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Allopentaploids and Allohexaploids between *Rana nigromaculata* and *Rana plancyi chosenica*

By

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ABSTRACT

Allopentaploids and allohexaploids were produced between *Rana nigromaculata,* (N)NN, and *Rana plancyi chosenica,* (C)CC, in order to clarify their viability, sexual ratio and reproductive capacity. The allopentaploids, (N)NNNCC or (N)NNCCC, were obtained from female amphidiploids, (N)NNCC, between these two species by suppressing the extrusion of the second polar body by refrigeration of eggs after insemination with sperm of *R. nigromaculata* or *R. plancyi chosenica.* Allohexaploids, (N)NNNCCC, were obtained from amphidiploids, (N)NNCC, by suppressing the extrusion of the second polar body by refrigeration of eggs after insemination with sperm of male amphidiploids, (N)NNCC.

In eight experimental series, (N)NNCC ♀ × (N)NN ♂, with cold-treatment of eggs, 255(6.1%) of 4163 eggs became normally feeding tadpoles. Of 178 analyzed tadpoles, 138 (77.5%) were allopentaploids, (N)NNNCC, 100 completed metamorphosis and 52 attained sexual maturity. There were seven females and 45 males. In five experimental series, (N)NNCC ♀ × (C)CC ♂, with cold-treatment of eggs, 110 (7.4%) of 1491 eggs became normally feeding tadpoles. Of 77 analyzed tadpoles, 63 (81.8%) were allopentaploids, (N)NNCCC, 59 completed metamorphosis and 37 attained sexual maturity. There were 11 females and 26 males. The testes of 11 male allopentaploids including six (N)NNNCC and five (N)NNCCC had no normal spermatozoa. On the other hand, two of the seven female allopentaploids, (N)NNNCC, and nine of the 11 female allopentaploids, (N)NNCCC, contained many mature eggs. When these females were injected with bullfrog pituitaries, only two female allopentaploids, (N)NNCCC, laid 191 and 180 eggs, respectively. When inseminated with sperm of a male diploid *R. nigromaculata,* 122 (63.9%) and 55 (30.6%) eggs cleaved normally, respectively, and 12 (6.3%) and nine (5.0%) attained metamorphosis. The chromosomes of the offspring which were abnormal embryos and tadpoles or normal feeding tadpoles were examined.

In 11 experimental series, (N)NNCC ♀ × (N)NNCC ♂, with cold-treatment of eggs, 240 (4.3%) of 5603 eggs became normally feeding tadpoles. Of 137 analyzed tadpoles, 53 (38.7%) were allohexaploids, (N)NNNCCC; 41 of them completed metamorphosis and 22 attained sexual maturity. There were five females and 17 males. The testes of eight males examined contained no normal spermatozoa. The ovaries of four females were degenerated and contained no mature ova, while the other female had several large ova in the ovaries.

INTRODUCTION

The first fertile amphidiploid in vertebrate was produced between *Rana brevipoda* and *R. nigromaculata* by KAWAMURA and NISHIOKA (1960, 1963a). They obtained a male from an egg of *R. brevipoda* by transplanting a blastula nucleus of *R. nigromaculata*. Later, they (1963b) obtained the first female amphidiploid between *R. brevipoda* and *R. nigromaculata* and could establish an amphidiploid strain. This female amphidiploid was obtained together with six male amphidiploids from *R. brevipoda* eggs by cold-treatment after insemination with sperm of tetraploid males of *R. nigromaculata*. Another useful method of producing amphidiploids between *R. nigromaculata* and *R. brevipoda* was found by KAWAMURA, NISHIOKA and OKUMOTO (1983). Considerably many amphidiploids were obtained from females of reciprocal allotriploids between *R. nigromaculata* and *R. brevipoda* by mating with diploid males of *R. nigromaculata* or *R. brevipoda*. NISHIOKA (1983) also reported that males and females of reciprocal hybrids between *R. nigromaculata* and *R. plancyi chosenica* produced diploid and triploid offspring by backcrossing with the parental species. The allotriploid females consisting of two *R. nigromaculata* genomes and one *R. plancyi chosenica* genome produced numerous amphidiploids by mating with diploid male *R. plancyi chosenica*. NISHIOKA and OKUMOTO (1983) established an amphidiploid strain between *R. nigromaculata* and *R. plancyi chosenica* by repeating the matings from generation to generation between male and female amphidiploids.

