98.6%) | 621(88.1%) | 496(70.4%) | 440(62.4%) |
| | 4n, Nos. 1～3 | 846 | 829(98.0%) | 738(87.2%) | 527(62.3%) | 452(53.4%) |

b. Experimental matings, 2n 80♀, Nos. 4～6×4n 81♂, Nos. 1～3

Six matings were made between the three 2-year-old female diploids (2n 80♀, Nos. 4～6) used in the above control matings and three one-year-old male tetraploids (4n 81♂, Nos. 1～3). It was found that 75.3～98.1%, 785 (89.2%) of 880 eggs in total, cleaved normally, while 58 eggs did abnormally. After 29 of the normally cleaved eggs became abnormal neurulae owing to incomplete invagination at the gastrula stage and thereafter 84 others died of various abnormalities, 66.7～87.6%, 672 (76.4%) in total, hatched normally. While 137 tadpoles died without taking food, 51.9～68.4%, 535 (60.8%) in total, became normally feeding tadpoles (Table 14).

c. Experimental matings, 4n 81♀, Nos. 1 and 2×2n 80♂, Nos. 4 and 5

Two one-year-old female tetraploids (4n 81♀, Nos. 1 and 2) were mated with two 2-year-old male diploids (2n 80♂, Nos. 4 and 5). The results showed that 695 (98.6%) of 705 eggs cleaved normally, while only three cleaved abnormally.

After 74 of the normally cleaved eggs became abnormal neurulae owing to incomplete invagination and 125 others died of various abnormalities at the hatching stage, 496 (70.4%) hatched normally. While 56 tadpoles died without taking food, 440 (62.4%) became normally feeding tadpoles (Table 14).

d. Experimental matings, 4n 81♀, Nos. 1 and 2×4n 81♂, Nos. 1∼3

Five matings were made between two one-year-old female tetraploids (4n 81♀, Nos. 1 and 2) and three one-year-old male tetraploids (4n 81♂, Nos. 1∼3). It was found that 94.4∼99.4%, 829 (98.0%) of 846 eggs in total, cleaved normally, while only 12 eggs did abnormally. After 91 of the normally cleaved eggs became abnormal neurulae owing to incomplete invagination at the gastrula stage and 211 others died of various abnormalities at the hatching stage, 52.6∼84.9%, 527 (62.3%) in total, hatched normally. While 75 tadpoles died of edema or underdevelopment without taking food, 44.3∼69.8%, 452 (53.4%) in total, became normally feeding tadpoles (Table 14).

## 4. Chromosome number

a. Control matings, 2n 80♀, Nos. 4∼6×2n 80♂, Nos. 4 and 5

Chromosomes were examined in a total of 120 of 357 normally feeding tadpoles, including 50 of 94 produced from 2n 80♀, No. 4×2n 80♂, No. 4, 50 of 153

TABLE 15

Chromosome number of the offspring produced in 1982 from male or female first-generation tetraploids

| Parents | | Number of tadpoles | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Number of chromosomes | | | | | | | | | |
| Female | Male | Analyzed | 24 (2n) | 36 (3n) | 37∼42 (3n+) | 43∼47 (4n−) | 48 (4n) | 49∼54 (4n+) | 60 (5n) | 72 (6n) | Mosaics |
| 2n 80, No. 4 | 2n 80, No. 4 | 50 | 48 | 2 | | | | | | | |
| | 4n 81, No. 1 | 66 | | 63 | 1 | 1 | 1 | | | | |
| | 4n 81, No. 2 | 69 | | 66 | | | 3 | | | | |
| | 4n 81, No. 3 | 73 | | 72 | | | 1 | | | | |
| 2n 80, No. 5 | 2n 80, No. 4 | 50 | 50 | | | | | | | | |
| | 4n 81, No. 1 | 49 | | 48 | | 1 | | | | | |
| | 4n 81, No. 2 | 31 | | 30 | | | | | | | 1 |
| 2n 80, No. 6 | 2n 80, No. 5 | 20 | 20 | | | | | | | | |
| | 4n 81, No. 3 | 63 | | 63 | | | | | | | |
| 4n 81, No. 1 | 2n 80, No. 4 | 63 | | 62 | 1 | | | | | | |
| | 4n 81, No. 1 | 48 | | | | | 47 | | | | 1 |
| | 4n 81, No. 2 | 55 | | | | | 55 | | | | |
| 4n 81, No. 2 | 2n 80, No. 5 | 167 | | 158 | 2 | | | | 4 | | 3 |
| | 4n 81, No. 1 | 62 | | | | | 59 | 1 | | 1 | 1 |
| | 4n 81, No. 2 | 52 | | | | | 51 | | | | 1 |
| | 4n 81, No. 3 | 55 | | | | 1 | 53 | | | 1 | |
| 2n, Nos. 4∼6 | 2n, Nos. 4, 5 | 120 | 118 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 4n, Nos. 1∼3 | 351 | 0 | 342 | 1 | 2 | 5 | 0 | 0 | 0 | 1 |
| 4n, Nos. 1, 2 | 2n, Nos. 4, 5 | 230 | 0 | 220 | 3 | 0 | 0 | 0 | 4 | 0 | 3 |
| | 4n, Nos. 1∼3 | 272 | 0 | 0 | 0 | 1 | 265 | 1 | 0 | 2 | 3 |

produced from 2n 80♀, No. 5×2n 80♂, No. 4 and 20 of 110 produced from 2n 80♀, No. 6×2n 80♂, No. 5. The results indicated that 118 (98.3%) of them were diploids. The remaining two which had been obtained from 2n 80♀, No. 4×2n 80♂, No. 4 were triploids (Table 15).

