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# Reproductive Capacity and Progeny of Amphidiploids between *Rana nigromaculata* and *Rana plancyi chosenica*

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(With 15 Text-figures)

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## INTRODUCTION

In the vegetable kingdom, both natural and induced amphidiploids are innumerable and have played important roles in evolution and improvement of plants, respectively. Thus, there have so far been voluminous papers and monographs dealing with amphidiploids (GOTTSCHALK, 1976; LEWIS, 1980). The animal kingdom is in perfect contrast to the vegetable kingdom in this respect except amphibian amphidiploids produced by KAWAMURA and NISHIOKA (1960, 1963a, b, 1967, 1983), KAWAMURA, NISHIOKA and OKUMOTO (1983) and NISHIOKA (1963, 1971, 1983).

The first fertile amphidiploid was a single male obtained by KAWAMURA and NISHIOKA (1960, 1963a). This amphidiploid was produced from a fertilized *Rana brevipoda* egg by transplantation of a blastula nucleus of *Rana nigromaculata*. Although there were three other male amphidiploids, one of which was obtained from an unfertilized egg by transplantation with a blastula nucleus of an allotriploid and the other two were produced from *Rana brevipoda* eggs fertilized with *Rana nigromaculata* sperm by inflicting a heat shock immediately before the appearance of the first cleavage furrow, all of them died before their reproductive capacity was tested. A few years after the above male amphidiploids were obtained, the first female amphidiploid was produced together with six male amphidiploids from *Rana brevipoda* eggs inseminated with sperm of a tetraploid male *Rana nigromaculata* by cold-treatment after insemination (KAWAMURA and NISHIOKA, 1963b). On the basis of this experiment, KAWAMURA and NISHIOKA (1983) considered autotetraploid male *Rana nigromaculata* to be the most useful animals in producing male and female amphidiploids. By repeating this kind of experiments, they obtained several mature male and female amphidiploids and succeeded in establishing an amphidiploid strain, that is, an artificial species which is almost completely fertile and produces nearly an equal number of males and females.

KAWAMURA, NISHIOKA and OKUMOTO (1983) noticed about eight years ago that there was another useful method in producing amphidiploids between *Rana nigromaculata* and *Rana brevipoda*. It was found that considerably many amphidiploids were obtained from females of reciprocal allotriploids between these two species by mating with diploid male *Rana nigromaculata* or *Rana brevipoda*. Autotetraploids were also obtained from autotriploid females by mating with diploid males in each of the two species. A remarkable improvement in fertility of allotriploid females between *Rana nigromaculata* and *Rana brevipoda* occurred by changing their food from mosquitos and domestic flies to crickets in 1975 (NISHIOKA and MATSUURA, 1977). Before that time, these allotriploid females were believed to be almost sterile, although a few auxocytes were often found in their ovaries.

NISHIOKA (1983) reported that both males and females of reciprocal hybrids between *Rana nigromaculata* and *Rana plancyi chosenica* were somewhat fertile and produced diploid and triploid offspring by backcrossing with parental species.

Of the backcrosses, 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.* Although both male and female amphidiploids were produced by this method, males were far more numerous than females.

In the present study, matings between male and female amphidiploids were repeated from generation to generation for the purpose of establishing an amphidiploid strain between *Rana nigromaculata* and *Rana plancyi chosenica* by improvement in developmental capacity and normalization in sex ratio.

## MATERIALS AND METHODS

Male and female *Rana nigromaculata* HALLOWELL, (N)NN, were collected from the suburbs of Hiroshima, while male and female *Rana plancyi chosenica* OKADA, (C)CC, were collected from Suwon, Korea. These frogs and their offspring were used in the present study as materials together with two male and one female amphidiploids (NISHIOKA, 1983), which had been produced from the two species by the following methods.

1. In 1973, diploid hybrids, (N)NC, were produced from a cross between a field-caught female *Rana nigromaculata* and a field-caught male *Rana plancyi chosenica*, (N)NN♀, No. 6 × (C)CC♂, No. 6.
2. In 1975, allotriploids, (N)NNC, were produced from large eggs of female hybrid, (N)NC 73♀, No. 8, by backcrossing with sperm of a male *Rana nigromaculata*, (N)NN 73♂, No. 2.
3. In 1978, 11 mature amphidiploids were produced from a female allotriploid, (N)NNC 75♀, No. 5, obtained in 1975, by mating with a male *Rana plancyi chosenica*, (C)CC 76♂, No. 1. Of these amphidiploids, a female, (N)NNCC 78♀, No. 1, and two males, (N)NNCC 78♂, Nos. 1 and 2, were used in the present study.

Ovulation of females was accelerated by injecting suspension of bullfrog pituitaries. Eggs were always fertilized by the artificial fertilization method. The chromosomes of tadpoles were observed in the tail-tips of tadpoles which were more than 30 mm in total length by the water-pretreatment squash method (MAKINO and NISHIMURA, 1952) modified by NISHIOKA (1972a). In this case, the tadpoles had not been treated with colchicine, as it was neccessary to rear them continuously. Those of mature frogs were examined by the blood culture method (VOLPE and GEBHARDT, 1968; WU and YANG, 1980). The mitotic chromosomes in the testes were prepared by the method of SCHMID, OLERT and KLETT (1979).

Tadpoles were fed on boiled spinach or chard which was thoroughly washed with water. Frogs were fed on crickets, *Gryllus bimaculatus* DE GEER. The testes of mature males were fixed in NAVASHIN's fluid, sectioned at 12 $\mu$, and stained with HEIDENHAIN's iron hematoxylin.

The following abbreviations are used in the present paper.

N ...........A set of *Rana nigromaculata* chromosomes

C ............A set of *Rana plancyi chosenica* chromosomes
(N)............*Rana nigromaculata* cytoplasm
(C)............*Rana plancyi chosenica* cytoplasm
(N)NN ......Diploid *Rana nigromaculata*
(C)CC ......Diploid *Rana plancyi chosenica*
(N)NC ......Diploid hybrid, *Rana nigromaculata* ♀ × *Rana plancyi chosenica* ♂
(C)CN ......Diploid hybrid, *Rana plancyi chosenica* ♀ × *Rana nigromaculata* ♂
(N)NNC ...Allotriploid consisting of two *Rana nigromaculata* genomes and one *Rana plancyi chosenica* genome
(N)NCC ...Allotriploid consisting of one *Rana nigromaculata* genome and two *Rana plancyi chosenica* genomes
(N)NNCC...Amphidiploid consisting of two *Rana nigromaculata* genomes and two *Rana plancyi chosenica* genomes

## OBSERVATION

### *I. Reproductive capacity of parental amphidiploids examined in 1980*

#### 1. Origin of parental amphidiploids

A diploid female (N)NC hybrids obtained in 1973 from a cross, *Rana nigromaculata* ♀, No. 6 × *Rana plancyi chosenica* ♂, No. 6, was sexually matured in 1975. From large eggs of this female, (N)NNC allotriploids were produced by inseminating with sperm of a male *Rana nigromaculata* in this year. As females of these allotriploids were sexually matured in the breeding season of 1978, eight (Nos. 1∼8) of them were mated with a male *Rana plancyi chosenica*. Fourteen (N)NNCC amphidiploids were produced from these matings and 11 of them attained sexual maturity in the breeding season of 1980. Of the mature amphidiploids, 10 were males and the remainder was a female.

#### 2. Females

a. Female amphidiploid

A single female amphidiploid, (N)NNCC 78♀, No. 1, was 65.0 mm in body length at the age of two years. As ovulation began to occur 20 hours after pituitary injection, this female was thereafter kept at 17∼20°C for 24 hours. Then, 1585 eggs were pressed out from the cloaca on glass slides and fertilized with sperm of two male amphidiploids and one male diploid *Rana nigromaculata*. The eggs were almost uniform in size. When 50 eggs were measured, they were $2.26 \pm 0.01$ mm in diameter.

b. Diploid female hybrids

A diploid female hybrid, (C)CN 76♀, No. 21, obtained in 1976 from a mating, *Rana plancyi chosenica* ♀, No. 8 × *Rana nigromaculata* ♂, No. 8, was 62.5 mm in body length at the age of four years in 1980. This female laid 1096 eggs which were divided into two groups of 146 large and 950 normal-sized eggs. While 50 of the large eggs were 2.21±0.01 mm in diameter, 50 of the normal-sized eggs were 1.74±0.01 mm in diameter. On the other hand, a female of the reciprocal hybrid, (N)NC 76♀, No. 16, obtained in 1976 from a mating, *Rana nigromaculata*♀, No. 10 × *Rana plancyi chosenica*♂, No. 10, was 63.0 mm in body length at the age of four years in 1980. This female laid 1241 eggs, of which 223 were large and the other 1018 were normal-sized. While 50 of the large eggs were 2.19±0.01 mm in diameter, 50 of the normal-sized eggs were 1.72±0.01 mm.

### 3. Males

Two of 10 mature male amphidiploids, (N)NNCC 78♂, Nos. 1 and 2, obtained in 1978 were 58.0 mm and 56.5 mm in body length at the age of two years in 1980. The left and right testes of one (No. 1) of the male amphidiploids were 5.5 mm × 4.5 mm and 5.0 mm × 4.0 mm in size, respectively. Those of the other (No. 2) were 5.0 mm × 3.5 mm and 4.5 mm × 4.0 mm in size. In contrast, a male diploid *Rana nigromaculata*, (N)NN 76♂, No. 4, was 60.0 mm in body length at the age of four years. The left and right testes of this male were 6.0 mm × 2.5 mm and 6.0 mm × 3.0 mm in size, respectively. A 4-year-old male diploid *Rana plancyi chosenica*, (C)CC 76♂, No. 3, was 44.5 mm in body length. The left and right testes were 2.5 mm × 2.0 mm and 3.0 mm × 2.0 mm in size, respectively.

### 4. Crossing experiments

a. (N)NNCC 78♀, No. 1 × (N)NNCC 78♂, Nos. 1 and 2

In the breeding season of 1980, the female amphidiploid, (N)NNCC 78♀, No. 1, was mated with the two male amphidiploids, (N)NNCC 78♂, Nos. 1 and 2. The results showed that 328 (46.9%) of 700 eggs cleaved normally. Of the normally cleaved eggs, 57 died of incomplete invagination at the gastrula stage, while 271 (38.7%) became normal tail-bud embryos. Thereafter, 14 embryos died of edema or ill-development of gills by the hatching stage, while 257 (36.7%) hatched normally. Of the normally hatched tadpoles, 43 died of edema or underdevelopment without taking food and 28 others died of edema shortly after they began to eat or of emaciation owing to lack of appetite. Eventually, 186 (26.6%) eggs became normally feeding tadpoles which were more than 30 mm in total length.

The chromosomes of these feeding tadpoles were examined. Although all the tadpoles grew normally after the chromosome analysis, 32 tadpoles died of underdevelopment or edema immediately before or during metamorphosis. A total of 154 (22.0%) eggs attained completion of normal metamorphosis (Table 1).

The numbers of the normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs correspond to 82.6%, 78.4%, 56.7% and 47.0% of the normally cleaved eggs, respectively.

b. (N)NNCC 78♀, No. 1 × (N)NN 76♂, No. 4

The female amphidiploid, (N)NNCC 78♀, No. 1, was mated with a diploid

TABLE 1
Developmental capacity of the first-generation offspring produced from amphidiploids in 1980

| Parents: Female | Parents: Male | No. of eggs | No. of normal cleavages | No. of normal tail-bud embryos | No. of normally hatched tadpoles | No. of normally feeding tadpoles | No. of metamorphosed frogs |
|---|---|---|---|---|---|---|---|
| (N)NNCC 78, No. 1 | (N)NNCC 78, No. 1 | 311 | 203 (65.3%) | 185 (59.5%) | 178 (57.2%) | 119 (38.3%) | 108 (34.7%) |
| | (N)NNCC 78, No. 2 | 389 | 125 (32.1%) | 86 (22.1%) | 79 (20.3%) | 67 (17.2%) | 46 (11.8%) |
| | (N)NN 76, No. 4 | 368 | 332 (90.2%) | 200 (54.3%) | 183 (49.7%) | 179 (48.6%) | 130 (35.3%) |
| | (C)CC 76, No. 3 | 317 | 205 (64.7%) | 163 (51.4%) | 154 (48.6%) | 112 (35.3%) | 72 (22.7%) |
| (C)CN 76, No. 21 | (N)NNCC 78, No. 1 | 212 | 152 (71.7%) | 122 (57.5%) | 119 (56.1%) | 96 (45.3%) | 96 (45.3%) |
| | (N)NNCC 78, No. 2 | 186 | 15 ( 8.1%) | 14 ( 7.5%) | 14 ( 7.5%) | 7 ( 3.8%) | 6 ( 3.2%) |
| (N)NC 76, No. 16 | (N)NNCC 78, No. 1 | 163 | 22 (13.5%) | 22 (13.5%) | 22 (13.5%) | 16 ( 9.8%) | 16 ( 9.8%) |

male *Rana nigromaculata*, (N)NN 76♂, No. 4. Although 332 (90.2%) of 368 eggs cleaved normally, 21 of the normally cleaved eggs died at the blastula stage, 68 died of incomplete invagination at the gastrula stage and 43 died of edema or severe abnormality at the tail-bud stage. After 132 eggs in total died of various abnormalities, 200 (54.3%) eggs became normal tail-bud embryos. During the hatching stage, 17 embryos died of edema or some other abnormalities, while 183 (49.7%) eggs hatched normally. Of these normally hatched tadpoles, 179 (48.6%) became normally feeding tadpoles which were more than 30 mm in total length. The chromosomes of these feeding tadpoles were examined. Although 49 of them died of edema immediately before or during metamorphosis, the remaining 130 (35.3%) attained completion of metamorphosis.

The numbers of the normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs correspond to 60.2%, 55.1%, 53.9% and 39.2% of the normally cleaved eggs, respectively (Table 1).

c. (N)NNCC 78♀, No. 1×(C)CC 76♂, No. 3

The female amphidiploid, (N)NNCC 78♀, No. 1, was mated with a diploid male *Rana plancyi chosenica*, (C)CC 76♂, No. 3. It was found that 205 (64.7%) of 317 eggs cleaved normally. After three died of abnormality at the blastula stage, 16 of incomplete invagination at the gastrula stage and 23 of edema or severe abnormality at the tail-bud stage, 163 (51.4%) eggs became normal tail-bud embryos. While nine embryos died of edema at the hatching stage, the other 154 (48.6%) hatched normally. Of the normally hatched tadpoles, 30 and 12 died of underdevelopment or edema without taking food and shortly after beginning to eat, respectively. Eventually, 112 (35.3%) eggs became normally feeding tadpoles which were more than 30 mm in total length. The chromosomes of these feeding tadpoles were examined. After three tadpoles died soon after the chromosome examination, 23 others of edema immediately before or during metamorphosis and 14 during metamorphosis without showing any external abnormality, 72 (22.7%) normally completed metamorphosis (Table 1).

The numbers of the normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs correspond to 79.5%, 75.1%, 54.6% and 35.1% of the normally cleaved eggs, respectively.

d. (C)CN 76♀, No. 21×(N)NNCC 78♂, Nos. 1 and 2

The 4-year-old female hybrid, (C)CN 76♀, No. 21, obtained in 1976 from a cross, *Rana plancyi chosenica*♀ × *Rana nigromaculata*♂, was mated with two 2-year-old male amphidiploids, (N)NNCC 78♂, Nos. 1 and 2. Of 398 normal-sized eggs inseminated with sperm of the two male amphidiploids, 167 (42.0%) cleaved normally and 136 (34.2%) became normal tail-bud embryos, while 31 died of edema, underdevelopment, blisters or microcephaly by this stage. After three embryos died of edema at the hatching stage, 133 (33.4%) hatched normally. Of these normally hatched tadpoles, 103 (25.9%) became normally feeding tadpoles which were more than 30 mm in total length, while the other 30 died of edema without taking food or soon after beginning to eat. The chromosomes of the normally feeding tadpoles were examined. After one of these tadpoles died after the chromosome examination, the other 102 (25.6%) attained completion of metamorphosis (Table 1).