NISHIOKA (1983) found that about two-thirds of the backcrosses of the male reciprocal hybrids between *R. plancyi chosenica* and *R. nigromaculata* mated with female *R. nigromaculata* were triploids. The females of reciprocal hybrids between the same two species usually laid some large eggs, which became triploids by fertilization with sperm of *R. nigromaculata* or *R. plancyi chosenica*. From the triploid female backcrosses of diploid female hybrids between female *R. nigromaculata* and male *R. plancyi chosenica* mated with male *R. nigromaculata*, numerous tetraploids were produced by mating with male *R. plancyi chosenica*. The tetraploids occupied about two-thirds of all the tadpoles produced from this kind of matings. Most of the metamorphosed tetraploids were males.

In the present study, allopentaploids and allohexaploids were produced between *R. nigromaculata* and *R. plancyi chosenica* in order to compare them with amphidiploids in viability, sexual ratio and reproductive capacity.

MATERIALS AND METHODS

The animals used in this study are *Rana nigromaculata* HALLOWELL collected from the suburbs of Hiroshima City and *Rana plancyi chosenica* OKADA collected from Suwon, Korea, and their offspring. In addition, the first, second and third offspring of amphidiploids, (N)NNCC, between the two species which were first produced by NISHIOKA in 1978 (NISHIOKA, 1983; NISHIOKA and OKUMOTO, 1983) were used.

Ovulation was accelerated by injection of suspension of bullfrog pituitaries into the abdomen of females. Eggs were fertilized by the routine method. Allopentaploids were obtained from female amphidiploids, (N)NNCC, by suppressing the extrusion of the second polar body by refrigeration of the eggs for 2.5~3.0 hours at 0.5~2.0°C, 20~25 minutes after insemination with normal sperm of *Rana nigromaculata* or *R. plancyi chosenica* (Fig. 1). Allohexaploids were also obtained from female amphidiploids, (N)NNCC, by suppressing the extrusion of the second polar body by the same method as described above after insemination with sperm of male amphidiploids, (N)NNCC (Fig. 1). A part of fertilized eggs in each kind of experiments was reared as controls without refrigeration of the eggs.

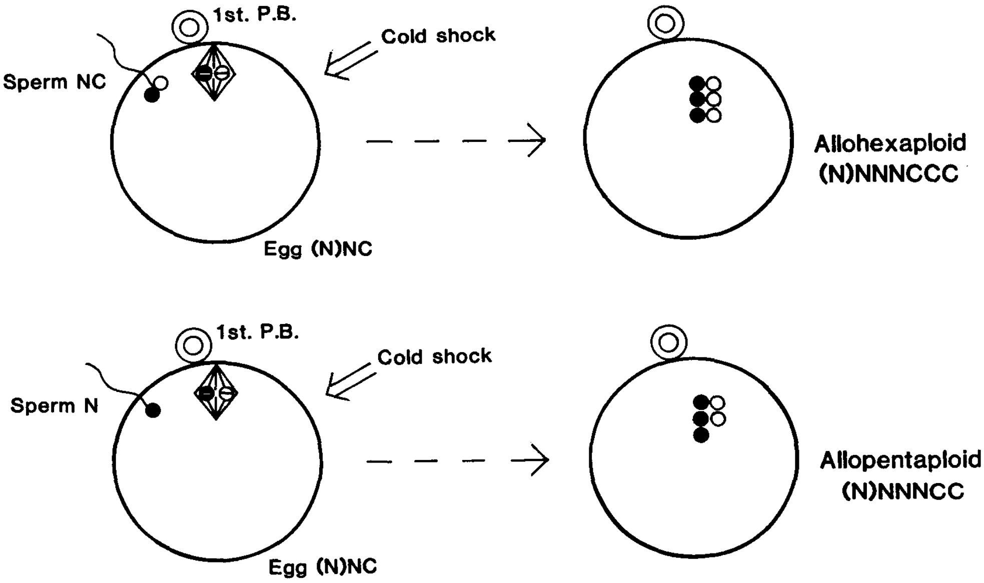

Fig. 1. Methods for producing allohexaploids and allopentaploids between *Rana nigromaculata*, (N)NN, and *Rana plancyi chosenica*, (C)CC.