b. Experimental matings, 2n 80♀, Nos. 4~6×4n 81♂, Nos. 1~3

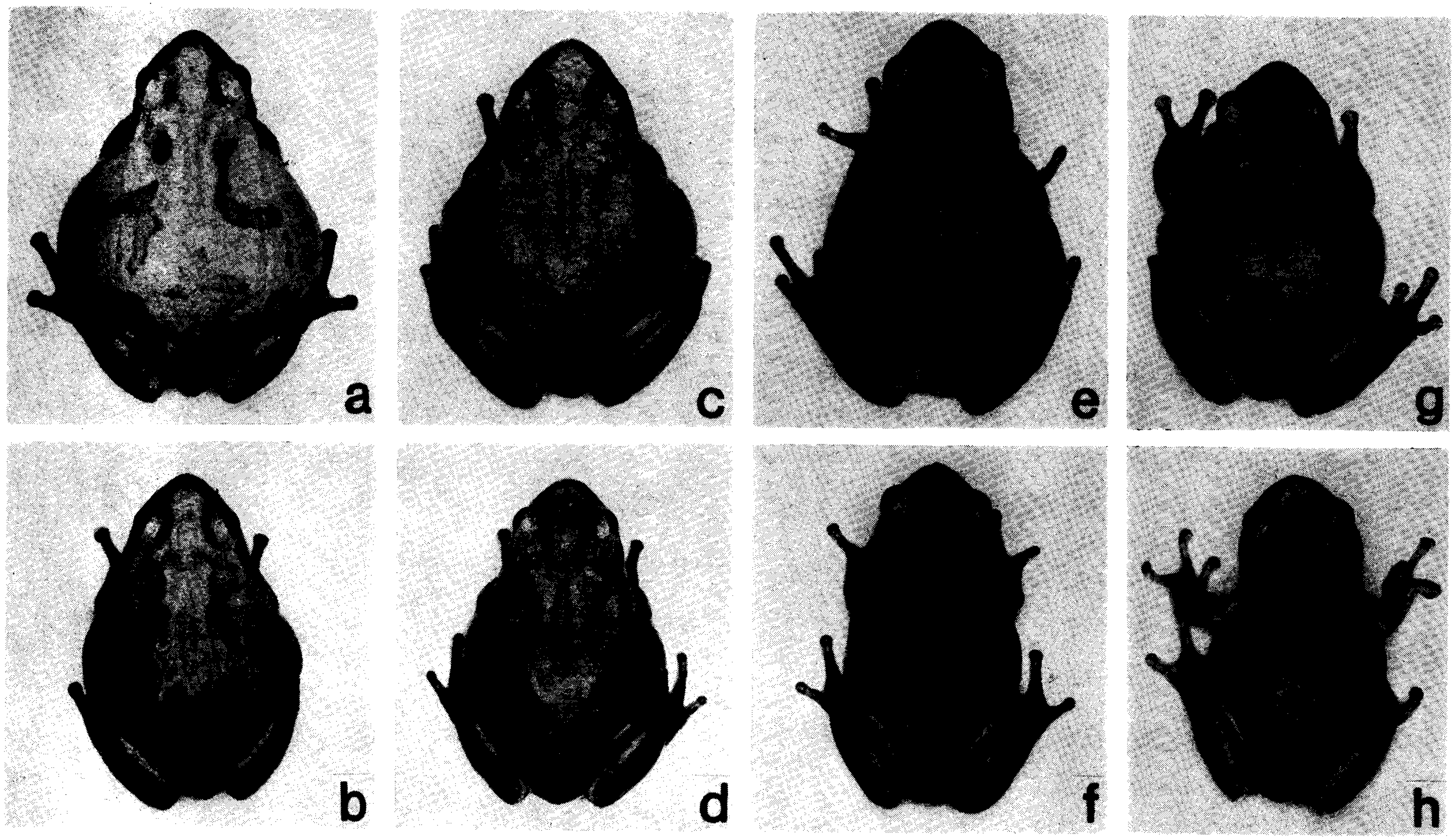
Chromosomes were examined in a total of 351 of 535 normally feeding tadpoles, including 66 of 102, 69 of 132 and 73 of 87 produced from 2n 80♀, No. 4×4n 81♂, Nos. 1~3, 49 of 69 and 31 of 42 produced from 2n 80♀, No. 5×4n 81♂, Nos. 1 and 2 and 63 of 103 produced from 2n 80♀, No. 6×4n 81♂, No. 3. It was found that 342 (97.4%) were triploids, five (1.4%) were tetraploids, three (0.9%) were aneuploids and the remainder was a chromosome mosaic (Table 15). Of the three aneuploids, one had 41 (3n+5) chromosomes and another had 46 (4n−2) chromosomes. These two were produced from a mating, 2n 80♀, No. 4×4n 81♂, No. 1. The remaining aneuploid had 46 (4n−2) chromosomes and had been produced from 2n 80♀, No. 5×4n 81♂, No. 1. The single mosaic consisted of a mixture of diploid and triploid cells and was produced from 2n 80♀, No. 5×4n 81♂, No. 2.

c. Experimental matings, 4n 81♀, Nos. 1 and 2×2n 80♂, Nos. 4 and 5

Chromosomes were analyzed in a total of 230 of 440 normally feeding tadpoles, including 63 of 129 produced from 4n 81♀, No. 1×2n 80♂, No. 4 and 167 of 311 produced from 4n 81♀, No. 2×2n 80♂, No. 5. It was found that 220 (95.7%) were triploids, four (1.7%) were pentaploids, three (1.3%) were aneuploids and the remaining three were mosaics (Table 15). Of the three aneuploids, one obtained from 4n 81♀, No. 1×2n 80♂, No. 4 had 38 (3n+2) chromosomes, while the other two obtained from 4n 81♀, No. 2×2n 80♂, No. 5, had 37 (3n+1) chromosomes. The three mosaics were all constructed of a mixture of triploid and pentaploid cells. They were produced from a mating, 4n 81♀, No. 2×2n 80♂, No. 5.

d. Experimental matings, 4n 81♀, Nos. 1 and 2×4n 81♂, Nos. 1~3

Chromosomes were analyzed in a total of 272 of 452 normally feeding tadpoles, including 48 of 59 produced from 4n 81♀, No. 1×4n 81♂, No. 1, 55 of 88 produced from 4n 81♀, No. 1×4n 81♂, No. 2, 62 of 113 produced from 4n 81♀, No. 2×4n 81♂, No. 1, 52 of 91 produced from 4n 81♀, No. 2×4n 81♂, No. 2 and 55 of 101 produced from 4n 81♀, No. 2×4n 81♂, No. 3. The results showed that 265 (97.4%) were tetraploids, two (0.7%) were hexaploids, two were aneuploids and the remaining three (1.1%) were mosaics (Table 15). Of the two aneuploids, one obtained from 4n 81♀, No. 2×4n 81♂, No. 1 had 50 (4n+2) chromosomes, while the other obtained from 4n 81♀, No. 2×4n 81♂, No. 3 had 46 (4n−2) chromosomes. The three mosaics were all constructed of a mixture of tetraploid and hexaploid cells. They were produced from three matings, 4n 81♀, No. 1×4n 81♂, No. 1, 4n 81♀, No. 2×4n 81♂, No. 1 and 4n 81♀, No. 2×4n 81♂, No. 2.




×1.2

Fig. 4. Diploid, triploid, tetraploid and pentaploid treefrogs, 1.5 years old.

a, b. Female and male diploids produced from 2n 80♀, No. 1×2n 80,♂ No. 1
c, d. Female and male triploids produced from 4n 80♀, No. 6×2n 80♂, No. 3
e, f. Female and male tetraploids produced from 4n 80♀, No. 6×4n 80♂, No. 3
g, h. Female and male pentaploids produced from 4n 80♀×4n 80♂

### 5. Metamorphosis

a. Control matings

i) 2n 80♀, No. 4 × 2n 80♂, No. 4

Of 48 of 50 diploid tadpoles and the two triploid ones produced from this mating, 38 and one completed metamorphosis, respectively.

ii) 2n 80♀, No. 5 × 2n 80♂, No.4

A total of 153 normally feeding tadpoles produced from this mating were all regarded as diploids and continuously reared, as 50 of them were confirmed to be diploids by chromosome analysis. It was found that 126 of them metamorphosed normally.

iii) 2n 80♀, No. 6 × 2n 80♂, No. 5

A total of 110 normally feeding tadpoles produced from this mating were all regarded as diploids and continuously reared, as 20 of them were confirmed to be diploids by chromosome analysis. The results showed that 81 of them completed metamorphosis.

b. Experimental matings, 2n 80♀, Nos. 4∼6 × 4n 81♂, Nos. 1∼3

A total of 254 of 342 normally feeding triploid tadpoles, including 46 of 63, 45 of 66 and 62 of 72 triploids produced from three matings, 2n 80♀, No. 4 × 4n 81♂, Nos. 1∼3, 31 of 48 and 22 of 30 triploids produced from two matings, 2n 80♀, No. 5 × 4n 81♂, Nos. 1 and 2, and 48 of 63 triploids produced from a mating, 2n 80♀, No. 6 × 4n 81♂, No. 3, metamorphosed normally.

c. Experimental matings, 4n 81♀, Nos. 1 and 2 × 2n 80♂, Nos. 4 and 5

A total of 151 of 220 normally feeding triploid tadpoles produced from two matings including 43 of 62 produced from 4n 81♀, No. 1 × 2n 80♂, No. 4, and 108 of 158 produced from 4n 81♀, No. 2 × 2n 80♂, No. 5, metamorphosed normally. Four pentaploids all completed metamorphosis.

d. Experimental matings, 4n 81♀, Nos. 1 and 2 × 4n 81♂, Nos. 1∼3

A total of 182 of 265 tetraploids, including 36 of 47 produced from 4n 81♀, No. 1 × 4n 81♂, No. 1, 38 of 55 produced from 4n 81♀, No. 1 × 4n 81♂, No. 2, 41 of 59 produced from 4n 81♀, No. 2 × 4n 81♂, No. 1, 28 of 51 produced from 4n 81♀, No. 2 × 4n 81♂, No. 2 and 39 of 53 produced from 4n 81♀, No. 2 × 4n 81♂, No. 3 metamorphosed normally.