The numbers of the normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs correspond to 81.4%, 79.6%, 61.7% and 61.1% of the normally cleaved eggs, respectively.

e. (N)NC 76♀, No. 16×(N)NNCC 78♂, No. 1

The 4-year-old female hybrid, (N)NC 76♀, No. 16, obtained in 1976 from a cross, *Rana nigromaculata*♀ × *Rana plancyi chosenica*♂, was mated with a 2-year-old male amphidiploid, (N)NNCC 78♂, No. 1. Of 163 normal-sized eggs inseminated with sperm of the male amphidiploid, only 22 (13.5%) cleaved normally. Of these normally cleaved eggs, 16 (9.8%) became normally metamorphosed frogs, while the other six died of edema at the feeding tadpole stage (Table 1).

### *II. Reproductive capacity of first-generation amphidiploids examined in 1981*

Of one-year-old amphidiploids of the first-generation offspring produced in 1980 from a mating, (N)NNCC 78♀, No. 1×(N)NNCC 78♂, No. 1, the best grown female, (N)NNCC 80♀, No. 1, and the two best grown males, (N)NNCC 80♂, Nos. 1 and 2, were used in mating experiments performed in 1981, together with a female *Rana nigromaculata*, (N)NN 77♀, No. 1 and a male *Rana nigromaculata*, (N)NN 77♂, No. 1, obtained in 1977 from a control mating, (N)NN♀ ×

(N)NN♂, and a female *Rana plancyi chosenica*, (C)CC 76♀, No. 1 and a male *Rana plancyi chosenica*, (C)CC 76♂, No. 4, obtained in 1976 from a mating, (C)CC♀ × (C)CC♂.

1. Females

a. Female amphidiploid, (N)NNCC 80♀, No. 1

One year old and 66.0 mm in body length. Laid 1674 eggs after pituitary injection. The eggs were almost uniform in size. When 50 eggs were measured, they were 2.33±0.01 mm in diameter.

b. Female diploid *Rana nigromaculata*, (N)NN 77♀, No. 1

Four years old and 63.0 mm in body length. Laid 2932 eggs after pituitary injection. The eggs were almost uniform in size. When 50 eggs were measured, they were 1.76±0.01 mm in diameter.

c. Female diploid *Rana plancyi chosenica*, (C)CC 76♀, No. 1

Five years old and 54.5 mm in body length. Laid 1326 eggs after pituitary injection. The eggs were almost uniform in size. When 50 eggs were measured, they were 1.63±0.01 mm in diameter.

2. Males

a. Male amphidiploid, (N)NNCC 80♂, No. 1

One year old and 58.0 mm in body length. The left and right testes were 5.5 mm × 4.5 mm and 5.5 mm × 4.0 mm in size, respectively.

b. Male amphidiploid, (N)NNCC 80♂, No. 2

One year old and 57.5 mm in body length. The left and right testes were 5.0 mm × 4.0 mm and 5.0 mm × 3.5 mm in size, respectively.

c. Diploid male *Rana nigromaculata*, (N)NN 77♂, No. 1

Four years old and 60.0 mm in body length. Both left and right testes were 6.0 mm × 3.0 mm in size.

d. Diploid male *Rana plancyi chosenica*, (C)CC 76♂, No. 4

Five years old and 43.5 mm in body length. Both left and right testes were 3.0 mm × 2.0 mm in size.

3. Crossing experiments

a. (N)NNCC 80♀, No. 1 × (N)NNCC 80♂, Nos. 1 and 2

The one-year-old female amphidiploid, (N)NNCC 80♀, No. 1, and 2 one-year-old male amphidiploids, (N)NNCC 80♂, Nos. 1 and 2, produced in 1980 from a mating between the parental female and male amphidiploids were mated with each other in the breeding season of 1981. It was found that 806 (94.7%) of 851 eggs cleaved normally and 620 (72.9%) became normal tail-bud embryos. Of the remainders of the normally cleaved eggs, 94 and 46 died of necrosis at the gastrula and neurula stage, respectively, after showing incomplete invagination, and 46 others died of edema or microcephaly at the tail-bud stage. After 137 eggs died of edema, blisters, microcephaly or ill-development of gills at the hatching stage, 483 (56.8%) hatched normally. Of the normally hatched tadpoles, 49 died without taking food or soon after beginning to eat. Eventually, 434 (51.0%) became normally feeding tadpoles which were more than 30 mm in total length. Of these tadpoles, 16 died of edema immediately before or during metamorphosis and 35 others died during metamorphosis without showing any external abnormality. A total of 383 (45.0%) completed metamorphosis (Table 2).

TABLE 2
Developmental capacity of the second-generation offspring produced from amphidiploids in 1981

| First-generation offspring | | No. of eggs | No. of normal cleavages | No. of normal tail-bud embryos | No. of normally hatched tadpoles | No. of normally feeding tadpoles | No. of metamorphosed frogs |
|---|---|---|---|---|---|---|---|
| Female | Male | | | | | | |
| (N)NNCC 80, No. 1 | (N)NNCC 80, No. 1 | 271 | 254 (93.7%) | 193 (71.2%) | 162 (59.8%) | 135 (49.8%) | 132 (48.7%) |
| | (N)NNCC 80, No. 2 | 580 | 552 (95.2%) | 427 (73.6%) | 321 (55.3%) | 299 (51.6%) | 251 (43.3%) |
| | (N)NN 77, No. 1 | 152 | 144 (94.7%) | 119 (78.3%) | 88 (57.9%) | 77 (50.7%) | 63 (41.4%) |
| | (C)CC 76, No. 4 | 173 | 62 (35.8%) | 51 (29.5%) | 49 (28.3%) | 33 (19.1%) | 30 (17.3%) |
| (N)NN 77, No. 1 | (N)NNCC 80, No. 1 | 139 | 28 (20.1%) | 18 (12.9%) | 10 ( 7.2%) | 8 ( 5.8%) | 8 ( 5.8%) |
| | (N)NNCC 80, No. 2 | 126 | 20 (15.9%) | 15 (11.9%) | 7 ( 5.6%) | 6 ( 4.8%) | 5 ( 4.0%) |
| (N)NN 77, No. 1 | (N)NN 77, No. 1 | 74 | 74 (100 %) | 70 (94.6%) | 69 (93.2%) | 62 (83.8%) | 55 (74.3%) |
| (C)CC 76, No. 1 | (C)CC 76, No. 4 | 104 | 83 (79.8%) | 76 (73.1%) | 72 (69.2%) | 66 (63.5%) | 59 (56.7%) |

The numbers of the normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs correspond to 76.9%, 59.9%, 53.8% and 47.5% of the normally cleaved eggs, respectively.

b. (N)NNCC 80♀, No. 1 × (N)NN 77♂, No. 1

The one-year-old female amphidiploid, (N)NNCC 80♀, No. 1 was mated with the 4-year-old male *Rana nigromaculata,* (N)NN 77♂, No. 1. Of 152 eggs, 144 (94.7%) cleaved normally and 119 (78.3%) became normal tail-bud embryos, while 25 died of incomplete invagination, edema or some other abnormalities by the tail-bud stage. At the hatching stage, 31 embryos died of edema, ill-development of gills or some other abnormalities, and 88 (57.9%) hatched normally. Of these normally hatched tadpoles, 77 (50.7%) became normally feeding tadpoles which were more than 30 mm in total length, while the other 11 died without taking food or soon after beginning to eat. Of the normally feeding tadpoles, 63 (41.4%) attained completion of metamorphosis, while 11 died of edema immediately before metamorphosis and three others died during metamorphosis without showing any external abnormality (Table 2).

The numbers of normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs correspond to 82.6%, 61.1%, 53.5% and 43.8% of the normally cleaved eggs, respectively.

c. (N)NNCC 80♀, No. 1×(C)CC 76♂, No. 4

The one-year-old female amphidiploid, (N)NNCC 80♀, No. 1, was mated with the 5-year-old male *Rana plancyi chosenica*, (C)CC 76♂, No. 4. Of 173 eggs, 62 (35.8%) cleaved normally and 51 (29.5%) became normal tail-bud embryos, while 11 died of incomplete invagination or edema by the tail-bud stage. After two embryos died of edema, 49 (28.3%) eggs hatched normally. Of the normally hatched tadpoles, 16 died of edema, underdevelopment or gill abnormality, while 33 (19.1%) became normally feeding tadpoles which were more than 30 mm in total length. While three of the feeding tadpoles died of edema immediately before metamorphosis, 30 (17.3%) attained completion of metamorphosis (Table 2).

The numbers of the normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs correspond to 82.3%, 79.0%, 53.2% and 48.4% of the normally cleaved eggs.

d. (N)NN 77♀, No. 1×(N)NNCC 80♂, Nos. 1 and 2

The 4-year-old diploid female *Rana nigromaculata*, (N)NN 77♀, No. 1, was mated with 2 one-year-old male amphidiploids, (N)NNCC 80♂, Nos. 1 and 2, which had been obtained from a mating between a female and a male amphidiploid. The result showed that only 48 (18.1%) of 265 eggs cleaved normally. The paucity of the normally cleaved eggs seemed to be attributable to large spermatozoa unsuitable for the eggs of the diploid female *Rana nigromaculata*. Of the normally cleaved eggs, 33 (12.5%) became normal tail-bud embryos, while 15 died of edema or underdevelopment by the tail-bud stage. Thereafter, 16 embryos died of edema, underdevelopment, gill abnormality, blisters or microcephaly, while 17 (6.4%) hatched normally. Eventually, 14 (5.3%) became normally feeding tadpoles which were more than 30 mm in total length. After one tadpole died during metamorphosis without showing any external abnormality, 13 (4.9%) attained completion of metamorphosis (Table 2).

The numbers of the normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs correspond to 68.8%, 35.4%, 29.2% and 27.1% of the normally cleaved eggs, respectively.

e. Control matings

In the control mating of *Rana nigromaculata*, (N)NN 77♀, No. 1×(N)NN 77♂, No. 1, 74 (100%) eggs cleaved normally. After four, one, seven and seven eggs died of various abnormalities at the stage of tail-bud, hatching, beginning to eat and immediately before or during metamorphosis, respectively, 55 (74.3%) normally completed metamorphosis.

On the other hand, 83 (79.8%) of 104 eggs in the control mating of *Rana plancyi chosenica*, (C)CC 76♀, No. 1×(C)CC 76♂, No. 4, cleaved normally. After seven, four, six and seven died of various abnormalities at the stages of tail-bud, hatching, beginning to eat and immediately before or during metamorphosis, respectively, 59 (56.7%) normally completed metamorphosis. The number of the normally metamorphosed frogs corresponds to 71.1% of the normally cleaved eggs (Table 2).

## *III. Reproductive capacity of second-generation amphidiploids examined in 1982*

A total of 25 mature female and 23 mature male amphidiploids of the second-generation offspring were produced in 1981 from a mating between a female and a male amphidiploid of the first-generation offspring, (N)NNCC 80♀, No. 1× (N)NNCC 80♂, No. 2. Of these one-year-old amphidiploids of the second-generation offspring, the best grown female, (N)NNCC 81♀, No. 1, and the best grown male, (N)NNCC 81♂, No. 1, were used in mating experiments performed in 1982 together with a 5-year-old female and a 5-year-old male *Rana nigromaculata*, (N)NN 77♀, No. 2 and (N)NN 77♂, No. 2, obtained in 1977 from a control mating, (N)NN♀×(N)NN♂, and a 6-year-old female and a 6-year-old male *Rana plancyi chosenica*, (C)CC 76♀, No. 2 and (C)CC 76♂, No. 5, obtained in 1976 from a control mating, (C)CC♀×(C)CC♂.

### 1. Females

a. Female amphidiploid, (N)NNCC 81♀, No. 1

One year old and 72.0 mm in body length. Laid 1946 eggs after pituitary injection. The eggs were almost uniform in size. When 50 eggs were measured, they were 2.31±0.01 mm in diameter.

b. Diploid female *Rana nigromaculata*, (N)NN 77♀, No. 2

Five years old and 70.0 mm in body length. Laid 2869 eggs after pituitary injection. The eggs were almost uniform in size. When 50 eggs were measured, they were 1.80±0.01 mm in diameter.

c. Diploid female *Rana plancyi chosenica*, (C)CC 76♀, No. 2

Six years old and 56.0 mm in body length. Laid 1416 eggs after pituitary injection. The eggs were almost uniform in size. When 50 eggs were measured, they were 1.67±0.01 mm in diameter.

### 2. Males

a. Male amphidiploid, (N)NNCC 81♂, No. 1

One year old and 62.5 mm in body length. The left and right testes were

6.0 mm × 4.0 mm and 5.5 mm × 4.0 mm in size, respectively.

b. Diploid male *Rana nigromaculata*, (N)NN 77♂, No. 2

Five years old and 64.5 mm in body length (Fig. 1). The left and right testes were 6.0 mm × 3.0 mm and 6.0 mm × 3.5 mm in size, respectively.

c. Diploid male *Rana plancyi chosenica*, (C)CC 76♂, No. 5

Six years old and 45.0 mm in body length (Fig. 2). The left and right testes were 3.0 mm × 2.0 mm and 3.5 mm × 2.5 mm in size, respectively.

### 3. Crossing experiments

a. (N)NNCC 81♀, No. 1 × (N)NNCC 81♂, No. 1

The one-year-old female amphidiploid, (N)NNCC 81♀, No. 1, produced in 1981, and the one-year-old male amphidiploid, (N)NNCC 81♂, No. 1, produced from a mating between a female and a male amphidiploid second-generation offspring were mated with each other in 1982. Of 445 eggs, 319 (71.7%) cleaved normally, 307 (69.0%) became normal tail-bud embryos, 275 (61.8%) hatched normally and 268 (60.2%) became normally feeding tadpoles which were more than 30 mm in total length. The others of the normally cleaved eggs died of edema or under-development at various developmental stages. Eventually, 257 (57.8%) normally completed metamorphosis.

The numbers of the normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs correspond to 96.2%, 86.2%, 84.0% and 80.6% of the normally cleaved eggs, respectively (Table 3).

TABLE 3

Developmental capacity of the third-generation offspring produced from amphidiploids in 1982

| Second-generation offspring | | No. of eggs | No. of normal cleavages | No. of normal tail-bud embryos | No. of normally hatched tadoples | No. of normally feeding tadpoles | No. of metamor-phosed frogs |
|---|---|---|---|---|---|---|---|
| Female | Male | | | | | | |
| (N)NNCC 81, No. 1 | (N)NNCC 81, No. 1 | 445 | 319 (71.7%) | 307 (69.0%) | 275 (61.8%) | 268 (60.2%) | 257 (57.8%) |
| | (N)NN 77, No. 2 | 96 | 65 (67.7%) | 63 (65.6%) | 63 (65.6%) | 61 (63.5%) | 58 (60.4%) |
| | (C)CC 76, No. 5 | 126 | 105 (83.3%) | 100 (79.4%) | 96 (76.2%) | 88 (69.8%) | 86 (68.3%) |
| (N)NN 77, No. 2 | (N)NNCC 81, No. 1 | 167 | 26 (15.6%) | 20 (12.0%) | 14 ( 8.4%) | 9 ( 5.4%) | 9 ( 5.4%) |
| (C)CC 76, No. 2 | (N)NNCC 81, No. 1 | 138 | 12 ( 8.7%) | 10 ( 7.2%) | 7 ( 5.1%) | 7 ( 5.1%) | 7 ( 5.1%) |

b. (N)NNCC 81♀, No. 1 × (N)NN 77♂, No. 2

The one-year-old female amphidiploid, (N)NNCC 81♀, No. 1, was mated with the five-year-old diploid male *Rana nigromaculata*, (N)NN 77♂, No. 2. Of 96 eggs, 65 (67.7%) cleaved normally and, eventually, 58 (60.4%) attained completion of metamorphosis, while seven died of edema or underdevelopment during the embryonic and tadpole stages. More specifically, 63 (65.6%) became normal tail-bud embryos and hatched normally and, thereafter, 61 (63.5%) became normally feeding tadpoles.