The chromosomes of tadpoles were examined in the tail-tips by the squash method with water-pretreatment (Nishioka, 1972). Those of mature frogs were examined by the blood culture method (Volpe and Gebhardt, 1968; Wu and Yang, 1980). The meiotic chromosomes in the testes were observed by the method of Okumoto (1980).

Tadpoles were fed on boiled spinach or chard. Frogs were fed on tropical crickets, *Gryllus bimaculatus* De Geer. The gonads of young or mature frogs were preserved in Navashin's fluid, embedded in paraffin, sectioned at 12μ, and stained with Heidenhain's iron hematoxylin.

The following abbreviations are used in this 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* Hallowell

(C)CC ··· Diploid *Rana plancyi chosenica* OKADA
(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
(N)NNNCC ··· Allopentaploid consisting of three *Rana nigromaculata* genomes and two *Rana plancyi chosenica* genomes
(N)NNCCC ··· Allopentaploid consisting of two *Rana nigromaculata* genomes and three *Rana plancyi chosenica* genomes
(N)NNNCCC ··· Allohexaploid consisting of three *Rana nigromaculata* genomes and three *Rana plancyi chosenica* genomes

OBSERVATION

I. Production of allopentaploids, (N)NNNCC

1. Control series, (N)NNCC♀ × (N)NN♂

Eight control matings were made between eight female amphidiploids, (N)NNCC, obtained from female *Rana nigromaculata* and male *Rana plancyi chosenica* and four male diploid *Rana nigromaculata,* (N)NN (Fig. 2), in 1981~1983. It was found that 700 (79.5%) of the 881 eggs in total cleaved normally, and 577 (65.5%) became normal tail-bud embryos, 535 (60.7%) became normally hatched tadpoles, and 511 (58.0%) began to take food normally. The number of these tadpoles corresponded to 73.0% of the normally cleaved eggs (Table 1).

When the chromosomes were examined in 450 of these feeding tadpoles, 444 (98.7%) were triploids, three others were pentaploids and the remaining three were 2n—5n mosaics (Table 1).

2. Experimental series, (N)NNCC♀ × (N)NN♂, with cold-treatment of eggs

Matings were made between the same eight female amphidiploids, (N)NNCC♀, Nos. 1~8, and the same four male diploid *Rana nigromaculata,* (N)NN♂, Nos. 1~4, as those used in the control series. When 4163 eggs in total were refrigerated after insemination, 19.5~74.6% of the respective numbers of eggs in the eight experimental series, 1405 (33.7%) eggs in total, cleaved normally. Thereafter, many embryos died of abnormalities and 3.4~39.8% of the eggs used in the eight series, 489 (11.7%) eggs in total, became normal tail-bud embryos. Many of these embryos died of various abnormalities and 2.4~33.0% of the respective number of eggs in the eight experimental series, 378 (9.1%) eggs in total, became normally hatched tadpoles. After many tadpoles died of ill-development of the gills or the other abnormalities without taking food, 1.8 ~22.6% of the respective numbers of eggs in the eight experimental series, 255

TABLE 1

Production of allotriploids, (N)NNC, and allopentaploids, (N)NNNCC, from female amphidiploids, (N)NNCC, between *Rana nigromaculata* and *Rana plancyi chosenica*, by mating with male diploid *Rana nigromaculata* after no- or cold-treatment of eggs