Two hexaploids, two aneuploids and three chromosome mosaics all could not complete metamorphosis.

## *V. Morphology of polyploids*

### 1. External characters

*Hyla arborea japonica* are remarkable in capacity of changing body color. They are mostly green or yellow-green in dorsal ground color and change into gray, grayish brown or dark brown in accordance with their environment. An irregular pattern of dark green or blackish brown is usually found on the back. This pattern is especially distinct when the dorsal ground color is gray or grayish brown and various in composition from simple to complicated. In some individuals, this pattern is scarcely found. The dorsal skin is smooth and often covered closely with minute granules. A black longitudinal stripe always exists on each side of the head. The ventral surface of the body is uniformly white or yellowish white and covered closely with minute granules. Males have a large external vocal sac on the throat. Males and females are 25～40 mm in body length.

a. Diploids and triploids produced in 1979

External characters were observed in 15 mature female and 39 mature male triploids, and three mature female and three mature male diploids obtained in 1979 from eggs of two female wild-type diploids (YK. Y, W♀, Nos. 1 and 2) by refrigeration after inseminating with sperm of a field-caught male (F 79♂, No. 1). They were also observed in five mature female and 11 mature male triploids produced in 1979 from eggs of a female colored-albinic diploid (YK. Y, C-A♀, No. 3) by refrigeration after inseminating with sperm of the same male as that used in the above mating. All these diploids and triploids examined were one year old. It was found that there were no differences in male and female external characters between the diploids and triploids.

b. Diploids, triploids and tetraploids produced in 1980

External characters were observed in 20 mature female and 20 mature male diploids obtained in 1980 from a mating between a field-caught female and a field-caught male and 36 mature female and 13 mature male tetraploids, and two mature female and one mature male triploids produced in 1980 from matings between three female triploids (3n 79♀, Nos. 1～3) and three field-caught males (F 80♂, Nos. 1～3). All these diploids, triploids and tetraploids were one year old.

There were no differences in male and female external characters among the diploids, triploids and tetraploids.

c. Triploids, tetraploids and pentaploids produced in 1981.

External characters were observed in 52 mature female and 49 mature male tetraploids produced in 1981 from matings between four female tetraploids (4n 80♀, Nos. 1, 3, 5 and 6) and three male tetraploids (4n 80♂, Nos. 1～3), in 28 mature female and 37 mature male triploids produced in 1981 from matings between three female tetraploids (4n 80♀, Nos. 2, 5 and 6) and the same three male diploids as those used in the above matings and in four mature female and six mature male pentaploids produced in 1981 from matings between seven female tetraploids (4n 80♀, Nos. 1～7) and the above three male diploids. All these triploids, tetraploids and pentaploids were one year old.

The results indicated that no differences were found in male and female external characters among the triploids, tetraploids and pentaploids.

The chromosomes of mature male diploid, triploid and tetraploid treefrogs obtained in 1981 are shown in Figs. 5～7.

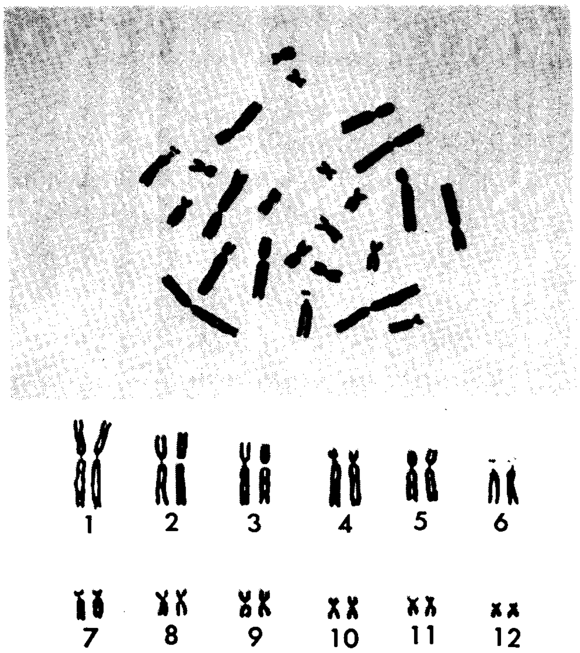



Fig. 5. Metaphase plate and the karyotype of a control diploid male. ×950

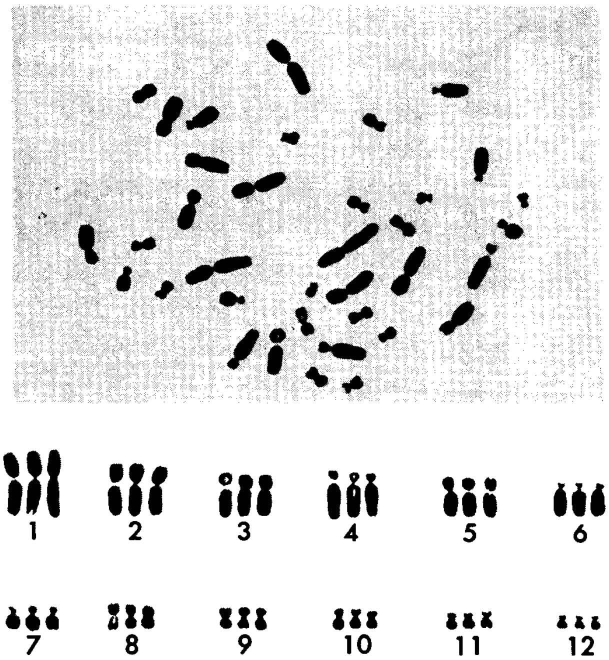



Fig. 6. Metaphase plate and the karyotype of a triploid male produced from 4n♀×2n♂. ×950

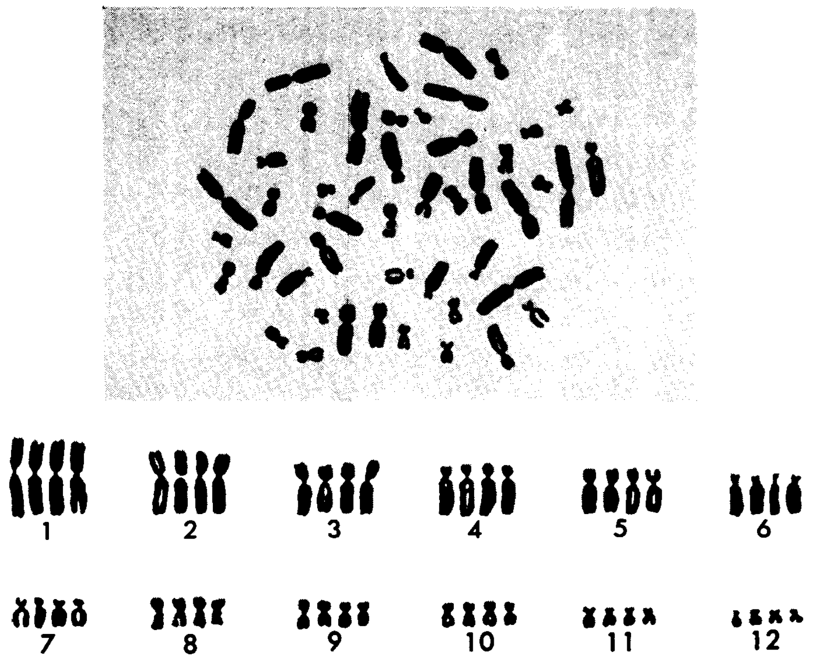



Fig. 7. Metaphase plate and the karyotype of a tetraploid male produced from 4n ♀×4n♂. ×950

## 2. Inner structure of testes

### a. Diploids

The inner structure of testes was observed in three one-year-old mature male diploids (2n 80♂, Nos. 1 ~ 3) produced in 1980 from a mating between a field-caught female (F 80♀, No. 1) and a field-caught male (F 80♂, No. 1) and in two 2-year-old mature male diploids (2n 80♂, Nos. 4 and 5) (Table 9). While one of the testes of each male was used in mating experiments, the other was fixed and sectioned in order to observe the inner structure.