The numbers of normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs correspond to 96.9%, 96.9%, 93.8% and 89.2% of the normally cleaved eggs, respectively (Table 3).

c. (N)NNCC 81♀, No. 1×(C)CC 76♂, No. 5

The one-year-old female amphidiploid, (N)NNCC 81♀, No. 1, was mated with the 6-year-old male *Rana plancyi chosenica*, (C)CC 76♂, No. 5. Of 126 eggs, 105 (83.3%) cleaved normally. Of the normally cleaved eggs, 19 died of edema, underdevelopment, blisters, curvature of the body or some other abnormalities before attaining the completion of metamorphosis, while 100 (79.4%), 96 (76.2%), 88 (69.8%) and 86 (68.3%) became normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs, respectively. These numbers correspond to 95.2%, 91.4%, 83.8% and 81.9% of the normally cleaved eggs, respectively (Table 3).

d. (N)NN 77♀, No. 2×(N)NNCC 81♂, No. 1

The 5-year-old female diploid *Rana nigromaculata*, (N)NN 77♀, No. 2 was mated with the one-year-old male amphidiploid, (N)NNCC 81♂, No. 1. Of 167 eggs, 26 (15.6%) cleaved normally, 20 became normal tail-bud embryos, 14 hatched normally and 9 (5.4%) became normally feeding tadpoles which were more than 30 mm in total length. Six, six and five other eggs died of edema at the stages of tail-bud, hatching and beginning to eat, respectively. The nine feeding tadpoles attained completion of metamorphosis.

The numbers of the normal tail-bud embryos, normally hatched tadpoles, normally feeding tadpoles and normally metamorphosed frogs correspond to 76.9%, 53.8%, 34.6% and 34.6% of the normally cleaved eggs, respectively (Table 3).

e. (C)CC 76♀, No. 2×(N)NNCC 81♂, No. 1

The 6-year-old female diploid *Rana plancyi chosenica* was mated with the one-year-old male amphidiploid, (N)NNCC 81♂, No. 1. Of 138 eggs, only 12 (8.7%) cleaved normally, 10 (7.2%) became normal tail-bud embryos and seven (5.1%) hatched normally and became normally metamorphosed frogs (Table 3).

f. Control matings

In the control mating of *Rana nigromaculata*, (N)NN 77♀, No. 2×(N)NN 77♂, No. 2, 267 (89.6%) of 298 eggs cleaved normally. All the normally cleaved eggs developed normally until the hatching stage. While 32 of them were continuously reared, the others were abandoned. On the other hand, 222 (89.2%) of 249 eggs in the control mating of *Rana plancyi chosenica*, (C)CC 76♀, No. 2×(C)CC 76♂, No. 5, cleaved normally. Of the normally cleaved eggs, 32 became abnormal and died before the hatching stage, while 190 hatched normally. Although many of the hatched tadpoles died of edema or underdevelopment without taking food, 124 became normally feeding tadpoles. Eventually, 106 (42.6%) attained completion of metamorphosis. This number of metamorphosed frogs corresponds to 47.7% of the normally cleaved eggs.

## *IV. Chromosomes*

### 1. First-generation offspring produced from amphidiploids in 1980

#### a. (N)NNCC 78♀, No. 1×(N)NNCC 78♂, Nos. 1 and 2

Chromosomes were examined in the tail-tips of 119 feeding tadpoles which were produced in 1980 from a mating between a 2-year-old female and a 2-year-old male amphidiploid, (N)NNCC 78♀, No. 1×(N)NNCC 78♂, No. 1, and were more than 30 mm in total length. These amphidiploids were those which were produced in 1978 from a mating between a female allotriploid, (N)NNC♀, and a diploid male *Rana plancyi chosenica*, (C)CC♂. It was found that 104 of the 119 tadpoles were tetraploids, two were diploids, four were hexaploids and the remaining nine were 2n-6n mosaics (Table 4).

TABLE 4

Chromosome number of the first-generation offspring produced from amphidiploids in 1980

| Parents | | No. of normally feeding tadpoles | Number of tadpoles | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Ana-lyzed | Number of chromosomes | | | | | | | |
| Female | Male | | | 26 (2n) | 39 (3n) | 52 (4n) | 65 (5n) | 78 (6n) | 26–52 (2n–4n) | 26–65 (2n–5n) | 26–78 (2n–6n) |
| (N)NNCC 78, No. 1 | (N)NNCC 78, No. 1 | 119 | 119 | 2 | | 104 | | 4 | | | 9 |
| | (N)NNCC 78, No. 2 | 67 | 67 | | | 63 | | 2 | | | 2 |
| | (N)NN 76, No. 4 | 179 | 179 | 1 | 176 | | | | | 2 | |
| | (C)CC 76, No. 3 | 112 | 112 | | 104 | | 8 | | | | |
| (C)CN 76, No. 21 | (N)NNCC 78, No. 1 | 96 | 96 | 3 | 86 | 4 | | | 3 | | |
| | (N)NNCC 78, No. 2 | 7 | 7 | | 7 | | | | | | |
| (N)NC 76, No. 16 | (N)NNCC 78, No. 1 | 16 | 16 | | 16 | | | | | | |

Chromosomes were also examined in the tail-tips of 67 feeding tadpoles which were produced in 1980 from a mating between the above female and another 2-year-old male amphidiploid, (N)NNCC 78♀, No. 1×(N)NNCC 78♂, No. 2, and were more than 30 mm in body length. The results showed that 63 were tetraploids, two were hexaploids and the remaining two were 2n-6n mosaics (Table 4).

#### b. (N)NNCC 78♀, No. 1×(N)NN 76♂, No. 4

Chromosomes were examined in the tail-tips of 179 feeding tadpoles which were produced in 1980 from a mating between the above female amphidiploid, (N)NNCC 78♀, No. 1, and a 4-year-old diploid male *Rana nigromaculata*, (N)NN

76♂, No. 4, and were more than 30 mm in total length. It was found that 176 were triploids, one was a diploid and the remaining two were 2n-5n mosaics (Table 4).

c. (N)NNCC 78♀, No. 1 × (C)CC 76♂, No. 3

Chromosomes were examined in the tail-tips of 112 feeding tadpoles which were produced in 1980 from a mating between the above female amphidiploid, (N)NNCC 78♀, No. 1, and a 4-year-old diploid male *Rana plancyi chosenica* (C)CC 76♂, No. 3, and were more than 30 mm in total length. It was found that 104 were triploids and the other eight were pentaploids (Table 4).

d. (C)CN 76♀, No. 21 × (N)NNCC 78♂, Nos. 1 and 2

Chromosomes were examined in the tail-tips of 103 feeding tadpoles which were produced in 1980 from matings between a 4-year-old diploid female hybrid, *Rana plancyi chosenica*♀ × *Rana nigromaculata*♂, and the above two 2-year-old male amphidiploids, (N)NNCC 78♂, Nos. 1 and 2, and were more than 30 mm in total length. It was found that 93 were triploids, three were diploids, four were tetraploids and the remaining three were 2n-4n mosaics (Table 4).

e. (N)NC 76♀, No. 16 × (N)NNCC 78♂, No. 1

Chromosomes were examined in the tail-tips of 16 feeding tadpoles which were produced in 1980 from a mating between a 4-year-old diploid female hybrid, *Rana nigromaculata*♀ × *Rana plancyi chosenica*♂, and one of the above two 2-year-old male amphidiploids, (N)NNCC 78♂, No. 1, and were more than 30 mm in total length. All the 16 tadpoles were triploids (Table 4).

2. Second-generation offspring produced from amphidiploids in 1981

a. (N)NNCC 80♀, No. 1 × (N)NNCC 80♂, Nos. 1 and 2

Chromosomes were examined in the tail-tips of 167 of 434 feeding tadpoles which were produced in 1981 from matings between a one-year-old female and two one-year-old male amphidiploids of the first-generation offspring, (N)NNCC 80♀, No. 1 and (N)NNCC 80♂, Nos. 1 and 2, of the parental amphidiploids and were more than 30 mm in total length. It was found that 166 were tetraploids and the

TABLE 5

Chromosome number of the second-generation offspring produced from amphidiploids in 1981

| First-generation offspring | | No. of normally feeding tadpoles | Number of tadpoles | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Analyzed | Number of chromosomes | | | | | | | |
| Female | Male | | | 26 (2n) | 39 (3n) | 52 (4n) | 65 (5n) | 78 (6n) | 26–52 (2n–4n) | 26–65 (2n–5n) | 26–78 (2n–6n) |
| (N)NNCC 80, No. 1 | (N)NNCC 80, No. 1 | 135 | 91 | | | 91 | | | | | |
| | (N)NNCC 80, No. 2 | 299 | 76 | | | 75 | | | 1 | | |
| | (N)NN 77, No. 1 | 77 | 20 | | 20 | | | | | | |
| | (C)CC 76, No. 4 | 33 | 10 | | 10 | | | | | | |
| (N)NN 77, No. 1 | (N)NNCC 80, No. 1 | 8 | 8 | | 8 | | | | | | |
| | (N)NNCC 80, No. 2 | 6 | 6 | | 5 | 1 | | | | | |
| (N)NN 77, No. 1 | (N)NN 77, No. 1 | 62 | 20 | 20 | | | | | | | |
| (C)CC 76, No. 1 | (C)CC 76, No. 4 | 66 | 20 | 20 | | | | | | | |

remainder was a 2n-4n mosaic (Table 5). The 2n-4n mosaic was an exceptional individual in that the body was the smallest and somewhat distorted. After the chromosome examination, this tadpole was stunted and died before metamorphosis.

As the remaining 267 feeding tadpoles including 44 derived from male (N)NNCC 80♂, No. 1 and 223 derived from male (N)NNCC 80♂, No. 2 were completely normal in size, shape and viability, they were all regarded as tetraploids, although their chromosomes were not observed.

b. (N)NNCC 80♀, No. 1 × (N)NN 77♂, No. 1

Chromosomes were examined in the tail-tips of 20 of 77 feeding tadpoles which were produced in 1981 from a mating between the above female amphidiploid, (N)NNCC 80♀, No. 1, and a 4-year-old diploid male *Rana nigromaculata*, (N)NN 77♂, No. 1, and were more than 30 mm in total length. All the 20 tadpoles were triploids (Table 5). Although the chromosomes of the other 57 feeding tadpoles were not observed, they were regarded as triploids, as they were completely normal in size, shape and viability.

c. (N)NNCC 80♀, No. 1 × (C)CC 76♂, No. 4

Chromosomes were examined in the tail-tips of 10 of 33 feeding tadpoles which were produced in 1981 from a mating between the above female amphidiploid, (N)NNCC 80♀, No. 1, and a 5-year-old diploid male *Rana plancyi chosenica*, (C)CC 76♂, No. 4, and were more than 30 mm in total length. All the 10 tadpoles were triploids (Table 5). The other 23 feeding tadpoles were regarded as triploids without observing chromosomes, as they were completely normal in size, shape and viability.

d. (N)NN 77♀, No. 1 × (N)NNCC 80♂, Nos. 1 and 2

Chromosomes were examined in the tail-tips of 14 feeding tadpoles which were produced in 1981 from matings between a 4-year-old diploid female *Rana nigromaculata*, (N)NN 77♀, No. 1, and the above two male amphidiploids, (N)NNCC 80♂, Nos. 1 and 2, and were more than 30 mm in total length. It was found that 13 were triploids and the remainder was a tetraploid (Table 5).

e. Control matings

Chromosomes were examined in the tail-tips of 20 of 62 feeding tadpoles which were produced in 1981 from the control mating between a 4-year-old diploid female and a 4-year-old diploid male *Rana nigromaculata*, (N)NN 77♀, No. 1 × (N)NN 77♂, No. 1, and were more than 30mm in total length. All the 20 tadpoles were diploids (Table 5). On the other hand, chromosomes were examined in the tail-tips of 20 of 66 feeding tadpoles which were produced in 1981 from the control mating between a 5-year-old diploid female and a 5-year-old diploid male *Rana plancyi chosenica*, (C)CC 76♀, No. 1 × (C)CC 76♂, No. 4, and were more than 30 mm in total length. In this case, all the 20 tadpoles were also diploids.

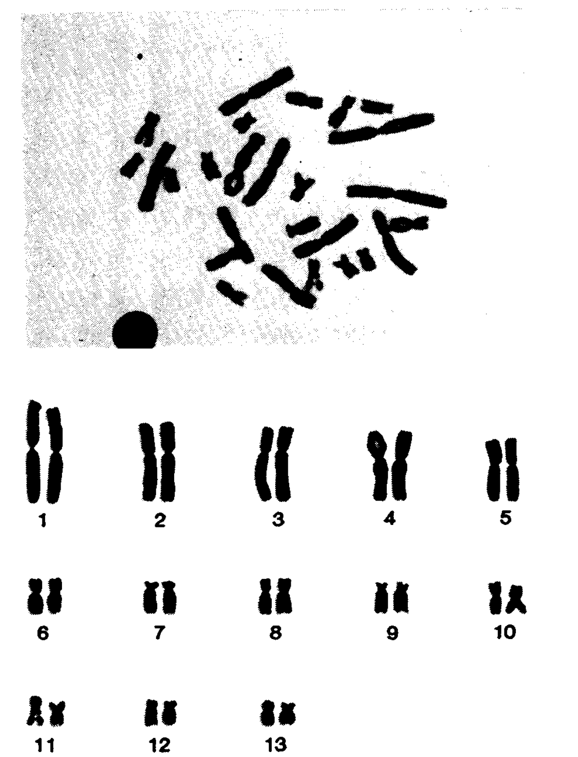


Fig. 1. Metaphase plate and the karyotype of a control diploid *Rana nigromaculata*. ×900

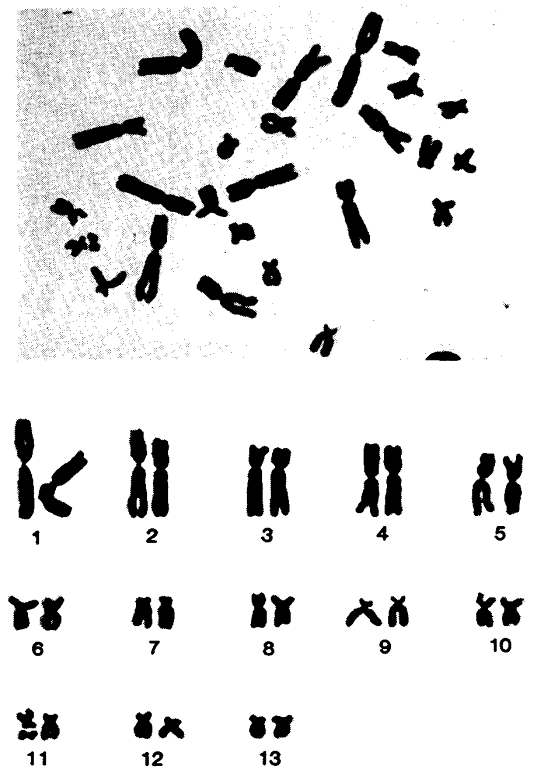


Fig. 2. Metaphase plate and the karyotype of a control diploid *Rana plancyi chosenica*. ×900