Year	Parents		Treat-ment of eggs	No. of eggs	No. of normal cleavages	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally feeding tadpoles	Number of tadpoles					
									Ana-lyzed	Number of chromosomes				
	Female	Male								39 (3n)	65 (5n)	13–65 (n–5n)	26–65 (2n–5n)	Others
1981	(N)NNCC 80, No. 1	(N)NN 80, No. 1	No	125	107 (85.6%)	102 (81.6%)	96 (76.8%)	95 (76.0%)	86	84			2	
1982	(N)NNCC 81, No. 2	(N)NN 80, No. 2	No	96	55 (57.3%)	53 (55.2%)	51 (53.1%)	46 (47.9%)	43	43				
	(N)NNCC 81, No. 3	(N)NN 80, No. 2	No	116	102 (87.9%)	97 (83.6%)	93 (80.2%)	88 (75.9%)	82	80	1		1	
	(N)NNCC 81, No. 4	(N)NN 80, No. 2	No	246	223 (90.7%)	176 (71.5%)	151 (61.4%)	147 (59.8%)	141	140	1			
1983	(N)NNCC 81, No. 5	(N)NN 81, No. 3	No	75	52 (69.3%)	23 (30.7%)	20 (26.7%)	19 (25.3%)	13	13				
	(N)NNCC 81, No. 6	(N)NN 81, No. 3	No	101	60 (59.4%)	39 (38.6%)	38 (37.6%)	36 (35.6%)	29	29				
	(N)NNCC 82, No. 7	(N)NN 81, No. 4	No	72	64 (88.9%)	55 (76.4%)	54 (75.0%)	48 (66.7%)	27	26	1			
	(N)NNCC 82, No. 8	(N)NN 81, No. 4	No	50	37 (74.0%)	32 (64.0%)	32 (64.0%)	32 (64.0%)	29	29				
	Total		No	881	700 (79.5%)	577 (65.5%)	535 (60.7%)	511 (58.0%)	450	444 (98.7%)	3 (0.7%)		3 (0.7%)	
1981	(N)NNCC 80, No. 1	(N)NN 80, No. 1	Cold	363	141 (38.8%)	63 (17.4%)	40 (11.0%)	32 (8.8%)	29	4	21	1	1	2
1982	(N)NNCC 81, No. 2	(N)NN 80, No. 2	Cold	279	208 (74.6%)	111 (39.8%)	92 (33.0%)	63 (22.6%)	35		31		3	1
	(N)NNCC 81, No. 3	(N)NN 80, No. 2	Cold	422	94 (22.3%)	50 (11.8%)	42 (10.0%)	28 (6.6%)	24		16	2	4	2
	(N)NNCC 81, No. 4	(N)NN 80, No. 2	Cold	437	145 (33.2%)	53 (12.1%)	38 (8.7%)	28 (6.4%)	28		27	1		
1983	(N)NNCC 81, No. 5	(N)NN 81, No. 3	Cold	882	172 (19.5%)	30 (3.4%)	21 (2.4%)	16 (1.8%)	12		10			2
	(N)NNCC 81, No. 6	(N)NN 81, No. 3	Cold	894	378 (42.3%)	64 (7.2%)	52 (5.8%)	35 (3.9%)	28	14	13	1		
	(N)NNCC 82, No. 7	(N)NN 81, No. 4	Cold	374	95 (25.4%)	42 (11.2%)	38 (10.2%)	21 (5.6%)	3	1	2			
	(N)NNCC 82, No. 8	(N)NN 81, No. 4	Cold	512	172 (33.6%)	76 (14.8%)	55 (10.7%)	32 (6.3%)	19	1	18			
	Total		Cold	4163	1405 (33.7%)	489 (11.7%)	378 (9.1%)	255 (6.1%)	178	20 (11.2%)	138 (77.5%)	5 (2.8%)	8 (4.5%)	7 (3.9%)

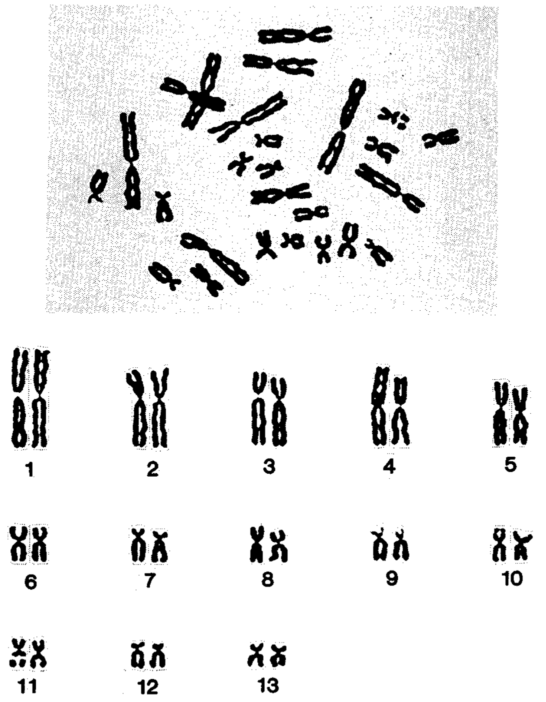

Fig. 2. Metaphase plate and the karyotype of a diploid *Rana nigromaculata*, (N)NN. ×900