The testes of all the male diploids were completely normal in inner structure. The seminiferous tubules were filled with compact bundles of normal spermatozoa. These bundles looked like petals in shape. Along the inner walls of seminiferous tubules, there were many isolated primary spermatogonia, whose nucleus had two or one nucleolus. Among these normal spermatogonia, there were a few large primary spermatogonia which seemed to be tetraploid. Tetraploid mitoses of spermatogonia were also found together with diploid mitoses, although they were scarce. In the peripheral parts of seminiferous tubules, there were some groups of secondary spermatogonia and many first and second spermatocytes at various stages of the meiotic divisions (Fig. 8a, b).

### b. Triploids

The inner structure of testes was observed in two one-year-old mature male triploids (3n 79♂, Nos. 1 and 2) produced in 1979 from eggs of a female (YK. Y, W♀, No. 1) by refrigeration after inseminating with sperm of a field-caught male (F 79♂, No. 1) and in a one-year-old mature male triploid (3n 79♂, No. 3) obtained from an egg of a colored-albinic female (YK. Y, C-A♀, No. 3) by refrigeration after inseminating with sperm of a field-caught male (F 79♂, No. 1). One of the testes of each male triploid was fixed and sectioned in order to observe the inner structure, while the other was used in mating experiments (cf. Table 9).

The testes of the male triploids were very abnormal in inner structure. In the seminiferous tubules, there were some coarse bundles of normally and abnormally shaped spermatozoa which were various in size. These bundles were surrounded with pycnotic nuclei and degenerating germ cells. In the peripheral parts of seminiferous tubules, there were abundant first spermatocytes, some of which were degenerating at the metaphase or anaphase stage. The first spermatocytes seemed to be somewhat larger than those of the diploids. Besides triploid spermatocytes, there were occasionally a few remarkably large spermatocytes which seemed to be hexaploid ones. Along the inner walls of the seminiferous tubules, there were many primary and secondary spermatogonia. Some spermatogonia were at the metaphase of mitosis. Besides triploid spermatogonia, there were a few remarkably large spermatogonia which seemed to be hexaploid (Fig. 8c, d). The meioses in male triploid treefrogs will be reported in detail in a separate paper.

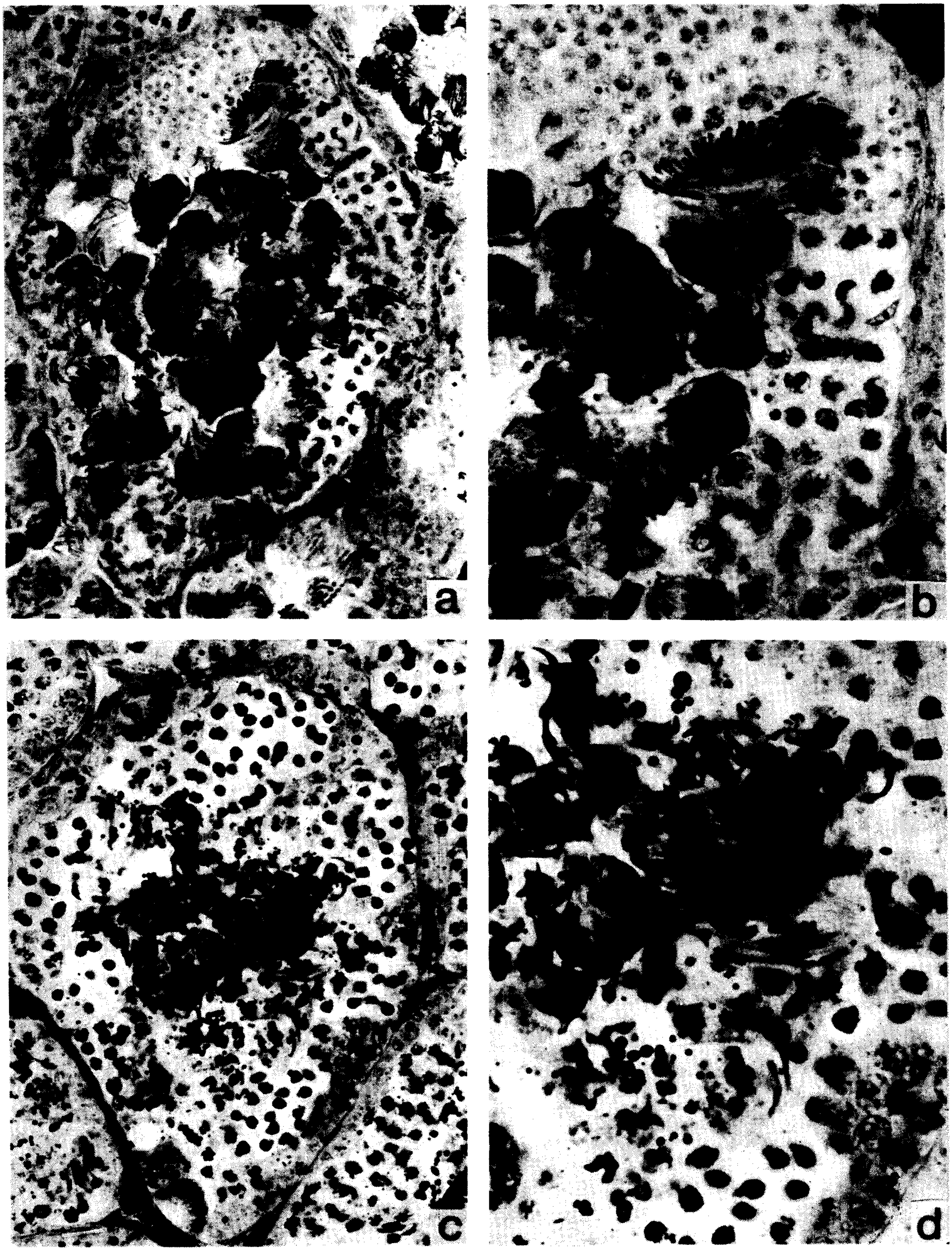



Fig. 8. Cross-sections of the testes of diploid and triploid males.

a. Control diploid, 2n 80 ♂, No. 1 ×300
b. Ditto ×600
c. Triploid, 3n 79 ♂, No. 3 ×300
d. Ditto ×600