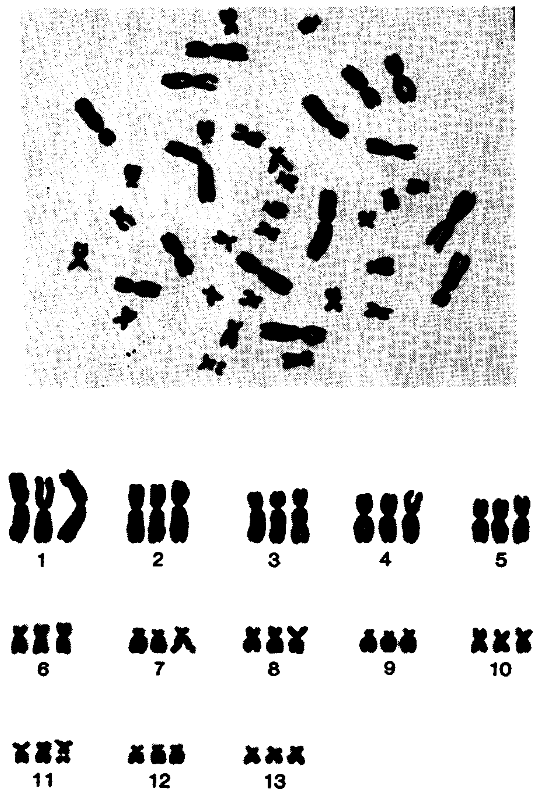


Fig. 3. Metaphase plate and the karyotype of a (N)NNC allotriploid male produced from (N)NNCC 78♀, No. 1×(N)NN 76♂, No. 4. ×900

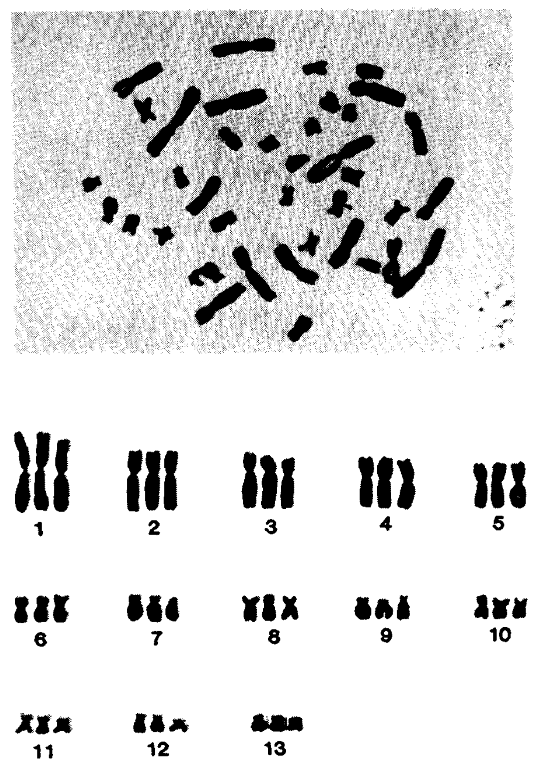


Fig. 4. Metaphase plate and the karyotype of a (N)NCC allotriploid male produced from (N)NNCC 78♀, No. 1×(C)CC 76♂, No. 3. ×900

### 3. Third-generation offspring produced from amphidiploids in 1982

Chromosomes were not examined in the tadpoles of the third-generation offspring produced in 1982 from a mating between a one-year-old female and a one-year-old male second-generation offspring of amphidiploids, (N)NNCC 81♀, No. 1 × (N)NNCC 81♂, No. 1, as almost all the second-generation offspring produced in 1981 from a female and two males of the first-generation offspring of amphidiploids were tetraploids. A male and a female third-generation offspring which were about 40 mm and 60 mm in body length, respectively, and about four months after metamorphosis were completely tetraploids, when their chromosomes were examined by the blood culture method (Fig. 5).

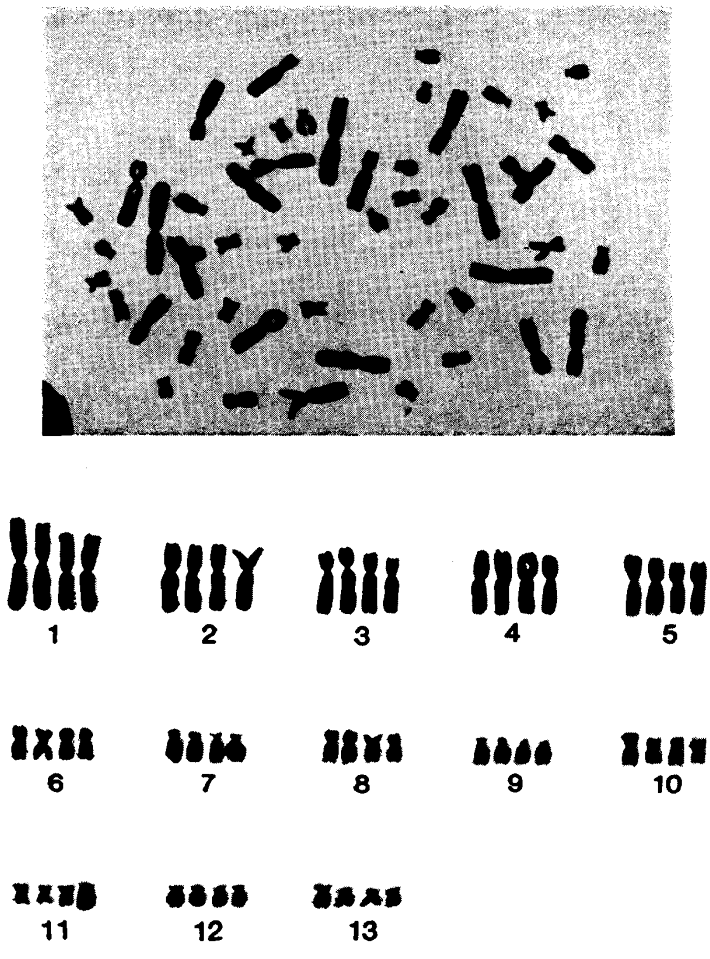


Fig. 5. Metaphase plate and the karyotype of a (N)NNCC amphidiploid male produced from (N)NNCC 81♀, No. 1 × (N)NNCC 81♂, No. 1. ×1150

## *V. Metamorphosis, viability and sex*

### 1. First-generation offspring produced from amphidiploids in 1980

a. (N)NNCC 78♀, No. 1 × (N)NNCC 78♂, No. 1

Of 104 feeding tetraploid tadpoles which were produced in 1980 from a mating between a 2-year-old female and a 2-year-old male amphidiploid, (N)NNCC 78♀, No. 1×(N)NNCC 78♂, No. 1, and were more than 30 mm in total length, 93 completed metamorphosis at the age of 54~98 days, 69.0 days on the average. Of these metamorphosed tetraploid frogs, 49 died within three months after metamorphosis. While the sex of 20 dead frogs was not identified owing to their postmortem changes, seven of the other 29 tetraploids were females and 22 were males. Of 34 mature tetraploid frogs, eight were females and 26 were males. The tetraploid frogs totalled 15 females and 48 (76.2%) males.

Two diploid tadpoles completed metamorphosis at the age of 55 days and 58 days, 56.5 days on the average. These two were sexually matured and became males. Four hexaploid tadpoles completed metamorphosis at the age of 61~66 days, 65.1 days on the average. While two of them died during the first hibernation, the other two were sexually matured. All these four hexaploids were males. Nine 2n-6n mosaic tadpoles completed metamorphosis at the age of 61~66 days, 65.1 days on the average. Four of these mosaic frogs were retarded in growth and were less than 20 mm in body length three months after metamorphosis, while the other five were normal in growth and were 35~45 mm at the same time. Although the nine mosaic frogs were sexually matured, all of them died nearly at the age of one year. They were all males (Table 6).

b. (N)NNCC 78♀, No. 1×(N)NNCC 78♂, No. 2

Of 63 feeding tetraploid tadpoles which were produced in 1980 from a mating between the above female and another 2-year-old male amphidiploid, (N)NNCC 78♀, No. 1×(N)NNCC 78♂, No. 2, and were more than 30 mm in total length, 42 completed metamorphosis at the age of 54~92 days, 64.8 days on the average. Fifteen of them died within three months after metamorphosis. Of the dead tetraploids, three were females and four were males. The sex of the other eight tetraploids could not be identified owing to their postmortem changes. Of 27 mature tetraploids, 16 were females and 11 were males. The tetraploid frogs totalled 19 females and 15 (44.1%) males.

Two hexaploid tadpoles completed metamorphosis at the age of 62 days and 65 days, 63.5 days on the average. Both hexaploids were males and died within 10 months after metamorphosis. Two 2n-6n mosaic tadpoles completed metamorphosis at the age of 61 days and 70 days, 65.5 days on the average. They lived for about one year and were sexually mature males (Table 6).

c. (N)NNCC 78♀, No. 1×(N)NN 76♂, No.4

Of 176 triploid tadpoles which were produced in 1980 from a mating between the above female amphidiploid, (N)NNCC 78♀, No. 1, and a 4-year-old male *Rana nigromaculata*, (N)NN 76♂, No. 4, and were more than 30 mm in total length, 127 completed metamorphosis at the age of 54~82 days, 59.3 days on the average. Of these metamorphosed frogs, 65 died within three months after metamorphosis. While the sex of 29 of the dead frogs were not identified owing to their postmortem changes, 18 and 18 of the other 36 were females and males, respectively. Of 62 mature frogs, 33 were females and 29 were males (Fig. 3). The metamorphosed triploids totalled 51 females and 47 (48.0%) males.

A single diploid tadpole completed metamorphosis at the age of 71 days. This frog was a male and died three weeks after metamorphosis. Two 2n-5n mosaic tadpoles completed metamorphosis at the age of 77 days and 84 days, 80.5 days on the average. They were males and died during hibernation (Table 6).

d. (N)NNCC 78♀, No. 1×(C)CC 76♂, No. 3

Of 104 triploid tadpoles which were produced in 1980 from a mating between the above female amphidiploid, (N)NNCC 78♀, No. 1, and a 4-year-old male *Rana plancyi chosenica*, (C)CC 76♂, No. 3, and were more than 30 mm in total length, 64 completed metamorphosis at the age of 54～84 days, 63.5 days on the average. Of these triploid frogs, 34 died within three months after metamor-

TABLE 6
Sex of the first-, second- and third-generation offspring produced from amphidiploids

| | Parents | | Ploidy | No. of metamorphosed frogs |
|---|---|---|---|---|
| | Female | Male | | |
| First-generation offspring (1980) | (N)NNCC 78, No. 1 | (N)NNCC 78, No. 1 | 4n | 93 |
| | | | 6n | 4 |
| | | | 2n | 2 |
| | | | 2n–6n | 9 |
| | | (N)NNCC 78, No. 2 | 4n | 42 |
| | | | 6n | 2 |
| | | | 2n–6n | 2 |
| | | (N)NN 76, No. 4 | 3n | 127 |
| | | | 2n | 1 |
| | | | 2n–5n | 2 |
| | | (C)CC 76, No. 3 | 3n | 64 |
| | | | 5n | 8 |
| | (C)CN 76, No. 21 | (N)NNCC 78, Nos. 1,2 | 3n | 92 |
| | | | 4n | 4 |
| | | | 2n | 3 |
| | | | 2n–4n | 3 |
| | (N)NC 76, No. 16 | (N)NNCC 78, No. 1 | 3n | 16 |
| Second-generation offspring (1981) | (N)NNCC 80, No. 1 | (N)NNCC 80, No. 1 | 4n | 132 |
| | | (N)NNCC 80, No. 2 | 4n | 251 |
| | | (N)NN 77, No. 1 | 3n | 63 |
| | | (C)CC 76, No. 4 | 3n | 30 |
| | (N)NN 77, No. 1 | (N)NNCC 80, Nos. 1,2 | 3n | 13 |
| | (N)NN 77, No. 1 | (N)NN 77, No. 1 | 2n | 55 |
| | (C)CC 76, No. 1 | (C)CC 76, No. 4 | 2n | 59 |
| Third-generation offspring (1982) | (N)NNCC 81, No. 1 | (N)NNCC 81, No. 1 | 4n | 257 |
| | | (N)NN 77, No. 2 | 3n | 58 |
| | | (C)CC 76, No. 5 | 3n | 86 |
| | (N)NN 77, No. 2 | (N)NNCC 81, No. 1 | 3n | 9 |
| | (C)CC 76, No. 2 | (N)NNCC 81, No. 1 | 3n | 7 |
| | (N)NN 77, No. 2 | (N)NN 77, No. 2 | 2n | 30 |
| | (C)CC 76, No. 2 | (C)CC 76, No. 5 | 2n | 106 |

phosis. While the sex of 17 of them was not identified owing to their postmortem changes, 10 of the other 17 were females and seven were males. Of 30 mature triploids, 12 were females (Fig. 4) and 18 were males. The triploid frogs totalled 22 females and 25 (53.2%) males.

Eight pentaploid tadpoles completed metamorphosis at the age of 56~85 days, 61.8 days on the average. While six of them died within three months after metamorphosis, the other two lived for about one year and attained sexual maturity. All these pentaploid frogs were males (Table 6).

e. (C)CN 76♀, No. 21 × (N)NNCC 78♂, Nos. 1 and 2

Of 93 triploid tadpoles which were produced in 1980 from matings between a 4-year-old female hybrid, (C)CC♀ × (N)NN♂, and the above two male amphidiploids, (N)NNCC 78♂, Nos. 1 and 2, and were more than 30 mm in total length,

in 1980, 1981 and 1982, respectively

| Sex of immature frogs | | | Sex of one-year-old frogs | | | Sex of all frogs examined | | |
|---|---|---|---|---|---|---|---|---|
| No. of frogs | ♀ | ♂ | No. of frogs | ♀ | ♂ | No. of frogs | ♀ | ♂ (%) |
| 29 | 7 | 22 | 34 | 8 | 26 | 63 | 15 | 48 (76.2) |
| 2 | 0 | 2 | 2 | 0 | 2 | 4 | 0 | 4 |
| — | — | — | 2 | 0 | 2 | 2 | 0 | 2 |
| — | — | — | 9 | 0 | 9 | 9 | 0 | 9 |
| 7 | 3 | 4 | 27 | 16 | 11 | 34 | 19 | 15 (44.1) |
| 2 | 0 | 2 | — | — | — | 2 | 0 | 2 |
| — | — | — | 2 | 0 | 2 | 2 | 0 | 2 |
| 36 | 18 | 18 | 62 | 33 | 29 | 98 | 51 | 47 (48.0) |
| 1 | 0 | 1 | — | — | — | 1 | 0 | 1 |
| 2 | 0 | 2 | — | — | — | 2 | 0 | 2 |
| 17 | 10 | 7 | 30 | 12 | 18 | 47 | 22 | 25 (53.2) |
| 6 | 0 | 6 | 2 | 0 | 2 | 8 | 0 | 8 |
| 26 | 14 | 12 | 57 | 28 | 29 | 83 | 42 | 41 (49.4) |
| — | — | — | 4 | 1 | 3 | 4 | 1 | 3 (75.0) |
| 3 | 2 | 1 | — | — | — | 3 | 2 | 1 (33.3) |
| 3 | 2 | 1 | — | — | — | 3 | 2 | 1 (33.3) |
| — | — | — | 13 | 7 | 6 | 13 | 7 | 6 (46.2) |
| 68 | 22 | 46 | 32 | 20 | 12 | 100 | 42 | 58 (58.0) |
| 121 | 33 | 88 | 48 | 25 | 23 | 169 | 58 | 111 (65.7) |
| 23 | 7 | 16 | 29 | 18 | 11 | 52 | 25 | 27 (51.9) |
| 24 | 12 | 12 | — | — | — | 24 | 12 | 12 (50.0) |
| 13 | 7 | 6 | — | — | — | 13 | 7 | 6 (46.2) |
| — | — | — | 53 | 26 | 27 | 53 | 26 | 27 (50.9) |
| — | — | — | 59 | 27 | 32 | 59 | 27 | 32 (54.2) |
| 14 | 9 | 5 | 81 | 42 | 39 | 95 | 51 | 44 (46.3) |
| — | — | — | 27 | 14 | 13 | 27 | 14 | 13 (48.1) |
| — | — | — | 44 | 12 | 32 | 44 | 12 | 32 (72.7) |
| 9 | 5 | 4 | — | — | — | 9 | 5 | 4 (44.4) |
| 7 | 3 | 4 | — | — | — | 7 | 3 | 4 (57.1) |
| — | — | — | 29 | 15 | 14 | 29 | 15 | 14 (48.3) |
| — | — | — | 88 | 48 | 40 | 88 | 48 | 40 (45.5) |

93 completed metamorphosis at the age of 71~93 days, 81.5 days on the average. Of these triploid frogs, 36 died within three months after metamorphosis. Although the sex was not identified owing to postmortem changes in 10 of the dead frogs, 14 of the other frogs were females and 12 were males. Of 57 mature triploids, 28 were females and 29 were males. The triploid frogs totalled 42 females and 41 (49.4%) males.