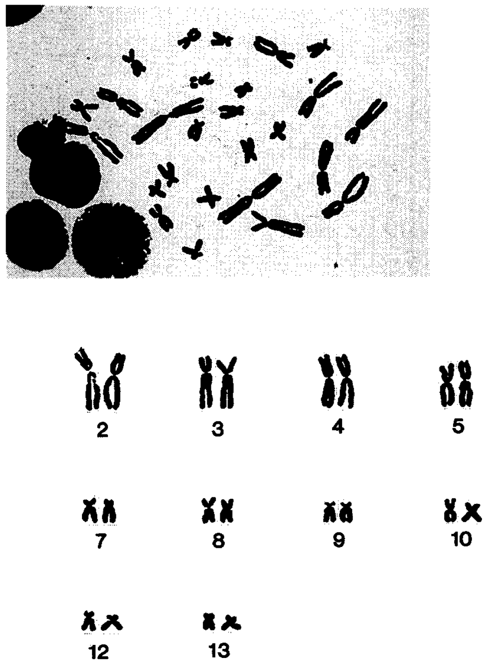

Fig. 3. Metaphase plate and the karyotype of a diploid *Rana plancyi chosenica*, (C)CC. ×900

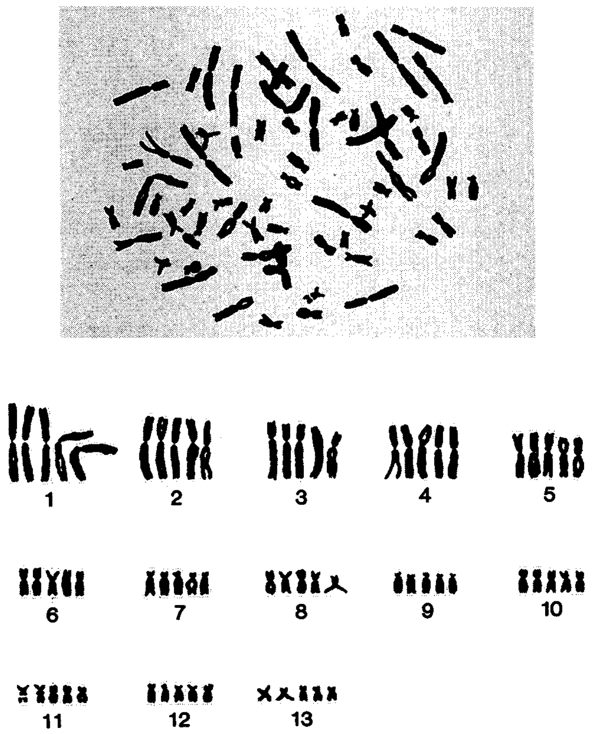

Fig. 4. Metaphase plate and the karyotype of an allopentaploid, (N)NNNCC, between *Rana nigromaculata* and *R. plancyi chosenica*. ×600