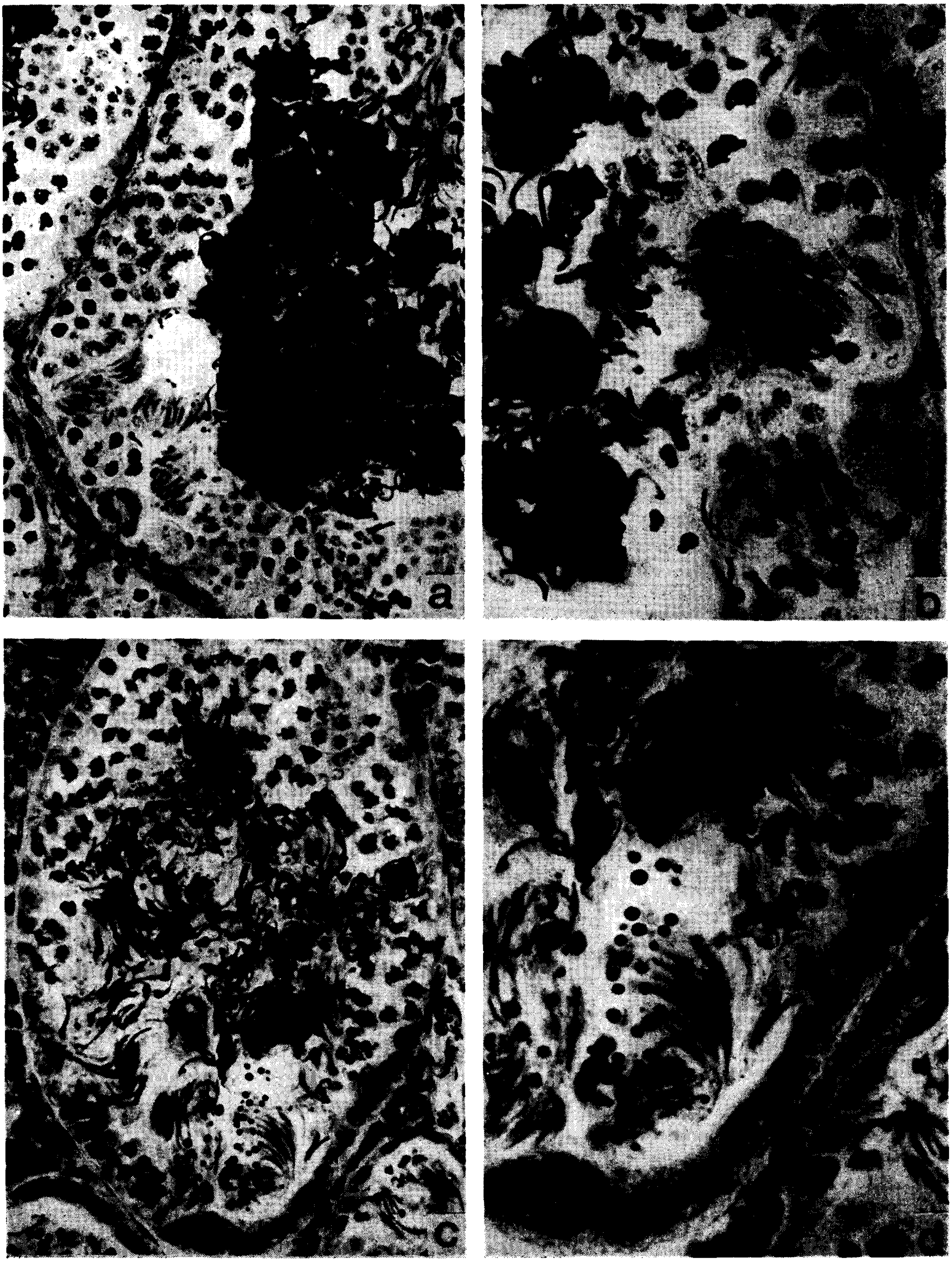



Fig. 9. Cross-sections of the testes of tetraploid and pentaploid males.

| | | |
|---|---|---|
| a. | Tetraploid, 4n 80♂, No. 1, produced from 3n 79♀, No. 3 × F 80♂, No. 3 | ×300 |
| b. | Ditto | ×600 |
| c. | Pentaploid, 5n 81♂, No. 1, produced from 4n 80♀, No. 6 × 2n 80♂, No. 3 | ×300 |
| d. | Ditto | ×600 |

c. Tetraploids

The inner structure of testes was observed in three one-year-old mature male tetraploids (4n 80♂, Nos. 1～3) obtained in 1980 from a mating between a female triploid (3n 79♀, No. 3) and a field-caught male (F 80♂, No. 3) and in three one-year-old mature male tetraploids (4n 81♂, Nos. 1～3) obtained in 1981 from a mating between a female tetraploid (4n 80♀, No. 3) and a male tetraploid (4n 80♂, No. 2). One of the testes of each male tetraploid was fixed and sectioned in order to examine the inner structure, while the other was used in mating experiments in 1981 or 1982 (cf. Table 9).

It was found that the testes of these six male tetraploids were almost normal. The seminiferous tubules were filled with many compact bundles of normally shaped spermatozoa which were remarkably larger than those of male diploids. The peripheral parts of seminiferous tubules were occupied by first and second spermatocytes at various meiotic stages. Along the inner walls of seminiferous tubules, there were some primary and secondary spermatogonia. Mitotic figures of tetraploid spermatogonia at the metaphase were occasionally found. Very large spermatogonia which seemed to be octoploid were rarely found besides tetraploid spermatogonia (Fig. 9a, b).

d. Pentaploids

The inner structure of testes was observed in three one-year-old mature male pentaploids (5n 81♂, Nos. 1～3) produced in 1981 from among matings between female tetraploids (4n 80♀, Nos. 1～7) and male diploids (2n 80♂, Nos. 1～3). It was found that the testes of these three male pentaploids were somewhat normal. The seminiferous tubules were roughly filled with coarse bundles of normally and abnormally shaped spermatozoa which were various in size. These bundles were surrounded with many pycnotic nuclei. The peripheral parts of seminiferous tubules were occupied by first and second spermatocytes at various meiotic stages. Along the inner walls of seminiferous tubules, there were many primary and secondary spermatogonia. There were a few mitotic figures of pentaploid spermatogonia. Giant spermatogonia were rarely found besides the pentaploid ones (Fig. 9c, d).

### 3. Occurrence of albinic tetraploids

Thirty and 56 triploid tadpoles produced in 1979 from two matings between two wild-type $f^cF$ or $fF$ diploid females (YK. Y, W♀, Nos. 1 and 2) and a field-caught $FF$ male (F 79♂, No. 1) by refrigeration of inseminated eggs were $f^cf^cF$, $ffF$, $FFF$, $f^cFF$ or $fFF$ in gene constitution and of wild type. The latter two ($f^cFF$ and $fFF$) are those which may occur by crossing-over at the meiosis. Twenty-four triploid tadpoles produced in the same season from a mating between a colored-albinic $f^cf$ diploid female (YK. Y, C-Alb♀, No. 3) and the above male by refrigeration of inseminated eggs were $f^cf^cF$, $ffF$ or $f^cfF$ in gene constitution and also of wild type (cf. Table 2).

Five, eight and 102, 115 in total, tetraploid tadpoles obtained in 1980 from three matings between three wild-type triploid females (3n 79♀, Nos. 1～3) and three field-caught *FF* males (F 80♂, Nos. 1～3) were all of wild type (cf. Table 5). It was conceivable that triploid female 3n 79♀, No. 3 was $f^c fF$ in gene constitution and this gene constitution was produced by crossing-over at the meiosis in an egg of the colored-albinic $f^c f$ diploid female (YK. Y, C-Alb♀, No. 3), as there were both colored albinos and red-eyed albinos among the first-generation offspring of female and male tetraploids obtained from the triploid female (3n 79♀, No. 3). If the 102 tetraploid tadpoles were raised from non-reduced triploid eggs ($f^c fF$) of triploid female parent (3n 79♀, No. 3), all of them should be $f^c fFF$ in gene constitution and prepare four kinds of diploid gametes, $f^c f$, $f^c F$, $fF$ and $FF$, at the ratio of 1: 2: 2: 1. Thus, in the tetraploid offspring from these tetraploids by brother and sister matings, there should be a mixture of wild-type tadpoles and colored-albinos at the ratio of 35: 1. There should be no case that all the tadpoles are of wild type or any red-eyed albino is produced.