Four tetraploid tadpoles completed metamorphosis at the age of 73~79 days, 75.5 days on the average, and attained sexual maturity. Of these tetraploid frogs, one was a female and three were males. Three diploid tadpoles completed metamorphosis at the age of 76~82 days, 79.0 days on the average. When their sex was examined about one month after metamorphosis, two were females and one was a male. Three 2n-4n mosaic tadpoles completed metamorphosis at the age of 72~76 days, 74.7 days on the average. These mosaic frogs were killed about one month after metamorphosis to examine their sex. Two of them were females and one was a male (Table 6).

f. (N)NC 76♀, No. 16×(N)NNCC 78♂, No. 1

Sixteen triploid tadpoles which were produced in 1980 from a mating between a 4-year-old diploid female hybrid, *Rana nigromaculata*♀ ×*Rana plancyi chosenica*♂, and one of the above male amphidiploids, (N)NNCC 78♂, No. 1, completed metamorphosis at the age of 68~78 days, 71.5 days on the average. Although three of them died immediately after metamorphosis and their sex was not identified owing to their postmortem changes, the other 13 attained sexual maturity. Seven of them were females and six were males (Table 6).

2. Second-generation offspring produced from amphidiploids in 1981

a. (N)NNCC 80♀, No. 1×(N)NNCC 80♂, No. 1

Of 135 tetraploid tadpoles which were produced in 1981 from a mating between a one-year-old female and a one-year-old male amphidiploid of the first-generation offspring, (N)NNCC 80♀, No. 1×(N)NNCC 80♂, No. 1, and were more than 30 mm in total length, 132 completed metamorphosis at the age of 56~82 days, 63.7 days on the average. Of these amphidiploid frogs, 100 died within three months after metamorphosis. Of the dead frogs, 22 were females and 46 were males. The sex of the other 32 frogs was not identified owing to their postmortem changes. Of 32 mature frogs, 20 were females and 12 were males. The tetraploid frogs totalled 42 females and 58 (58.0%) males (Table 6).

b. (N)NNCC 80♀, No. 1×(N)NNCC 80♂, No. 2

Of 299 tetraploid tadpoles which were produced in 1981 from a mating between the above female amphidiploid and another one-year-old male amphidiploid of the first-generation offspring, (N)NNCC 80♀, No. 1×(N)NNCC 80♂, No. 2, and were more than 30 mm in total length, 251 completed metamorphosis at the age of 56~82 days, 62.9 days on the average. Of the tetraploid frogs, 203 died from sudden cold during hibernation, as they had been reared outdoors. When the sex of these dead frogs was examined, 33 were females, 88 were males and the remaining 82 were undetermined owing to their postmortem changes. Of 48 mature frogs, 25 were females and 23 were males. The amphidiploids totalled 58 females and 111 (65.7%) males (Table 6).

c. (N)NNCC 80♀, No. 1×(N)NN 77♂, No. 1

Of 77 triploid tadpoles which were produced in 1981 from a mating between the above female amphidiploid, (N)NNCC 80♀, No. 1, and a 4-year-old diploid male *Rana nigromaculata*, (N)NN 77♂, No. 1, and were more than 30 mm in total length, 63 completed metamorphosis at the age of 61~90 days, 73.0 days on the average. Of these triploid frogs, 34 died within three months after metamorphosis. Although the sex of 11 of the dead frogs was not identified owing to their postmortem changes, seven of the others were females and 16 were males. Of 29 mature triploids, 18 were females and 11 were males. The triploid frogs totalled 25 females and 27 (51.9%) males (Table 6).

d. (N)NNCC 80♀, No. 1×(C)CC 76♂, No. 4

Of 33 triploid tadpoles which were produced in 1981 from a mating between the above female amphidiploid, (N)NNCC 80♀, No. 1, and a 5-year-old diploid male *Rana plancyi chosenica*, (C)CC 76♂, No. 4, and were more than 30 mm in total length, 30 completed metamorphosis at the age of 46~104 days, 71.4 days on the average. However, all these triploid frogs died within three months after metamorphosis. Of the dead frogs, 12 were females, 12 others were males and the sex of the remaining six was not identified owing to their postmortem changes (Table 6).

e. (N)NN 77♀, No. 1×(N)NNCC 80♂, Nos. 1 and 2

Thirteen triploid tadpoles which were produced in 1981 from a mating between a 4-year-old diploid female *Rana nigromaculata*, (N)NN 77♀, No. 1, and the above two male amphidiploid, (N)NNCC 80♂, Nos. 1 and 2, and were more than 30 mm in total length completed metamorphosis at the age of 50~61 days, 55.1 days on the average. When all these triploid frogs were killed about three months after metamorphosis to examine their sex, seven were females and six were males (Table 6).

f. Control matings

Of 62 diploid tadpoles which were produced in 1981 from the control mating between a female and a male diploid *Rana nigromaculata*, (N)NN 77♀, No. 1× (N)NN 77♂, No. 1 and were more than 30 mm in total length, 55 completed metamorphosis at the age of 56~82 days, 64.5 days on the average. Of 53 mature frogs, 26 were females and 27 were males (Table 6).

Of 66 diploid tadpoles which were produced in 1981 from the control matings between a female and a male diploid *Rana plancyi chosenica*, (C)CC 76♀, No. 1×

(C)CC 76♂, No. 4 and were more than 30 mm in total length, 59 completed metamorphosis at the age of 46~102 days, 71.4 days on the average. All these diploid frogs attained sexual maturity and were found to consist of 27 females and 32 males (Table 6).

3. Third-generation offspring produced from amphidiploids in 1982

a. (N)NNCC 81♀, No. 1×(N)NNCC 81♂, No. 1

Of 268 amphidiploid tadpoles which were produced in 1982 from a mating between a one-year-old female and a one-year-old male amphidiploid of the second-generation offspring, (N)NNCC 81♀, No. 1×(N)NNCC 81♂, No. 1, and were more than 30 mm in total length, 257 completed metamorphosis at the age of 59~104 days, 69.1 days on the average. Three of them died within one week after metamorphosis. The sex of these frogs was not identified owing to their postmortem changes. Four months after metamorphosis, 81 frogs were sexually matured in appearance, while the other 173 were immature. Of the mature frogs, 42 were females and 39 were males. Of the immature frogs, 14 were killed to examine their sex. It was found that nine were females and five were males. The amphidiploids totalled 51 females and 44 (46.3%) males. The sex of the remaining 159 immature frogs was unknown, as they were still living (Table 6).

b. (N)NNCC 81♀, No. 1×(N)NN 77♂, No. 2

Of 61 triploid tadpoles which were produced in 1982 from a mating between the above female amphidiploid, (N)NNCC 81♀, No. 1, and a 5-year-old diploid male *Rana nigromaculata*, (N)NN 77♂, No. 2, and were more than 30 mm in total length, 58 completed metamorphosis at the age of 61~91 days, 67.1 days on the average. Of these triploid frogs, 27 were sexually matured in appearance four months after metamorphosis. It was found that 14 of them were females and 13 were males (Table 6).

c. (N)NNCC 81♀, No. 1×(C)CC 76♂, No. 5

Of 88 triploid tadpoles which were produced in 1982 from a mating between the above female amphidiploid, (N)NNCC 81♀, No. 1, and a 6-year-old diploid male *Rana plancyi chosenica*, (C)CC 76♂, No. 5, and were more than 30 mm in total length, 86 completed metamorphosis at the age of 59~101 days, 67.9 days on the average. Of these triploid frogs, 44 were sexually matured in appearance four months after metamorphosis. It was found that 12 of them were females and 32 were males (Table 6).

d. (N)NN 77♀, No. 2×(N)NNCC 81♂, No. 1

Nine triploid tadpoles which were produced in 1982 from a mating between a 5-year-old diploid female *Rana nigromaculata*, (N)NN 77♀, No. 2, and the above male amphidiploid, (N)NNCC 81♂, No. 1, and were more than 30 mm in total length completed metamorphosis at the age of 59~93 days, 67.6 days on the average. All these triploids died within three weeks after metamorphosis. Five of them were females and four were males (Table 6).

e. (C)CC 76♀, No. 2×(N)NNCC 81♂, No. 1

Seven triploid tadpoles which were produced in 1982 from a mating between a 6-year-old diploid female *Rana plancyi chosenica*, (C)CC 76♀, No. 2, and the above male amphidiploid, (N)NNCC 81♂, No. 1, and were more than 30 mm in total length completed metamorphosis at the age of 63～105 days, 68.0 days on the average. All these triploids died within three weeks after metamorphosis. Three of them were females, while the other four were males (Table 6).

f. Control matings

Of 32 diploid tadpoles which were produced in 1982 from the control mating between a diploid female and a diploid male *Rana nigromaculata*, (N)NN 77♀, No. 2×(N)NN 77♂, No. 2, and were more than 30 mm in total length, 30 completed metamorphosis at the age of 59～67 days, 65.6 days on the average. One of these frogs died within one week after metamorphosis. The sex of this frog was not identified owing to its postmortem changes. The others were sexually matured in appearance four months after metamorphosis. Fifteen of them were females and the other 14 were males (Table 6).

Of 124 diploid tadpoles which were produced in 1982 from the control mating between a diploid female and a diploid male *Rana plancyi chosenica*, (C)CC 76♀, No. 2×(C)CC 76♂, No. 5, and were more than 30 mm in total length, 106 completed metamorphosis at the age of 59～81 days, 69.7 days on the average. Of these diploid frogs, 88 were sexually matured in appearance four months after metamorphosis. It was found that 48 of them were females and 40 (45.5%) were males. The other frogs were immature. The sex of these immature frogs was not identified, as they were still living (Table 6).

## *VI. Size and appearance of mature offspring of amphidiploids*

The first-, second- and third-generation offspring of amphidiploids at the age of 2.5 years, 1.5 years and 0.5 year, respectively, were compared with allotriploids, diploid hybrids and the control *Rana nigromaculata* and *Rana plancyi chosenica* in body length and external appearance (Fig. 6).

### 1. Body length

a. First-generation offspring produced in 1980

There were statistically no significant differences ($p>0.05$) among the females or males of the following three kinds of frogs at the age of 2.5 years.

i) Amphidiploids, (N)NNCC♀, produced from matings, (N)NNCC 78♀, No. 1×(N)NNCC 78♂, Nos. 1 and 2

ii) Allotriploids, (N)NNC♀, produced from a mating, (N)NNCC 78♀, No. 1×(N)NN 76♂, No. 4

iii) Allotriploids, (N)NCC♀, produced from a mating, (N)NNCC 78♀,

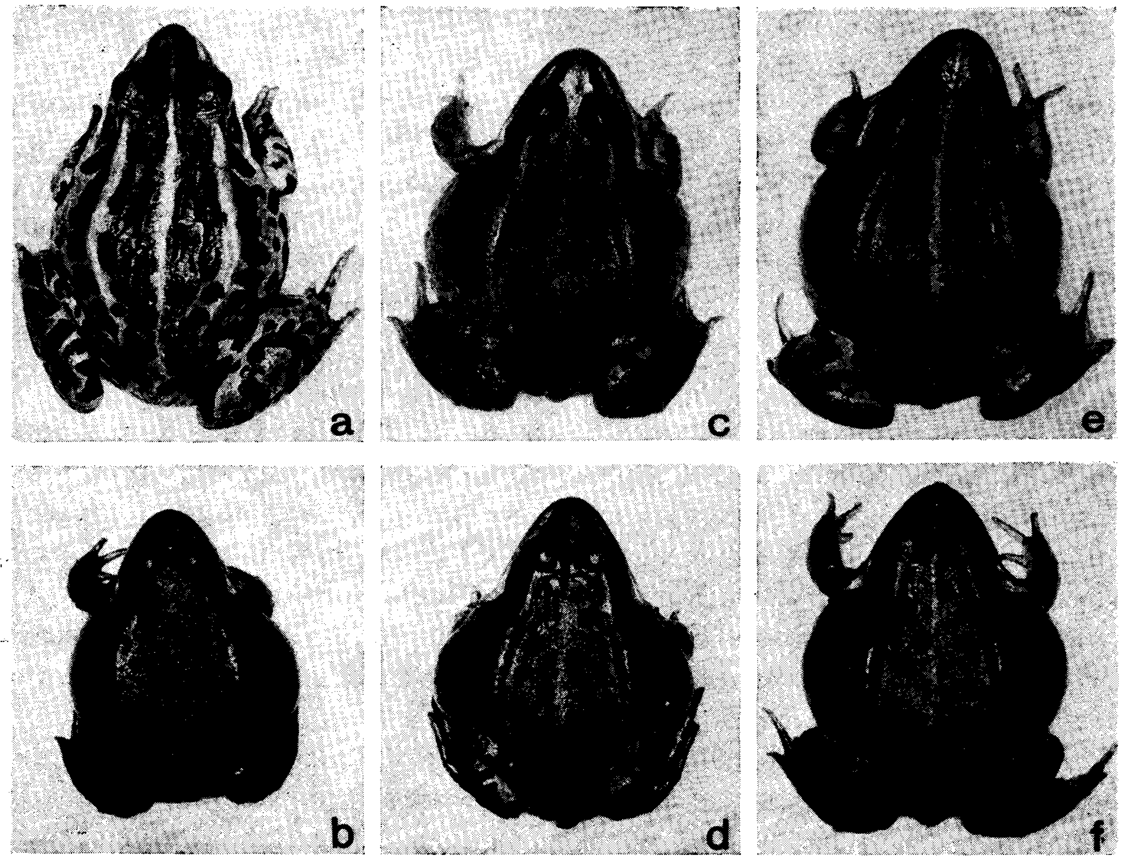

Fig. 6. Two-year-old reciprocal diploid hybrids and allotriploids between *Rana nigromaculata* and *Rana plancyi chosenica*, and the control diploids. ×0.6

a. Diploid female *Rana nigromaculata* produced from (N)NN 77♀, No. 1×(N)NN 77♂, No. 1
b. Diploid female *Rana plancyi chosenica* produced from (C)CC 76♀, No. 1×(C)CC 76♂, No. 4
c. Diploid female hybrid, (N)NC 76♀, No. 16
d. Diploid female hybrid, (C)CN 76♀, No. 21
e. Female (N)NNC allotriploid produced from (N)NNCC 78♀, No. 1×(N)NN 76♂, No. 4
f. Female (N)NCC allotriploid produced from (N)NNCC 78♀, No. 1×(C)CC 76♂, No. 3