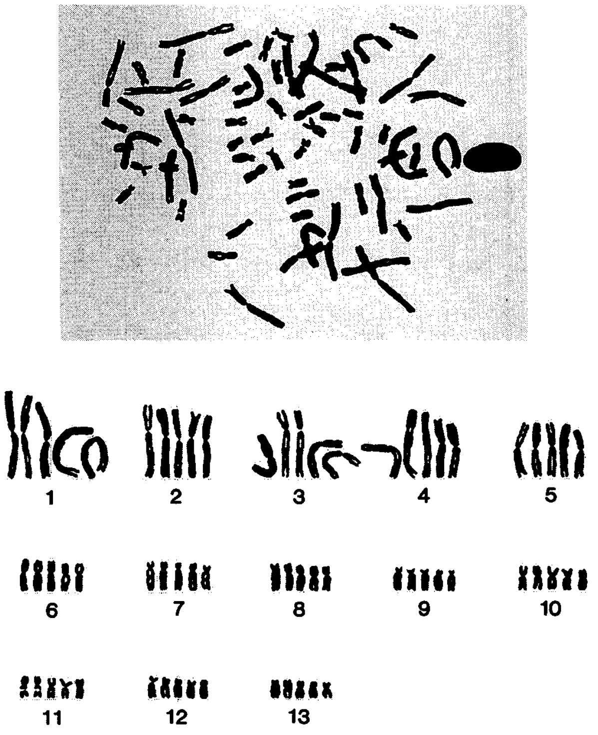

Fig. 5. Metaphase plate and the karyotype of an allopentaploid, (N)NNCCC, between *Rana nigromaculata* and *R. plancyi chosenica*. ×600

(6.1%) eggs in total, became normally feeding tadpoles. This number of tadpoles corresponded to 18.1% of the normally cleaved eggs (Table 1).

The chromosome numbers were counted in 178 of the feeding tadpoles. It was found that 138 (77.5%) of these tadpoles were pentaploids, (N)NNNCC (Fig. 4), 20 (11.2%) were triploids, five (2.8%) were n—5n mosaics, eight (4.5%) were 2n—5n mosaics, and the remaining seven (3.9%) had the other chromosome numbers. The triploid tadpoles were produced from four female amphidiploids, (N)NNCC 80, No. 1, (N)NNCC 81, No. 6, (N)NNCC 82, No. 7 and (N)NNCC 82, No. 8, while they were not produced from the other four female amphidiploids. The n—5n mosaics were produced from four female amphidiploids, (N)NNCC 80, No. 1, and (N)NNCC 81, Nos. 3, 4 and 6. The 2n—5n mosaics were produced from three female amphidiploids, (N)NNCC 80, No. 1 and (N)NNCC 81, Nos. 2 and 3. One tadpole was a haploid, produced from a female amphidiploid, (N)NNCC 80, No. 1, two tadpoles were diploids, produced from a female amphidiploid, (N)NNCC 81, No. 3, and three others were tetraploids, produced from two female amphidiploids, (N)NNCC 80, No. 1 and (N)NNCC 81, No. 5. The remainder was an aneuploid (2n+1), produced from a female amphidiploid, (N)NNCC 81, No. 2 (Table 1).

II. Production of allopentaploids, (N)NNCCC

1. Control series, (N)NNCC♀×(C)CC♂

Five control matings were made between five female amphidiploids, (N)NNCC, obtained from female *Rana nigromaculata* and male *Rana plancyi chosenica* and five male diploid *Rana plancyi chosenica*, (C)CC (Fig. 3), in 1981~1983. It was found that 259 (74.0%) of the 350 eggs in total cleaved normally, 217 (62.0%) hatched normally and 207 (59.1%) tadpoles began to take food normally. This number of tadpoles corresponded to 79.9% of the normally cleaved eggs (Table 2).

When the chromosome numbers were counted in 128 of these 207 feeding tadpoles, it was found that 123 (96.1%) were triploids, three were pentaploids, another was an n—5n mosaic, and the remainder was a 2n—5n mosaic (Table 2).

2. Experimental series, (N)NNCC♀×(C)CC♂, with cold-treatment of eggs

Matings were made between the same five female amphidiploids, (N)NNCC♀, Nos. 1, 2, 9, 10 and 11, and the same five male diploid *Rana plancyi chosenica*, (C)CC♂, Nos. 1~5, as those used in the control series. A total of 1491 eggs obtained from the female amphidiploids was refrigerated for 2.5~3.0 hours at 0.5~2.0°C, 20~25 minutes after insemination with sperm of the five male diploid *Rana plancyi chosenica* to suppress the extrusion of the second polar body. It was found that 7.6~53.2% of the respective numbers of eggs in the five experimental series, 392 (26.3%) eggs in total, cleaved normally. Many eggs died of abnormalities, and 175 (11.7%) eggs in total became normal tail-bud embryos. Thereafter, 2.4~15.2% of the respective numbers of eggs in the five experimental series, 139 (9.3%) eggs in total, became normally hatched tadpoles. Most of these