The results of seven brother and sister matings between seven female tetraploids (4n 80♀, Nos. 1～7) and three male tetraploids (4n 80♂, Nos. 1～3) obtained from a mating, 3n 79♀, No. 3 × F 80♂, No. 3 (Table 10), did not always occur as such a supposition. It was found that 327 and 208 normally hatched tadpoles produced from two matings, 4n 80♀, No. 1 × 4n 80♂, No. 1 and 4n 80♀, No. 3 × 4n 80♂, No. 2, were all of wild type. However, 10 of 108, 3 of 107, 22 of 349 and 11 of 89 normally hatched tadpoles produced from four matings, 4n 80♀, No. 2 × 4n 80♂, No. 1, 4n 80♀, No. 4 × 4n 80♂, No. 2, 4n 80♀, No. 6 × 4n 80♂, No. 3 and 4n 80♀, No. 7 × 4n 80♂, No. 3, respectively, were colored-albinos. The other 98, 104, 327 and 78 tadpoles from the four matings were of wild type. Of 234 normally hatched tadpoles produced from the remaining mating, 4n 80♀, No. 5 × 4n 80♂, No. 3, 14 were colored-albinos and 10 were red-eyed albinos, while the other 210 were of wild type. In total, of 1422 offspring produced from tetraploid parents by seven brother and sister matings, 1352 were

TABLE 16
Ratios of albinic tadpoles in the first-generation offspring of female and male tetraploids

| Parents | | No. of normally hatched tadpoles | No. of tadpoles | | |
|---|---|---|---|---|---|
| Female | Male | | Wild type | Colored-albino | Red-eyed albino |
| 4n 80, No. 1 | 4n 80, No. 1 | 327 | 327 | 0 | 0 |
| 4n 80, No. 2 | 4n 80, No. 1 | 108 | 98 | 10 | 0 |
| 4n 80, No. 3 | 4n 80, No. 2 | 208 | 208 | 0 | 0 |
| 4n 80, No. 4 | 4n 80, No. 2 | 107 | 104 | 3 | 0 |
| 4n 80, No. 5 | 4n 80, No. 3 | 234 | 210 | 14 | 10 |
| 4n 80, No. 6 | 4n 80, No. 3 | 349 | 327 | 22 | 0 |
| 4n 80, No. 7 | 4n 80, No. 3 | 89 | 78 | 11 | 0 |
| Total | | 1422 | 1352 | 60 | 10 |

of wild type, 60 were colored albinos and 10 were red-eyed albinos (Table 16).

On the other hand, 886 normally hatched tadpoles produced from seven matings between the above seven female tetraploids (4n 80♀, Nos. 1～7) and three male diploids (2n 80♂, Nos. 1～3) were all of wild type (Table 10).

From the findings stated above, it is believed that the triploid eggs of the female triploid (3n 79♀, No. 3: $f^cfF$) were formed by reduction divisons of hexaploid oocytes whose chromosomes were doubled at the oogonium stage. More specifically, triploid oogonia ($f^cfF$) of the triploid female parent become hexaploid ($f^cf^cffFF$) by doubling of chromosomes. Then, seven kinds of mature eggs ($f^cf^cf$, $f^cff$, $f^cf^cF$, $f^cfF$, $ffF$, $f^cFF$ and $fFF$) are prepared at the ratio of 1: 1: 1: 4: 1: 1: 1 after reduction divisions. When these eggs are fertilized with sperm ($F$) of a field-caught $FF$ male, seven kinds of tetraploids which are $f^cf^cfF$, $f^cffF$, $f^cf^cFF$, $f^cfFF$, $ffFF$, $f^cFFF$ and $fFFF$ in gene constitution are produced at the ratio of 1: 1: 1: 4: 1: 1: 1.

The results of the seven brother and sister matings using these various kinds of tetraploids may be explained as follows.

Mating 1. 4n 80♀, No. 1 × 4n 80♂, No. 1 ······ 327 were all of wild type.

Mating 2. 4n 80♀, No. 2 × 4n 80♂, No. 1 ······ 98 were of wild type and 10 were colored albinos.

Female 4n 80♀, No. 1 should be $f^cFFF$ or $fFFF$. Female 4n 80♀, No. 2 should be $f^cf^cfF$, $f^cffF$, $f^cf^cFF$, $f^cfFF$ or $ffFF$. If female 4n 80♀, No. 2 is $f^cf^cfF$, male 4n 80♂, No. 1 should be $f^cf^cFF$, $f^cfFF$ or $ffFF$. If female 4n 80♀, No. 2 is $f^cffF$, male 4n 80♂, No. 1 should be $f^cf^cFF$ or $f^cfFF$. If female 4n 80♀, No. 2 is $f^cf^cFF$ or $f^cfFF$, male 4n 80♂, No. 1 should be $f^cffF$ or $f^cf^cfF$. If female 4n 80♀, No. 2 is $ffFF$, male 4n 80♂, No. 1 should be $f^cf^cfF$.

Mating 3. 4n 80♀, No. 3 × 4n 80♂, No. 2 ······ 208 were all of wild type.

Mating 4. 4n 80♀, No. 4 × 4n 80♂, No. 2 ······ 104 were of wild type and three were colored albinos.

Female 4n 80♀, No. 3 should be $f^cFFF$ or $fFFF$. Female 4n 80♀, No. 4 should be $ffFF$, $f^cf^cFF$ or $f^cfFF$. If female 4n 80♀, No. 4 is $ffFF$, male 4n 80♂, No. 2 should be $f^cf^cFF$ or $f^cfFF$. If female 4n 80♀, No. 4 is $f^cf^cFF$ or $f^cfFF$, male 4n 80♂, No. 2 should be $f^cf^cFF$, $f^cfFF$ or $ffFF$.

Mating 5. 4n 80♀, No. 5 × 4n 80♂, No. 3 ······ 210 were of wild type, 14 were colored albinos and 10 were red-eyed albinos. These three kinds of tetraploids were considered to be at the ratio of 33: 2: 1.

Mating 6. 4n 80♀, No. 6 × 4n 80♂, No. 3 ······ 327 were of wild type and 22 were colored albinos. These two kinds of tetraploids were considered to be at the ratio of 11: 1.

Mating 7. 4n 80♀, No. 7 × 4n 80♂, No. 3 ······ 78 were of wild type and 11 were colored albinos. These two kinds of tetraploids were considered to be at the ratio of 11: 1.

As a condition producing wild-type frogs, colored albinos and red-eyed albinos at the ratio of 33: 2: 1 from mating 5, female 4n 80♀, No. 5 and male 4n 80♂, No. 3 should be $ffFF$ and $f^cffF$ or $f^cffF$ and $ffFF$, respectively. If male 4n

80♂, No. 3 is $ffFF$, females 4n 80♀, Nos. 6 and 7 should be $f^{c}f^{c}fF$. In this case, female 4n 80♀, No. 5 should be $f^{c}ffF$ in gene constitution. If male 4n 80♂, No. 3 is $f^{c}ffF$, female 4n 80♀, No. 5 should be $ffFF$ and females 4n 80♀, Nos. 6 and 7 should be $f^{c}f^{c}FF$ or $f^{c}fFF$.

In 1982, a total of 527 normally hatched tetraploid tadpoles were produced from matings between two female tetraploids (4n 81♀, Nos. 1 and 2) obtained in 1981 from a mating, 4n 80♀, No. 6 × 4n 80♂, No. 3, and three male tetraploids (4n 81♂, Nos. 1 ~ 3) obtained in 1981 from a mating, 4n 80♀, No. 3 × 4n 80♂, No. 2. All these tadpoles were of wild type (Table 14).

## DISCUSSION

It is more than 40 years ago that triploid amphibians were efficiently produced by cold or heat-shock treatment of unsegmented eggs by Fankhauser and Griffiths (1939), and Fankhauser and Watson (1942) in an American newt *Notophthalmus viridescens* and by Kawamura (1941a, b) in a Japanese pond frog *Rana nigromaculata*. The triploidy is established by union of the female and male pronuclei with the nucleus of the second polar body whose extrusion is suppressed by extremes of temperature. In the present study, 371 (60.8%) of 610 eggs exposed to low temperature cleaved normally and 178 (29.2%) became normally feeding tadpoles. When chromosomes were examined in these tadpoles, 110 (86.6%) of 127 analyzed ones were triploids, another was a tetraploid, 12 were diploids and the remaining four were 2n-3n mosaics. These triploids and diploids were nearly the same in development and growth. They scarcely differed in body length immediately after metamorphosis as well as at the mature adult stage.