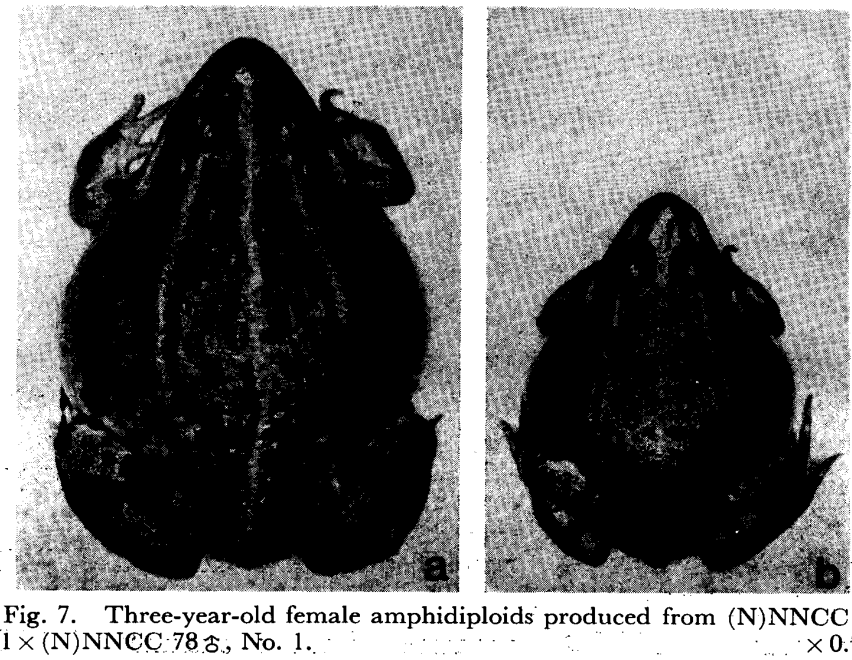

Fig. 7. Three-year-old female amphidiploids produced from (N)NNCC 78♀, No. 1×(N)NNCC 78♂, No. 1. ×0.7

a. Largest individual b. Smallest individual

No. 1 × (C)CC 76♂, No. 3

However, it must be noted that some of large amphidiploids were removed in order to use them in some experiments before the age of 2.5 years, while no allotriploids were removed. The largest of the one-year-old female amphidiploids produced in 1980 from the mating, (N)NNCC 78♀, No. 1 × (N)NNCC 78♂, No. 1, was a frog, (N)NNCC 80♀, No. 1, which was used in mating experiments of 1981. This female was 66.0 mm in body length at the age of one year and became 87.5 mm at the age of 2.5 years (Fig. 7a). The second largest female amphidiploid, (N)NNCC 80♀, No. 2, produced in 1980 was 64.5 mm in body length at the age of one year. This amphidiploid was not used in mating experiment, because the eggs obtained after pituitary injection were already overripe. Although this female was 80.5 mm in body length at the age of two years, she could not live until the age of 2.5 years. This female died during the breeding season after laying eggs. It was remarkable that some of the female amphidiploids were very speedy in growth. Such large frogs as found in the female amphidiploids were never found in the two kinds of (N)NNC and (N)NCC allotriploids (Table 7).

Two male amphidiploids, (N)NNCC 80♂, Nos. 1 and 2, produced from a

TABLE 7

Body lengths of the first-, second- and third-generation offspring produced from amphidiploids, allotriploids and the controls

| | Parents | | Constitution | Age (years) | Female | | Male | |
|---|---|---|---|---|---|---|---|---|
| | Female | Male | | | No. of frogs | Body length (Mean ±) mm | No. of frogs | Body length (Mean ±) mm |
| First-generation offspring (1980) | (N)NNCC 78, No. 1 | (N)NNCC 78, No. 1 | (N)NNCC | 2.5 | 7 | 59.0~87.5 (65.7±3.22) | 12 | 47.7~58.3 (53.2±1.00) |
| | | (N)NN 76, No. 4 | (N)NNC | 2.5 | 6 | 52.9~68.0 (62.9±2.21) | 9 | 54.0~59.8 (56.6±0.63) |
| | | (C)CC 76, No. 3 | (N)NCC | 2.5 | 12 | 56.0~69.3 (63.8±1.21) | 11 | 49.2~62.9 (57.1±1.42) |
| Second-generation offspring (1981) | (N)NNCC 80, No. 1 | (N)NNCC 80, No. 1 | (N)NNCC | 1.5 | 16 | 52.4~65.8 (61.0±0.88) | 10 | 47.9~57.9 (53.3±1.05) |
| | | (N)NNCC 80, No. 2 | (N)NNCC | 1.5 | 18 | 51.3~69.5 (60.9±1.56) | 18 | 45.6~59.6 (52.5±0.78) |
| | | (N)NN 77, No. 1 | (N)NNC | 1.5 | 16 | 51.5~67.9 (60.2±1.18) | 4 | 52.7~62.2 (56.9±1.97) |
| | (N)NN 77, No. 1 | (N)NN 77, No. 1 | (N)NN | 1.5 | 18 | 43.0~68.5 (57.2±0.99) | 18 | 47.0~53.0 (49.3±0.42) |
| | (C)CC 76, No. 1 | (C)CC 76, No. 4 | (C)CC | 1.5 | 26 | 41.3~54.4 (49.3±0.73) | 11 | 37.6~46.6 (40.2±0.75) |
| Third-generation offspring (1982) | (N)NNCC 81, No. 1 | (N)NNCC 81, No. 1 | (N)NNCC | 0.5[1)] | 20 | 59.5~63.7 (61.3±0.65) | 20 | 49.0~62.2 (57.5±0.72) |
| | | | (N)NNCC | 0.5 | 22 | 43.0~62.0 (49.1±1.23) | 19 | 38.5~60.5 (49.1±1.25) |
| | | (N)NN 77, No. 2 | (N)NNC | 0.5 | 14 | 43.0~53.0 (47.8±0.94) | 13 | 41.5~55.0 (49.7±0.98) |
| | | (C)CC 76, No. 5 | (N)NCC | 0.5 | 12 | 39.0~49.6 (44.2±1.24) | 32 | 32.1~49.4 (39.3±0.78) |
| | (N)NN 77, No. 2 | (N)NN 77, No. 2 | (N)NN | 0.5 | 15 | 41.0~49.0 (44.1±0.67) | 14 | 40.3~50.5 (43.6±0.71) |
| | (C)CC 76, No. 2 | (C)CC 76, No. 5 | (C)CC | 0.5 | 16 | 34.0~42.3 (37.9±0.73) | 15 | 30.0~36.7 (33.6±0.68) |

1) Fifty individuals were reared in a lage plastic case (65 cm × 45 cm × 15 cm) equipped with a water pipe and a drainpipe. In all the other series of the third-generation offspring, 40~50 individuals were reared in enamel pans (46 cm × 32 cm × 11 cm).

mating, (N)NNCC 78♀, No. 1×(N)NNCC 78♂, No. 1, and used in mating experiments of 1981 were the largest males, being 58.0 mm and 57.5 mm in body length. These males were killed at the age of onc year immediately after they were used in the mating experiments of 1981. The two second largest male amphidiploids, (N)NNCC 80♂, Nos. 3 and 4, produced in 1980 were 57.5 mm and 56.5 mm in body length, respectively. These males were also killed in the breeding season of 1981. Such large males were never found in the two kinds of allotriploids at the age of one year. Five other male amphidiploids produced in 1980 were 57.0~58.5 mm in body length at the age of two years and were killed without being reared until the age of 2.5 years. Twelve male amphidiploids which were living and measured at the age of 2.5 years included, moreover, some individuals being somewhat retarded in growth (Table 7).

b. Second-generation offspring produced in 1981

The second-generation offspring produced from matings between a female and two male amphidiploid first-generation offspring, (N)NNCC 80♀, No. 1×(N)NNCC 80♂, Nos. 1 and 2, were compared with (N)NNC allotriploids, diploid *Rana nigromaculata*, and diploid *Rana plancyi chosenica* in body length at the age of 1.5 years. While female amphidiploids statistically differed ($p<0.05$) from female *Rana plancyi chosenica* in size, they did not differ ($p>0.05$) from female *Rana nigromaculata* and female (N)NNC allotriploids. On the other hand, male amphidiploids statistically ($p<0.05$) differed from both male *Rana nigromaculata* and male *Rana plancyi chosenica*, although they did not differ ($p>0.05$) from male (N)NNC allotriploids (Table 7).

However, the largest four of 20 female amphidiploids produced in 1981 from a mating between a female and a male amphidiploid, (N)NNCC 80♀, No. 1×(N)NNCC 80♂, No. 1, were 63.5~67.0 mm in body length at the age of one year. The largest two of 12 males produced from the same mating were 56.0 mm and 57.0 mm in body length at the age of one year. These four females and two males were used in some experiments and excluded from the measurements at the age of 1.5 years. The largest, (N)NNCC 81♀, No. 1, of 25 female amphidiploids produced from the other mating between a female and a male amphidiploid, (N)NNCC 80♀, No. 1×(N)NNCC 80♂, No. 2, was 72.0 mm in body length at the age of one year. The largest, (N)NNCC 81♂, No. 1, of 23 male amphidiploids produced from the same mating was 62.5 mm in body length at the age of one year. These largest female and male were used in mating experiments performed in the breeding season of 1982 to produce third-generation offspring (Table 3). Such a large female or male was not found in the (N)NNC allotriploids, diploid *Rana nigromaculata* and diploid *Rana plancyi chosenica*. Four other females, (N)NNCC 81♀, Nos. 2~5, which were 67.5~70.0 mm in body length, and four other males, (N)NNCC 81♂, Nos. 2~5, which were 57.0~59.5 mm in body length, were killed at the age of one year together with the largest female and male. As these five large female and five large male amphidiploids were excluded from the measurements at the age of 1.5 years, it was evident that amphidiploids were somewhat larger than those presented in Table 7.

c. Third-generation offspring produced in 1982

Of 257 third-generation offspring produced in 1982 from a mating between a female and a male amphidiploid of the second-generation offspring, (N)NNCC 81♀, No. 1 × (N)NNCC 81♂, No. 1, 40～50 were reared in a enamel pan (46 cm × 32 cm × 11 cm), while the others were reared in plastic cases (65 cm × 45 cm × 15 cm), each of which contained about 50 frogs. Each plastic case was equipped with a water pipe and a drainpipe. Two kinds of allotriploids, (N)NNC and (N)NCC, and the control diploid *Rana nigromaculata* and *Rana plancyi chosenica*, (N)NN and (C)CC, were reared in enamel pans (46 cm × 32 cm × 11 cm), each of which contained 30～50 frogs. Five kinds of (N)NNCC, (N)NNC, (N)NCC, (N)NN and (C)CC were compared with each other after they had been reared in a similar condition for about six months. It was found that the male and female amphidiploids were statistically larger in body length than the (N)NCC allotriploids and diploid *Rana nigromaculata* and *Rana plancyi chosenica*, while they did not statistically differ ($p>0.05$) from female (N)NNC allotriploids. On the other hand, male amphidiploids were statistically larger ($p<0.05$) in body length than three other kinds of male (N)NCC, (N)NN and (C)CC frogs, while they did not differ ($p>0.05$) from male (N)NNC allotriploids (Table 7).

The male and female amphidiploids reared in plastic cases were kept for about six months in a better condition than the other kinds of frogs. It was found that they grew rapidly and became about the same in size as those reared for 1.5 years in an ordinary condition.

## 2. External characters

*Rana nigromaculata* and *Rana plancyi chosenica* distinctly differ from each other in many external characters. As one of the most remarkable differences, *Rana nigromaculata* shows a definite sexual dimorphism in color and pattern during the breeding season in contrast to *Rana plancyi chosenica*. Since the male of *Rana nigromaculata* reveals a distinct nuptial color, the two species and three kinds of (N)NNC, (N)NCC and (N)NNCC frogs were compared with one another by using females of these frogs. The results are presented in Table 8.

As found in Table 8, the amphidiploids were intermediate between *Rana nigromaculata* and *Rana plancyi chosenica* in external character as a whole. They were very similar in appearance to the (N)NNC allotriploids, consisting of two *Rana nigromaculata* genomes and one *Rana plancyi chosenica* genome, although this kind of allotriploids was intermediate between (N)NN and (N)NNCC in many external characters. The (N)NCC allotriploids, consisting of two *Rana plancyi chosenica* genomes and one *Rana nigromaculata* genome were also very similar in appearance to *Rana plancyi chosenica*, although they were intermediate between (C)CC and (N)NNCC in many external characters, as found in (N)NNC.

TABLE 8

External characters of female amphidiploids and allotriploids between *Rana nigromaculata* and *Rana plancyi chosenica*

| Kinds | (N)NN | (C)CC | (N)NNC | (N)NCC | (N)NNCC |
|---|---|---|---|---|---|
| Body shape | Somewhat slender | Somewhat dumpy | Intermediate between (N)NN and (N)NNCC | Somewhat dumpy | Intermediate between (N)NN and (C)CC |
| Dorsal tubercles: | | | | | |
| Shape | Elongated rods | Dots | 〃 | Intermediate between (C)CC and (N)NNCC | 〃 |
| Number | Many | Few | 〃 | 〃 | 〃 |
| Dorsal ground color | Pale brown | Green | Green or pale brown | Green | Green or pale brown |
| Dorsal black spots: | | | | | |
| Outlines | Clear-cut | Obscure | Clear-cut | Clear-cut | Clear-cut |
| Shape | Rod | Round | Intermediate between (N)NN and (C)CC | Round | Intermediate between (N)NN and (C)CC |
| Size | Large | Small | Intermediate between (N)NN and (N)NNCC | Intermediate between (C)CC and (N)NNCC | 〃 |
| Number | Many | Few | 〃 | 〃 | 〃 |
| Median stripe | Present, silvery | Absent | Present | Faintly present | Present |
| Dorsolateral folds: | | | | | |
| Elevation | Steep | Gentle | Intermediate between (N)NN and (C)CC | Gentle | Intermediate between (N)NN and (C)CC |
| Width | Narrow | Wide | 〃 | Wide | 〃 |
| Color | Pale, golden | Dark, coppery | Near (N)NN | Near (C)CC | 〃 |
| Color of ventral surface | White | Yellow, white or dysky with grey | White | White | White |
| Hind limbs | Long | Short | Intermediate between (N)NN and (N)NNCC | Short | Intermediate between (N)NN and (C)CC |
| Inner metatarsal tubercle | Small | Large | Intermediate between (N)NN and (N)NNCC | Large | Intermediate between (N)NN and (C)CC |

## *VII. Structure of testes and spermatogenesis in male offspring of amphidiploids*

The testes of eight mature male amphidiploids including three first- and five second-generation offspring were observed in terms of inner structure and spermatogenesis during the breeding season of 1982. The three first-generation offspring, (N)NNCC 80♂, Nos. 3~5, were produced in 1980 from a mating, (N)NNCC 78♀, No. 1 × (N)NNCC 78♂, No. 1, while the five second-generation offspring were produced in 1981 from a mating, (N)NNCC 80♀, No. 1 × (N)NNCC 80♂, No. 2. These testes were completely normal in inner structure except that the spermatozoa were remarkably larger and somewhat fewer than those of the control diploid *Rana nigromaculata* and *Rana plancyi chosenica*. The seminiferous tubules were almost filled with bundles of normal-shaped spermatozoa. In the peripheral parts, there were first and second spermatocytes and primary and secondary spermatogonia. Of these germ cells, first spermatocytes at the meiotic prophase were most abundant. Along the inner walls of seminiferous tubules, a few primary spermatogonia were sparsely distributed. The spermatocytes and spermatogonia were distinctly larger in size than those of the control diploid frogs (Fig. 8).