TABLE 2

Production of allotriploids, (N)NCC, and allopentaploids, (N)NNCCC, from female amphidiploids, (N)NNCC, between *Rana nigromaculata* and *Rana plancyi chosenica*, by mating with male diploid *Rana plancyi chosenica* after no- or cold-treatment of eggs

Year	Parents		Treat-ment of eggs	No. of eggs	No. of normal cleavages	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally feeding tadpoles	Number of tadpoles				
	Female	Male							Ana-lyzed	Number of chromosomes			
										39 (3n)	65 (5n)	13–65 (n–5n)	Others
1981	(N)NNCC 80, No. 1	(C)CC 76, No. 1	No	152	110 (72.4%)	104 (68.4%)	96 (63.2%)	92 (60.5%)	53	49	3	1	
1982	(N)NNCC 81, No. 2	(C)CC 80, No. 2	No	52	46 (88.5%)	40 (76.9%)	35 (67.3%)	31 (59.6%)	25	24			1
1983	(N)NNCC 82, No. 9	(C)CC 80, No. 3	No	58	51 (87.9%)	44 (75.9%)	44 (75.9%)	44 (75.9%)	24	24			
	(N)NNCC 82, No. 10	(C)CC 80, No. 4	No	40	29 (72.5%)	25 (62.5%)	25 (62.5%)	25 (62.5%)	17	17			
	(N)NNCC 82, No. 11	(C)CC 80, No. 5	No	48	23 (47.9%)	17 (35.4%)	17 (35.4%)	15 (31.3%)	9	9			
	Total		No	350	259 (74.0%)	230 (65.7%)	217 (62.0%)	207 (59.1%)	128	123 (96.1%)	3 (2.3%)	1 (0.8%)	1 (0.8%)
1981	(N)NNCC 80, No. 1	(C)CC 76, No. 1	Cold	366	63 (17.2%)	31 (8.5%)	26 (7.1%)	21 (5.7%)	18	2	11	4	1
1982	(N)NNCC 81, No. 2	(C)CC 80, No. 2	Cold	216	115 (53.2%)	43 (19.9%)	32 (14.8%)	20 (9.3%)	16	4	9	2	1
1983	(N)NNCC 82, No. 9	(C)CC 80, No. 3	Cold	455	179 (39.3%)	85 (18.7%)	69 (15.2%)	59 (13.0%)	34		34		
	(N)NNCC 82, No. 10	(C)CC 80, No. 4	Cold	340	26 (7.6%)	10 (2.9%)	8 (2.4%)	7 (2.1%)	7		7		
	(N)NNCC 82, No. 11	(C)CC 80, No. 5	Cold	114	9 (7.9%)	6 (5.3%)	4 (3.5%)	3 (2.6%)	2		2		
	Total		Cold	1491	392 (26.3%)	175 (11.7%)	139 (9.3%)	110 (7.4%)	77	6 (7.8%)	63 (81.8%)	6 (7.8%)	2 (2.6%)

tadpoles developed normally, and 2.1~13.0% of the respective numbers of eggs in the five experimental series, 110 (7.4%) eggs in total, became normally feeding tadpoles. This number of tadpoles corresponded to 28.1% of the normally cleaved eggs (Table 2).

In 77 of the 110 feeding tadpoles produced from the five experimental series, their chromosome numbers were counted. It was found that 63 (81.8%) of them were pentaploids, (N)NNCCC (Fig. 5). All the tadpoles other than the pentaploids were produced from two female amphidiploids, (N)NNCC , Nos. 1 and 2. Six of them were triploids, six others were n—5n mosaics and the remaining two were diploids (Table 2).

III. Production of allohexaploids, (N)NNNCCC

1. Control series, (N)NNCC ♀ × (N)NNCC ♂

In 1981~1983, 11 control matings were made between 11 female and six male amphidiploids which were produced from *Rana nigromaculata* and *Rana plancyi chosenica*. It was found that 666 (71.9%) of the 926 eggs in total cleaved normally, 528 (57.0%) eggs developed into normal tail-bud embryos, and 509 (55.0%) eggs became normally hatched tadpoles. Thereafter, 482 (52.1%) tadpoles began to take food normally. This number of feeding tadpoles corresponded to 72.4% of the normally cleaved eggs (Table 3).