The production of tetraploids from triploid females mated with diploid males has been reported by Humphrey and Fankhauser (1949), and Fankhauser and Humphrey (1950) in the axolotl. According to Humphrey and Fankhauser (1956), tetraploid axolotls were produced with a frequency of 3 to 4% in the spawnings of triploid females mated with diploid males, probably as a result of failure of the meiosis in the egg. Beetschen (1962) obtained four tetraploid females from among 3179 eggs of five triploid females mated with diploid males in *Pleurodeles waltl*. Later, he (1967) reported that 14 (0.33%) tetraploid larvae were produced from among 4204 eggs of six triploid females mated with diploid males during the period from 1959 to 1966.

In contrast to triploid females, no tetraploids nor higher polyploids were produced from triploid males by mating with diploid females. Kawamura (1951a, b) reported in *Rana nigromaculata* and *Cynops pyrrhogaster* that the offspring between diploid females and triploid males were aneuploids between diploid and triploid in chromosome number except a single diploid in each species. A similar finding was reported by Fankhauser and Humphrey (1954) in numerous offspring between diploid females and triploid males of axolotls.

In *Rana,* Kawamura (1941a, b) and Humphrey, Briggs and Fankhauser

(1950) could not obtain offspring from triploid females of *Rana nigromaculata* and *Rana pipiens*, respectively, owing to underdevelopment or degeneration of the ovaries. However, the assumption by KAWAMURA that the meager reproductive capacity would be increased by betterment of trophic conditions of the frogs was thereafter confirmed by KAWAMURA, NISHIOKA and OKUMOTO (1983) in *Rana nigromaculata* and *Rana brevipoda*. In the matings between triploid females and diploid males of *Rana nigromaculata*, 104 (3.4%) of 3044 eggs became feeding tadpoles. Twelve (12.4%) of 97 analyzed tadpoles were tetraploids, while the other 75 were diploids, triploids, aneuploids and others. In the same kind of matings in *Rana brevipoda*, 199 (4.7%) of 4208 eggs became feeding tadpoles. Of 192 analyzed tadpoles, 28 (14.6%) were tetraploids, while the other 164 were diploids, triploids, aneuploids and others. On the other hand, NISHIOKA (1983) reported that allotriploid females consisting of two *Rana nigromaculata* genomes and one *Rana plancyi chosenica* genome produced numerous amphidiploids by mating with diploid male *Rana plancyi chosenica*. Of 7791 eggs, 4233 (54.3%) cleaved normally, 505 (6.5%) became feeding tadpoles. Of 466 analyzed tadpoles, 291 (62.4%) were tetraploids, while the other 175 were triploids, diploids and aneuploids.

In the present study, 1149 (92.7%) of 1240 eggs obtained from three triploid females cleaved normally and 159 (12.8%) became feeding tadpoles. Of 142 analyzed tadpoles, 115 (81.0%) were tetraploids and 27 were diploids, triploids and aneuploids. From matings between diploid females and triploid males, no tetraploids were produced. The offspring from this combination were mostly aneuploids between diploid and triploid, and besides there were fairly many diploids, a few triploids and several aneuploids other than those stated above. Of 89 metamorphosed tetraploids, 66 were females and 23 (25.8%) were males. The female preponderance in number seems to be attributed to sex reversal of some genetic males into females. The occurrence of sex reversal from male to female in treefrogs has been confirmed by TAKAHASHI (1958, 1959), KAWAMURA and NISHIOKA (1977), and NISHIOKA and UEDA (1977).

While six diploid females, 35.5 ~ 37.0 mm in body length, laid 673 ~ 1052 eggs which were 1.20 ~ 1.22 mm in diameter and uniform in size, 10 tetraploid females, 36.0 ~ 38.5 mm in body length, laid 521 ~ 1154 eggs which were 1.32 ~ 1.36 mm in mean diameter and uniform in size. In contrast to diploid and tetraploid females, three triploid females, 36.5 ~ 37.5 mm in body length, laid 164 ~ 832 eggs which were various in size and 1.30 ~ 1.33 mm in mean diameter. When seven tetraploid females were mated with diploid and tetraploid males, it was found that the tetraploid females were nearly normal in reproductive capacity, while the tetraploid males were slightly inferior to the diploid males in this respect. In the matings between seven tetraploid females and three tetraploid males, 3291 (96.5%) of 3412 eggs cleaved normally and 1131 (33.1%) became feeding tadpoles, while 1578 (93.5%) of 1687 eggs cleaved normally and 718 (42.6%) became feeding tadpoles in the matings of the same seven females with three diploid males. In the matings between the tetraploid females and males, 563 (85.0%) of 662 analyzed tadpoles were tetraploids, 47 (7.1%) were hexaploids and the remaining

52 were aneuploids and others, while in the matings between the tetraploid females and three diploid males, 271 of 348 analyzed tadpoles were triploids, 59 were pentaploids and the remaining 18 were aneuploids and others. Of these triploids, tetraploids, pentaploids and hexaploids, 178, 351, 40 and 3 attained the completion of metamorphosis, respectively. The metamorphosed triploids and tetraploids were nearly the same in growth after metamorphosis. There were nearly equal numbers of males and females in each of these two kinds of frogs. In contrast to these, the pentaploids were smaller in body size, although 10 of them were sexually matured. Of 37 pentaploids whose sex was examined, 17 were females and 20 (54.1%) were males. The metamorphosed hexaploids were stunted in growth and died without taking food after metamorphosis.

The tetraploid offspring obtained from matings between male and female tetraploids seemed to have remarkably increased in reproductive capacity. Of 846 eggs of two tetraploid females mated with three tetraploid males, 829 (98.0%) cleaved normally and 452 (53.4%) became normally feeding tadpoles, while of 705 eggs of the same females mated with two diploid males, 695 (98.6%) cleaved normally and 440 (62.4%) became normally feeding tadpoles (Table 14). When three diploid females were mated with the above three tetraploid males, 785 (89.2%) of 880 eggs cleaved normally and 535 (60.8%) became normally feeding tadpoles. In the control matings between the two diploid females and the three diploid males, 381 (89.6%) of 425 eggs cleaved normally and 357 (84.0%) became normally feeding tadpoles. Of 272 tadpoles obtained from matings of tetraploid females and tetraploid males, 265 (97.4%) were tetraploids, two were hexaploids and the remaining five were aneuploids and others, while of 581 tadpoles obtained from reciprocal matings between diploids and tetraploids, 562 (96.7%) were triploids, five were tetraploids, four were pentaploids and the remaining 10 were aneuploid and others. In the control matings of diploid females and diploid males, 118 (98.3%) of 120 tadpoles were diploids and two were triploids.

These findings seem to indicate that autotetraploids in *Hyla arborea japonica* remarkably differ in reproductive capacity from those of axolotls (HUMPHREY and FANKHAUSER, 1946), *Xenopus laevis* (GURDON, 1959), *Rana japonica* (KAWAMURA, NISHIOKA and MYOREI, 1963), *Rana nigromaculata* (KAWAMURA and NISHIOKA, 1963b, 1983; NISHIOKA, 1971), whereas they are very similar to amphidiploids between *Rana nigromaculata* and *Rana brevipoda* (KAWAMURA and NISHIOKA, 1963a, 1983; KAWAMURA, NISHIOKA and OKUMOTO, 1983; NISHIOKA, 1971) and between *Rana nigromaculata* and *Rana plancyi chosenica* (NISHIOKA, 1983; NISHIOKA and OKUMOTO, 1983).

As the males of *Hyla arborea japonica* are considered to be heterogametic (XY) by KAWAMURA and NISHIOKA (1977), male and female tetraploids are probably XXXY and XXXX in sex chromosome constitution. It is supported that the male tetraploids produce almost regularly XX and XY spermatozoa in equal numbers, because nearly equal numbers of males and females were produced by matings between tetraploid females and tetraploid males.

The present study seems to support the assumption by BOGART and WASSERMAN (1972) that the tetraploid *Hyla versicolor* would arise independently in diploid *Hyla chrysoscelis* through an intermediate triploid stage. Production of spontaneous triploids from fertilized eggs have been reported in *Notophthalmus viridescens*, *Eurycea bislineata* et al. (FANKHAUSER, 1945), although such triploids are very scarce in anurans. If a mature triploid female was raised from an egg by any cause, this female would produce male and female tetraploids by mating with a normal diploid male. These male and female tetraploids may establish a population of tetraploid frogs, if they were geographically isolated from the original diploid population.