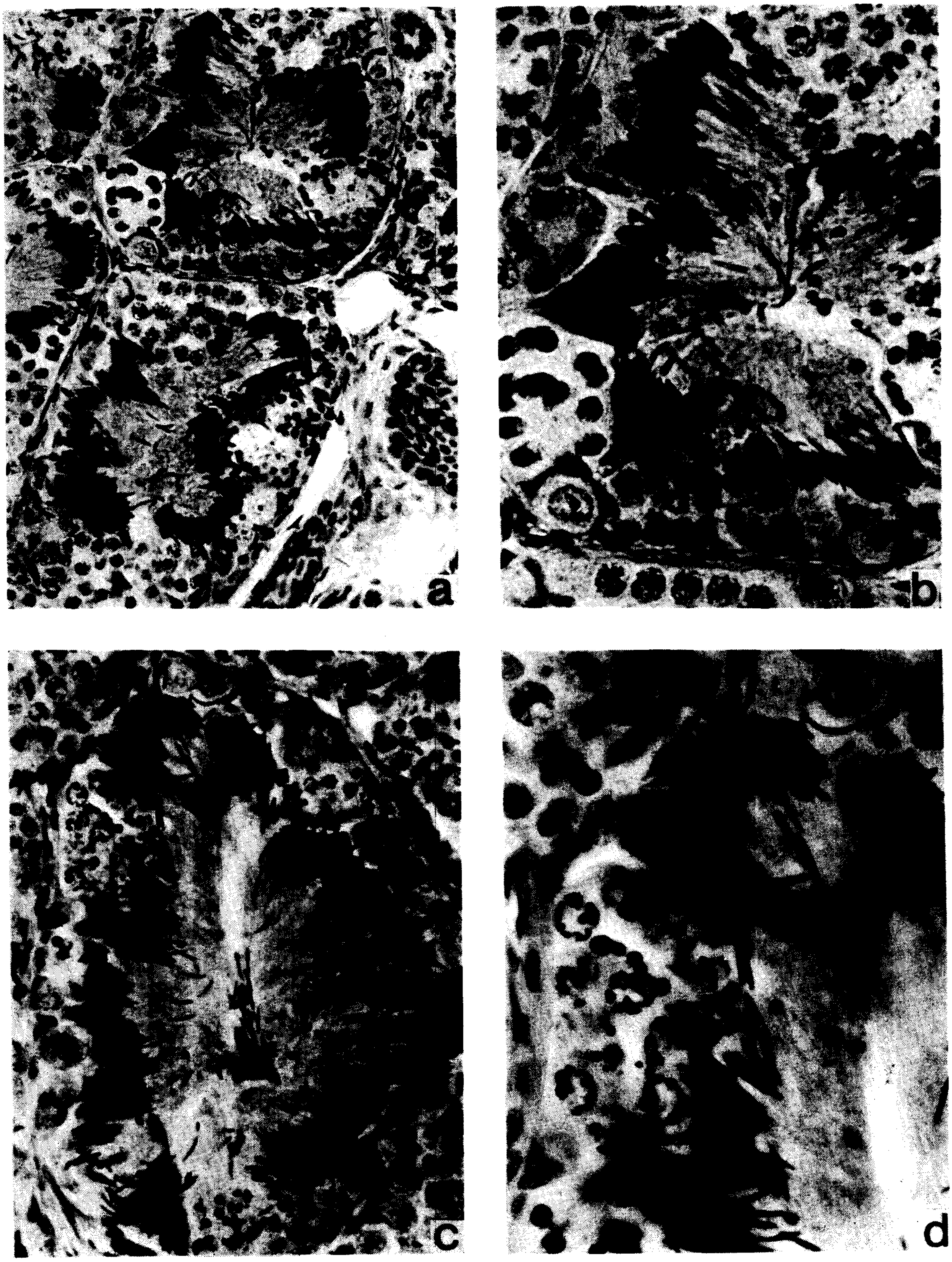


Fig. 8. Cross-sections of the testes of a male amphidiploid and the control diploid.

a. Control diploid *Rana nigromaculata* produced from (N)NN 77♀, No. 1 × (N)NN 77♂, No. 1 ×300

b. Ditto ×600

c. (N)NNCC amphidiploid produced from (N)NNCC 80♀, No. 1 × (N)NNCC 80♂, No. 2 ×300

d. Ditto ×600

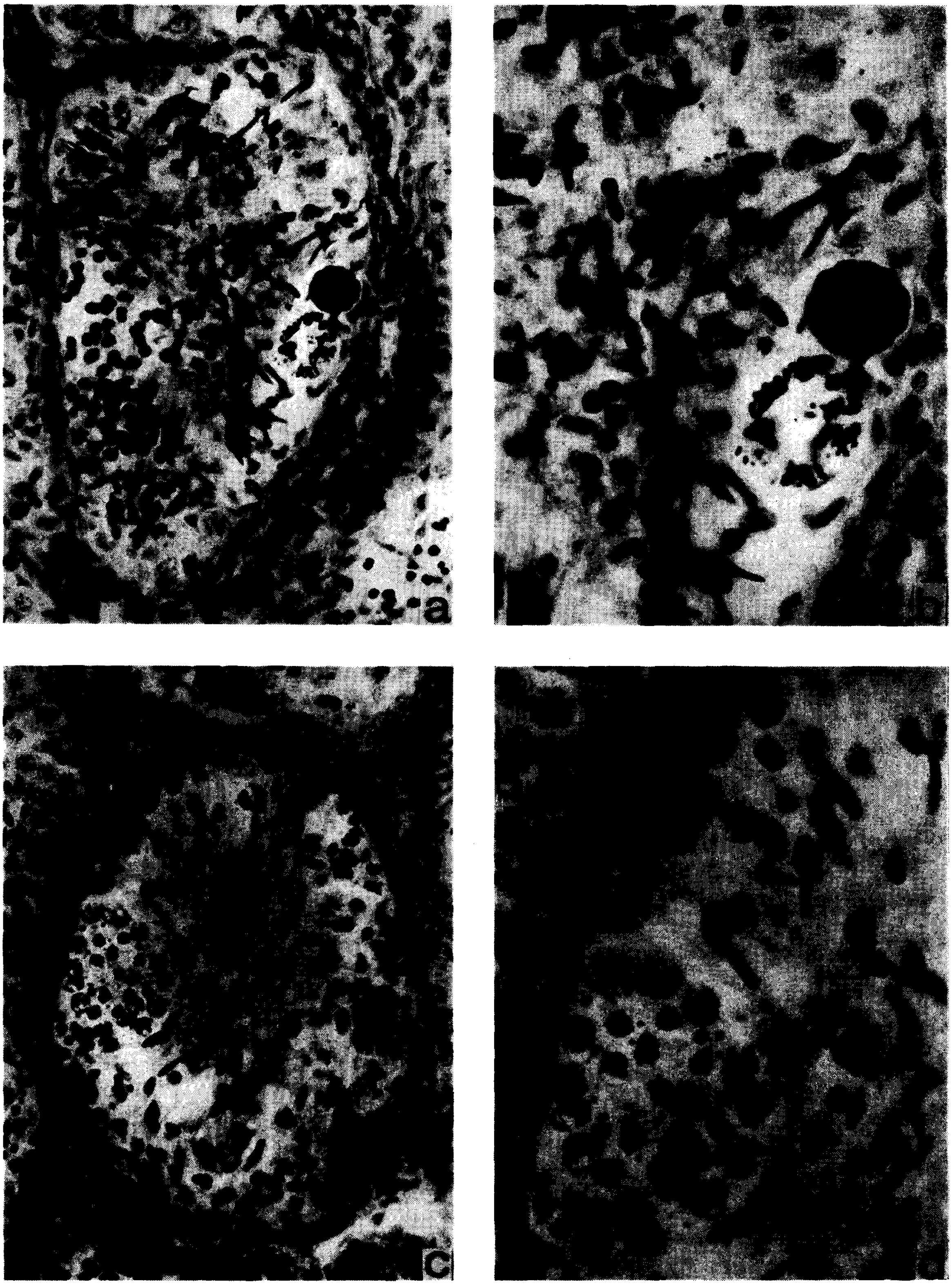

Fig. 9. Cross-sections of the testes of male allotriploids produced from a female amphidiploid by mating with a male *Rana nigromaculata* or *Rana plancyi chosenica*.

a. (N)NNC allotriploid produced from (N)NNCC 78♀, No. 1 × (N)NN 76♂, No. 4 ×300

b. Ditto ×600

c. (N)NCC allotriploid produced from (N)NNCC 78♀, No. 1 × (C)CC 76♂, No. 3 ×300

d. Ditto ×600

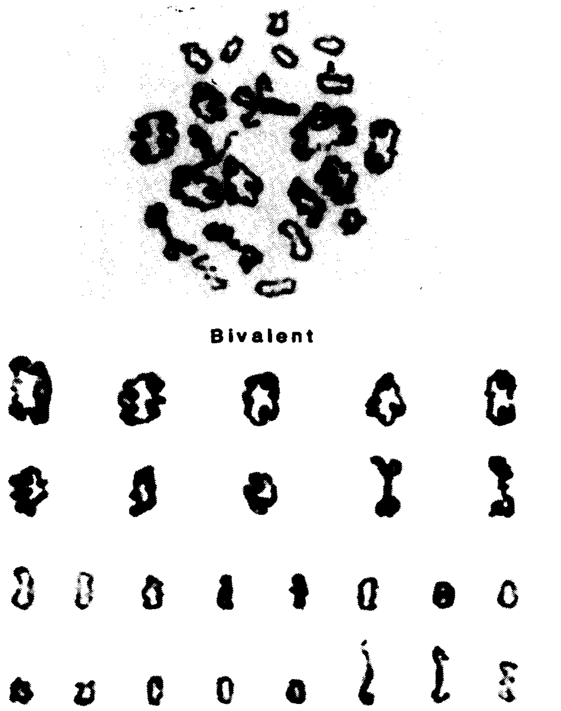


Fig. 10. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 10 large and 16 small bivalents in a male amphidiploid produced from (N)NNCC 81♀, No. 1 × (N)NNCC 81♂, No. 1. ×600

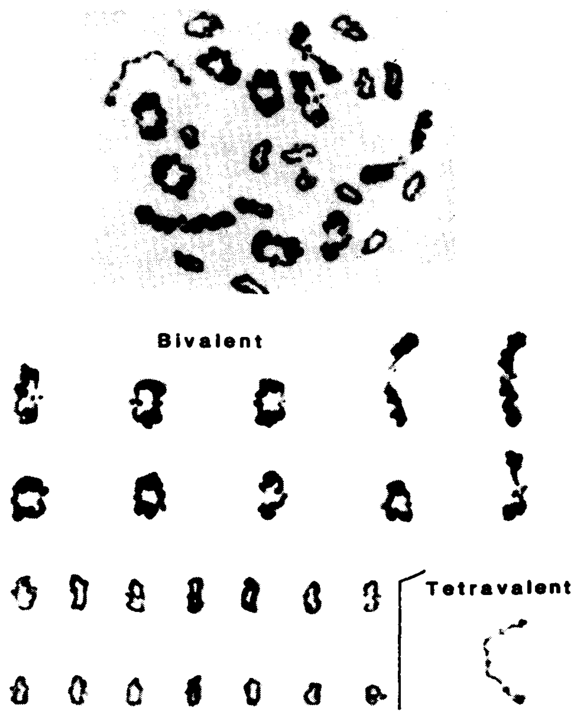


Fig. 11. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 10 large and 14 small bivalents and one tetravalent in a male amphidiploid produced from (N)NNCC 81♀, No. 1 × (N)NNCC 81♂, No. 1. ×600

In contrast to the testes of the male amphidiploids, those of the two kinds of male (N)NNC and (N)NCC allotriploids were very abnormal in inner structure. They had no normal spermatozoa. The seminiferous tubules contained abnormal spermatozoa and pycnotic nuclei in place of normal spermatozoa. However, there were abundant spermatocytes and spermatogonia in the peripheral parts (Fig. 9).

Spermatogenesis was observed in five mature male amphidiploids of the third-generation offspring produced in 1982 from the above mating, (N)NNCC 81♀, No. 1 × (N)NNCC 81♂, No. 1, by the method of SCHMID et al. (1979). It was found that the first meiotic divisions had usually 26 bivalents (Fig. 10). However, a few of them had various combinations of bivalents and tetravalents or rarely contained a few univalents besides bivalents and tetravalents (Fig. 11). A few octoploid spermatocytes having bivalents and tetravalents or having bivalents, trivalents and tetravalents were found among tetraploid spermatocytes.

Spermatogenesis was very abnormal in the testes of the two kinds of male (N)NNC and (N)NCC allotriploids produced in 1982 from two matings between a female amphidiploid, (N)NNCC 81♀, No. 1, and a male diploid *Rana nigromaculata*, (N)NN 77♂, No. 2 and a male diploid *Rana plancyi chosenica*, (C)CC 76♂, No. 5. Most of the first meiotic divisions had a combination of 13 bivalents and 13 univalents (Fig. 12), while others had some combinations of bivalents and

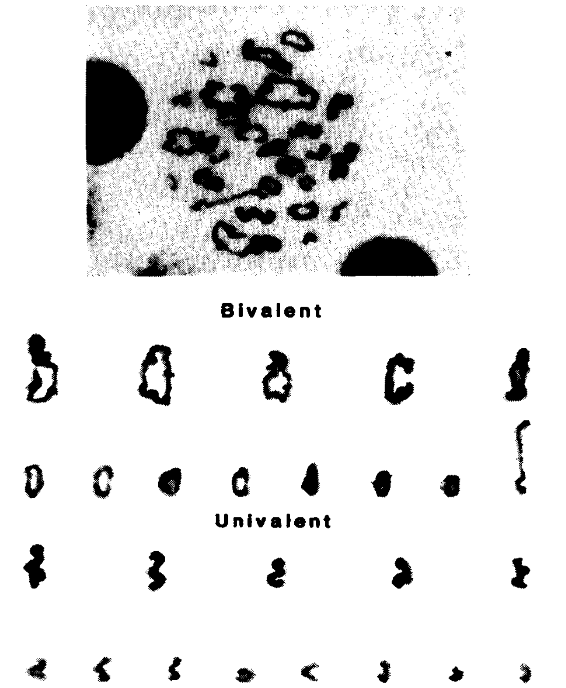


Fig. 12. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 5 large and 8 small bivalents and 13 univalents in a (N)NNC allotriploid male produced from (N)NNCC 81♀, No. 1×(N)NN 77♂, No. 2. ×600

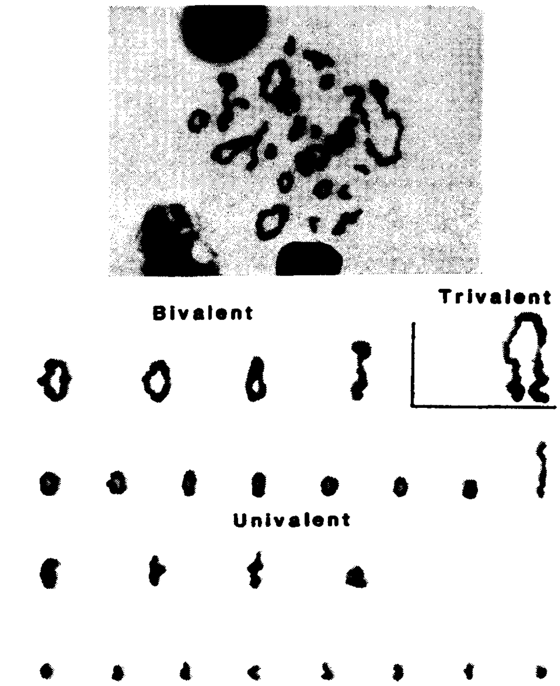


Fig. 13. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 4 large and 8 small bivalents, 12 univalents and one trivalent in a (N)NCC allotriploid male produced from (N)NNCC 81♀, No. 1×(C)CC 76♂, No. 5. ×600

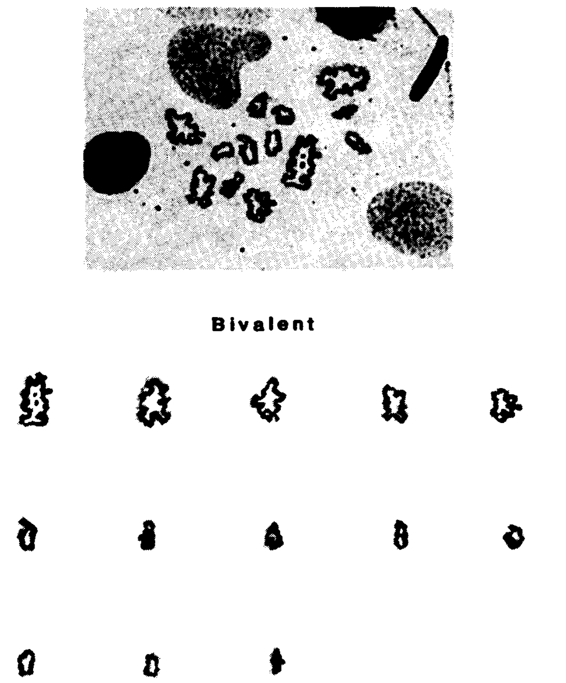


Fig. 14. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 5 large and 8 small bivalents in a diploid *Rana nigromaculata*. ×600

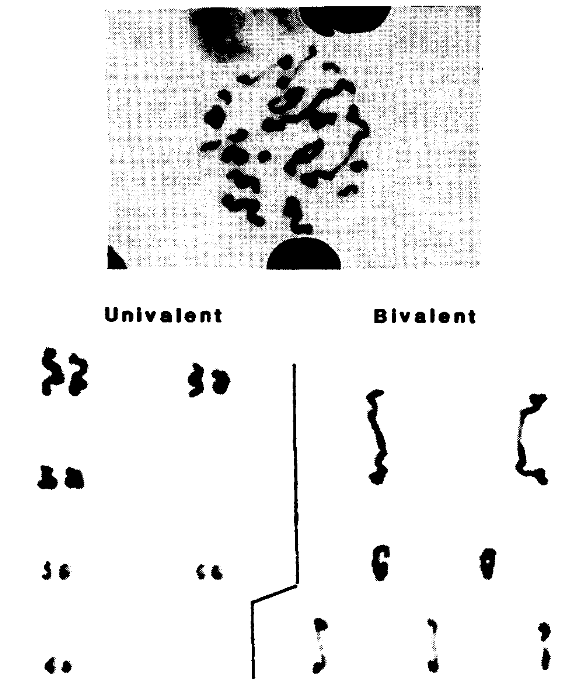


Fig. 15. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 2 large and 5 small bivalents and 12 univalents in a male (N)NC hybrid between *Rana nigromaculata* and *Rana plancyi chosenica*. ×600

univalents other than the latter or various combinations of univalents, bivalents and trivalents (Fig. 13). There were also some first meioses having chromosome complements which were hexaploid or more higher in ploidy.

The first meiotic divisions in the testes of the control diploid *Rana nigromaculata* and *Rana plancyi chosenica* generally contained 13 bivalents, while those in the testes of the diploid hybrids contained a mixture of bivalents and univalent chromosomes (Figs. 14, 15). However, tetraploid meioses were rarely found among the usual diploid ones.

## DISCUSSION

In the present study, developmental capacity was examined in the first-, second- and third-generation offspring of amphidiploids between *Rana nigromaculata* and *Rana plancyi chosenica*. The parental amphidiploids were produced from a female (N)NNC allotriploid by mating with a male diploid *Rana plancyi chosenica*. Normal cleavages occurred in 328 (46.9%) of a total of 700 eggs of a maternal amphidiploid mated with two paternal amphidiploids, in 806 (94.7%) of a total of 851 eggs of a female first-generation offspring mated with two brothers, and in 319 (71.7%) of 445 eggs of a female second-generation offspring mated with one brother. Normally feeding tadpoles were raised from 56.7%, 53.8% and 84.0% of the normally cleaved eggs, and metamorphosed frogs were from 47.0%, 47.5% and 80.6% in the first-, second- and third-generations, respectively. While sperm of five male amphidiploids fertilized 32.1～95.2%, 72.8% on the average, of the respective number of eggs of three female amphidiploids belonging to the three generations, sperm of three male amphidiploids of the first- and second-generation offspring fertilized only 8.7～20.1%, 15.1% on the average, of the respective number of eggs of two diploid female *Rana nigromaculata* and one diploid female *Rana plancyi chosenica*. It is noteworthy that the large spermatozoa of male amphidiploids could easily fertilize the large eggs of female amphidiploids, whereas they were considerably difficult to fertilize the normal-sized eggs of diploid females of the parental species.

In amphidiploids obtained from *Rana brevipoda* eggs by cold-treatment after insemination with sperm of autotetraploid male *Rana nigromaculata*, Kawamura and Nishioka (1983) reported that 30.9～86.5% of the respective number of eggs cleaved normally and 25.5～79.0% became feeding tadpoles in matings between five females and two males. The percentages of the feeding tadpoles corresponded to 44.7～93.5% of the normally cleaved eggs and were very similar to those in the matings between amphidiploid males and females in the present study.

In the first-generation offspring of amphidiploids between *Rana nigromaculata* and *Rana plancyi chosenica*, 167 (89.8%) of 186 feeding tadpoles whose chromosomes were analyzed were amphidiploids, while six of the other 19 were hexaploids, two were diploids and 11 were 2n-6n mosaics. The high percentage of amphidiploid offspring indicates that normal meiotic divisions occurred in almost all oocytes and spermatocytes. Hexaploids seem to have been produced from eggs with unreduced chromosomes by fertilization with diploid spermatozoa. It is very probable that tetraploid ova derived by normal meiotic divisions from octoploid oocytes whose chromosomes were doubled at the oogonium stage. The 2n-6n mosaics are considered to have been raised from eggs, in which the male pronucleus controlled the cleavage without fusing with the female pronucleus. At the first cleavage, each of the two diploid daughter nuclei of the male pronucleus entered into each blastomere, while the two diploid daughter nuclei of the female pronucleus remained in one of the blastomeres and fused with the daughter nucleus of the male pronucleus. The diploids seem to have gynogenetically developed by the diploid female pronucleus or androgenetically developed by the diploid male pronucleus.

In the second-generation offspring, 166 of 167 tadpoles obtained from matings between one female and two male amphidiploids were amphidiploids and the remainder was a 2n-4n mosaic. This finding seems to show that both male and female first-generation offspring of amphidiploids were almost completely normal in gametogenesis.

The sex of amphidiploids was examined in three generations. In the first-, second- and third-generation offspring, 63 (64.9%) of 97, 169 (62.8%) of 269 and 44 (46.3%) of 95 metamorphosed frogs were males. These percentages of males seem to indicate that the male amphidiploids are basically XXXY in sex chromosome constitution, as the normal diploid male of *Rana nigromaculata* is believed to be XY. The preponderance of males in the first- and second-generation offspring of amphidiploids seems to be attributable to sex reversal of some genetic females. It is well-known in anurans that sex reversal occurs by various causes such as overripeness of oviducal eggs (KUSCHAKEWITSCH, 1910; R. HERTWIG, 1921; WITSCHI, 1924), rearing under high temperature (WITSCHI, 1929), hybridization (DÜRKEN, 1935, 1938; KAWAMURA, 1943, 1950; KAWAMURA and KOBAYASHI, 1959, 1960; KAWAMURA and NISHIOKA, 1977a; KAWAMURA, NISHIOKA and UEDA, 1981), triploidy (HUMPHREY, BRIGGS and FANKHAUSER, 1950; KAWAMURA and TOKUNAGA, 1952; KAWAMURA and NISHIOKA, 1967), etc. KAWAMURA and NISHIOKA (1977a) reported that sex reversal occurred in a small number of gynogenetically produced diploids which were genetically females in *Rana nigromaculata, Rana brevipoda, Rana japonica, Rana tsushimensis* and *Rana rugosa*. KAWAMURA and NISHIOKA (1972, 1977b) confirmed that remarkable sex reversal occurred in seven consecutive generations derived from male nucleo-cytoplasmic hybrids consisting of *Rana ornativentris* cytoplasm and *Rana japonica* nuclei. A similar phenomenon of sex reversal was reported by NISHIOKA (1972b) in the second- and third-generation offspring derived from a female nucleo-cytoplasmic hybrid consisting of *Rana brevipoda* cytoplasm and *Rana nigromaculata* nuclei. Sex reversal of genetic females into phenotypic males was also described by KAWAMURA and NISHIOKA (1977a, 1978) and NISHIOKA (1978) in the descendants derived from X- or neutron-irradiated sperm or oviducal eggs of *Rana nigromaculata* and *Rana japonica*. All these factors giving rise to sex reversal seem to have been closely related to chromosome aberrations or abnormal combinations of chromosomes as well as to anatomical abnormalities of the body.

The process of sex reversal was observed in detail by WITSCHI (1929) in female tadpoles of *Rana sylvatica* exposed to high temperature. He confirmed that suppression of normal development first occurred in the cortical portions of the ovaries and gave rise to compensatory growth of the medullary portions, which some time later differentiated into the rete apparatus of testes. KAWAMURA and NISHIOKA (1978) and NISHIOKA (1978) have insisted that slight chromosome aberrations may bring about an abnormal metabolism of the body and in turn may suppress the normal development of the ovarian cortices, as the latter are very sensitive to general metabolism of the body.

It is very probable that the parental amphidiploids had some slight chromosome aberrations in such a degree that they can develop into mature frogs but can not build up the normal ovarian cortices in genetic females. The nearly equal number of males and females in the third-generation offspring may be attributed to vanishing of these slight chromosome aberrations, owing to selection of the most healthy amphidiploids in order to produce the following generation.

Some males and females of the first- and second-generation offspring of amphidiploids were remarkably larger than the male and female allotriploids produced from the same maternal amphidiploids and the control diploid *Rana nigromaculata* and *Rana plancyi chosenica*. The males and females of the third-generation offspring were also larger than the (N)NCC allotriploids produced from the same maternal amphidiploid and the control diploid *Rana plancyi chosenica*, while they did not statistically differ in body length from the (N)NNC allotriploids produced from the same maternal amphidiploid and the control *Rana nigromaculata*. However, some of the amphidiploids of the third-generation offspring were remarkably larger than all the others. Moreover, the amphidiploids of the third-generation offspring might become statistically larger than the (N)NNC allotriploids, if they were continuously reared over hibernation, as they were compared with the others at the age of six months. It is interesting that some of the first-, second- and third-generation offspring of amphidiploids at least grow remarkably larger as compared with allotriploids and the control diploid *Rana nigromaculata* and *Rana plancyi chosenica*.

KAWAMURA and NISHIOKA (1983) described that the amphidiploids produced from diploid female *Rana brevipoda* by mating with autotetraploid male *Rana nigromaculata* were slightly larger than the allotriploids obtained from the same males in mean total length of 50-day-old tadpoles and mean body length immediately after metamorphosis, while autotetraploids were not always larger than autotriploids. The amphidiploids obtained from seven of 11 female amphidiploids mated with male amphidiploids also seemed to be slightly larger than the allotriploids obtained from the same females, while those obtained from the other amphidiploids scarcely differed from the allotriploids of the same mothers in size.

Amphidiploids seem to be in conspicuous contrast to autotetraploids in growth rate. GURDON (1959) reported that there was no significant difference in growth rate between autotetraploids and diploids in *Xenopus laevis*. KAWAMURA, NISHIOKA and MYOREI (1963) also reported that diploid, triploid and tetraploid *Rana japonica* were nearly the same in the period of tadpole stage as well as in body length immediately after metamorphosis. According to JAYLET (1972), tetraploid larvae were slightly slower in growth than normal diploids and metamorphosed about one month later than the controls in *Pleurodeles waltl*. Thus, it seems evident that a kind of heterosis occurs in amphidiplids without being limited in one generation alone, owing to their almost complete repreductive capacity.

## SUMMARY

1. Reproductive capacity of a female and two male amphidiploids between *Rana nigromaculata* and *Rana plancyi chosenica* and of their first-, second- and third-generation offspring was examined. The parental amphidiploids were those which had been produced from a female (N)NNC allotriploid by mating with a diploid male *Rana plancyi chosenica*.

2. Of 700 eggs of the female amphidiploid mated with the two male amphidiploids, 328 (46.9%) cleaved normally, and 186 (26.6%) became feeding tadpoles. When the female amphidiploid was mated with a diploid male *Rana nigromaculata* or *Rana plancyi chosenica*, 48.6% or 35.3% of the respective number of eggs became feeding tadpoles.

3. Of 851 eggs of a female amphidiploid of the first-generation offspring mated with two brothers, 806 (94.7%) cleaved normally and 434 (51.0%) became feeding tadpoles. When the female amphidiploid was mated with a diploid male *Rana nigromaculata* or *Rana plancyi chosenica*, 50.7% or 19.1% of the respective number of eggs became feeding tadpoles.

4. Of 445 eggs of a female amphidiploid of the second-generation offspring mated with a brother, 319 (71.7%) cleaved normally and 268 (60.2%) became feeding tadpoles. When the female amphidiploid was mated with a diploid male *Rana nigromaculata* or *Rana plancyi chosenica*, 63.5% or 69.8% of the respective number of eggs became feeding tadpoles.

5. In the first-generation offspring of the parental amphidiploids, 167 (89.8%) of 186 tadpoles were tetraploids, two were diploids, six were hexaploids and the remaining 11 were 2n-6n mosaics. Of the tetraploid tadpoles, 135 completed metamorphosis and 61 attained sexual maturity. When the sex was examined in 97 tetraploid frogs, 34 were females and 63 (64.9%) were males.

In the offspring between the same female amphidiploid and a diploid male *Rana nigromaculata*, 176 (98.3%) of 179 tadpoles were triploids, one was a diploid and the remaining two were 2n-5n mosaics. In the offspring between this female and a diploid male *Rana plancyi chosenica*, 104 (92.9%) of 112 tadpoles were triploids, while the other eight were pentaploids.

6. In the second-generation offspring between a female and two male amphidiploids of the first-generation offspring, chromosomes were observed in 167 of the 434 feeding tadpoles. It was found that they were all tetraploids except one which was a 2n-4n mosaic. The other 267 tadpoles were also regarded as

amphidiploids without observing chromosomes, as they were completely normal in size, shape and viability. Twenty of 77 and 10 of 33 feeding tadpoles produced from the same female by mating with a diploid male *Rana nigromaculata* and a diploid male *Rana plancyi chosenica*, respectively, were all triploids. The other 57 and 23 tadpoles were also regarded as allotriploids without observing chromosomes, as they were completely normal in size, shape and viability.

Of the 433 tetraploid tadpoles, 383 metamorphosed normally and 80 attained sexual maturity. When the sex was examined in a total of 269 tetraploids, 100 were females and 169 (62.8%) were males.

7. In the third-generation offspring between a female and a male amphidiploids of the second-generation offspring, 257 of 268 amphidiploid tadpoles completed metamorphosis and 81 attained sexual maturity. When the sex was examined in 95 amphidiploid frogs, 51 were females and 44 (46.3%) were males.

8. Some males and females at least of the first-, second- and third-generation offspring of amphidiploids were remarkably larger than the male and female allotriploids produced from the same mother as well as the control diploid *Rana nigromaculata* and *Rana plancyi chosenica*. While the male and female amphidiploids of the third-generation offspring did not statistically differ in body length from the (N)NNC allotriploids produced from the same mother, they were larger than the (N)NCC allotriploids produced from the same mother as well as the control diploid *Rana nigromaculata* and *Rana plancyi chosenica*.

9. The amphidiploids were intermediate between *Rana nigromaculata* and *Rana plancyi chosenica* in external characters as a whole, while the allotriploids were intermediate between the amphidiploids and one of the two species.

10. The testes of male amphidiploids were completely normal in inner structure except that the spermatozoa, spermatocytes and spermatogonia were remarkably larger than those of the male diploid *Rana nigromaculata* and *Rana plancyi chosenica*. The first meiotic divisions usually contained 26 bivalent chromosomes.

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