Recently, a diploid-tetraploid cryptic species complex such as *Hyla versicolor* (2n=48) and *Hyla chrysoscelis* (2n=24) has been frequently reported in Hylidae, Ceratophrydidae, Pipidae, Ranidae and Bufonidae. Both diploids and tetraploids were found in *Odontophrynus americanus* (2n=26, 52) in South America (BARRIO and DE RUBEL, 1972; Beçak and Beçak, 1974), *Phyllomedusa burmeisteri* (2n=26, 52) in South America (BATISTIC, SOMA, BEÇAK and BEÇAK, 1975), *Pyxicephalus delalandii* (2n=26, 52) in South Africa and *Dicroglossus occipitalis* (2n=26, 52) in West and Central Africa (BOGART and TANDY, 1976), *Xenopus* (*laevis*) *borealis* (2n=36) and *Xenopus* (*laevis*) *bunyoniensis* (2n=72) in Central Aftica (TYMOWSKA and FISCHBERG, 1973), *Bufo viridis* (2n=22, 44) in Kirghizia (MAZIK, KADIROVA and TOKTOSUNOV, 1976) and *Bufo spp.* similar to *Bufo kerinyagae* (2n=20, 40) in East Africa (BOGART and TANDY, 1976). Beside these diploid-tetraploid cryptic species, there are diploid-tetraploid-hexaploid cryptic species consisting of *Xenopus fraseri* (2n=36), *Xenopus amieti* (2n=72) and *Xenopus ruwenzoriensis* (2n=104), in Central Africa (FISCHBERG and KOBEL, 1978; KOBEL, PASQUIER, FISCHBERG and GLOOR, 1980), and diploid-octoploid cryptic species *Ceratophrys ornata* (2n=26, 104) in South America (BARRIO and DE CHIERI, 1970).

In the present study, it was shown that 7.1% of the eggs of tetraploid females mated with tetraploid males became hexaploids, whereas 85.0% became tetraploids. Thus, it is very probable that the first tetraploid females (XXXX) and males (XXXY) in each of the above diploid-tetraploid cryptic species were produced from unreduced eggs (XXX) of a triploid female (XXX) by fertilization with spermatozoa (X or Y) of a diploid male (XY). The first hexaploid females (ZZZZZW) in *Xenopus* may be produced from unreduced eggs (ZZZW) of a tetraploid female (ZZZW) by fertilization with spermatozoa (ZZ) of a tetraploid male (ZZZZ). The mechanism how the octoploid population of *Ceratophrys ornata* was produced is unknown to the present authors. However, if the male is heterogametic, this population may have occurred from unreduced eggs (XXXXXX) of a hexaploid female (XXXXXX) by fertilization with spermatozoa (XX or XY) of a tetraploid male (XXXY), although both tetraploid and hexaploid populations of this species have not yet been discovered. It may be probable that the tetraploid and hexaploid populations were extinguished long time ago.

## SUMMARY

1. Many triploids, tetraploids and pentaploids were produced in Japanese treefrogs, *Hyla arborea japonica*. Developmental capacity, sex ratio and some other characters of these polyploids were examined.

2. When 610 eggs of three diploid females which were albinos or heterozygous for two albinic genes were refrigerated after fertilized with sperm of a field-caught male, 371 (60.8%) cleaved normally and 178 (29.2%) became normally feeding tadpoles. Chromosomes were observed in 127 of these tadpoles. It was found that 110 (86.6%) were triploids (3n=36), 12 were diploids, one was a tetraploid and the remaining four were 2n-3n mosaics.

3. Of the triploid feeding tadpoles, 94 metamorphosed normally and 76 attained sexual maturity. Of the latter, 20 were females and 56 (73.7%) were males.

4. Of 1092 eggs of three field-caught females mated with three triploid males, 740 (67.8%) cleaved normally and 98 (9.0%) became feeding tadpoles. Of 1240 eggs of three triploid females mated with three field-caught males, 1149 (92.7%) cleaved normally and 159 (12.8%) became feeding tadpoles. While no tetraploids were obtained from the triploid males, 115 tetraploids were produced from the triploid females, together with eight diploids, eight triploids and 11 aneuploids. This number of tetraploids corresponded to 81.0% of 142 feeding tadpoles whose chromosomes were analyzed. A total of 89 of these tetraploid tadpoles metamorphosed normally. Of the metamorphosed tetraploids, 66 were females and 23 (25.8%) were males.

5. Of 3412 eggs of seven female tetraploids mated with three male tetraploids, 3291 (96.5%) cleaved normally and 1131 (33.1%) became feeding tadpoles, while 1578 (93.5%) of 1687 eggs of the same females mated with three field-caught males cleaved normally and 718 (42.6%) became feeding tadpoles. Chromosomes were observed in 662 of the feeding tadpoles derived from the female and male tetraploids and in 348 of those derived from the female tetraploids and field-caught males. Of the former tadpoles, 563 (85.0%) were tetraploids, 47 (7.1%) were hexaploids and the other 52 were aneuploids and mosaics. Of the latter tadpoles, 271 (77.9%) were triploids, 59 (17.0%) were pentaploids and the other 18 were aneuploids and mosaics.

Of the tetraploid tadpoles, 351 metamorphosed normally and 143 attained sexual maturity. When sex was examined in 324 of the metamorphosed tetraploids, 168 were females and 156 (48.1%) were males. Only three of the hexaploid tadpoles could metamorphose but died without taking food. Their sex was unknown. Of the triploid tadpoles, 178 metamorphosed normally and 80 attained sexual maturity. When sex was examined in 172 metamorphosed triploids, 80 were females and 92 (53.5%) were males.

Forty of the pentaploid tadpoles metamorphosed normally and 10 attained sexual maturity. When sex was examined in 37 metamorphosed pentaploids, 17

were females and 20 (54.1%) were males.

6. Second-generation offspring of tetraploids were produced by brother and sister matings or by matings with diploid males or females. Of 846 eggs of two tetraploid females mated with three tetraploid males, 829 (98.0%) cleaved normally and 452 (53.4%) became feeding tadpoles, while 695 (98.6%) of 705 eggs of the same females mated with two diploid males cleaved normally and 440 (62.4%) became feeding tadpoles. When 880 eggs of two diploid females were inseminated with sperm of the above three tetraploid males, 785 (89.2%) cleaved normally and 535 (60.8%) became feeding tadpoles.

7. Of 272 feeding tadpoles obtained from the two tetraploid females by mating with the three tetraploid males, 265 (97.4%) were tetraploids, two were hexaploids and the other five were aneuploids and others. Of 230 of the feeding tadpoles obtained from the same females by mating with the two diploid males, 220 (95.7%) were triploids, four were pentaploids and the remaining six were aneuploids and others. Of 351 of the feeding tadpoles obtained from three diploid females by mating with the above tetraploid males, 342 (97.4%) were triploids, five were tetraploids and the remaining four were three aneuploids and a mosaic.

8. No differences were observed in external characters of males and females among diploids, triploids, tetraploids and pentaploids.

9. The testes of tetraploid males were almost normal in inner structure, although spermatozoa, spermatocytes and spermatogonia were distinctly larger than those of diploid males. While the testes of triploid males were very abnormal in inner structure, those of pentaploid males were somewhat normal. The seminiferous tubules of the pentaploid males were roughly filled with coarse bundles of normally and abnormally shaped spermatozoa which were various in size.

10. Of 1422 tetraploid tadpoles produced from tetraploid parents by seven borther and sister matings, 1352 were of wild type, 60 were colored albinos and 10 were red-eyed albinos. The origin of these albinic tetraploids was elucidated on the basis of gene constitution.

11. The origin of tetraploids in diploid-tetraploid cryptic species was considered.

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