The chromosome numbers were counted in 417 of the 482 feeding tadpoles obtained from the 11 control series. Of these tadpoles, 409 (98.1%) were tetraploids, three were diploids, one was a hexaploid and the remaining four were 2n—6n mosaics (Table 3).

2. Experimental series, (N)NNCC ♀ × (N)NNCC ♂, with cold-treatment of eggs

Matings were made between the same 11 female amphidiploids, (N)NNCC ♀, Nos. 1~3, 5, 6, 9, 10, and 12~15, and the same six male amphidiploids, (N)NNCC ♂, Nos. 1~6, as those used in the control series of 1981~1983. A total of 5603 eggs obtained from the female amphidiploids was refrigerated for 2.5~3.0 hours at 0.5~2.0°C, 20~25 minutes after insemination with sperm of the six male amphidiploids to suppress the extrusion of the second polar body. It was found that 8.2~51.5% of the respective numbers of eggs in the 11 experimental series, 1377 (24.6%) eggs in total, cleaved normally. Most of the normally cleaved eggs became abnormal and died. Eventually, only 2.3~25.3% of the respective numbers of eggs in the 11 experimental series, 525 (9.4%) eggs in total, became normal tail-bud embryos. Thereafter, many embryos died of abnormalities, and 1.9 ~19.3% of the respective numbers of eggs in the 11 experimental series, 356 (6.4%) tadpoles in total, hatched normally and became tadpoles. After many of the normally hatched tadpoles died of abnormalities, 1.3~10.6% of the respective numbers of eggs in the 11 experimental series, 240 (4.3%) eggs in total, became normally feeding tadpoles. This number of tadpoles corresponded to 17.4% of the normally cleaved eggs (Table 3).

TABLE 3

Production of amphidiploids, (N)NNCC, and allohexaploids, (N)NNNCCC, by mating between female and male amphidiploids, (N)NNCC, obtained from *Rana nigromaculata* and *Rana plancyi chosenica* after no- or cold-treatment of eggs

Year	Parents		Treatment of eggs	No. of eggs	No. of normal cleavages	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally feeding tadpoles	Number of tadpoles					
									Analyzed	Number of chromosomes				
	Female	Male								26 (2n)	52 (4n)	78 (6n)	26–78 (2n–6n)	Others
1981	(N)NNCC 80, No. 1	(N)NNCC 80, No. 1	No	121	102 (84.3%)	94 (77.7%)	92 (76.0%)	84 (69.4%)	82	1	79	1	1	
1982	(N)NNCC 81, No. 2	(N)NNCC 81, No. 2	No	51	41 (80.4%)	40 (78.4%)	37 (72.5%)	35 (68.6%)	34		34			
	(N)NNCC 81, No. 3	(N)NNCC 81, No. 2	No	62	55 (88.7%)	42 (67.7%)	41 (66.1%)	40 (64.5%)	36		36			
1983	(N)NNCC 81, No. 5	(N)NNCC 81, No. 3	No	134	61 (45.5%)	49 (36.6%)	46 (34.3%)	39 (29.1%)	25	1	24			
	(N)NNCC 81, No. 6	(N)NNCC 81, No. 3	No	146	73 (50.0%)	51 (34.9%)	46 (31.5%)	46 (31.5%)	38	1	37			
	(N)NNCC 82, No. 9	(N)NNCC 82, No. 4	No	49	26 (53.1%)	13 (26.5%)	13 (26.5%)	13 (26.5%)	13		13			
	(N)NNCC 82, No. 10	(N)NNCC 82, No. 4	No	57	51 (89.5%)	41 (71.9%)	40 (70.2%)	40 (70.2%)	30		29		1	
	(N)NNCC 82, No. 12	(N)NNCC 82, No. 5	No	59	50 (84.7%)	35 (59.3%)	34 (57.6%)	33 (55.9%)	25		25			
	(N)NNCC 82, No. 13	(N)NNCC 82, No. 5	No	118	87 (73.7%)	63 (53.4%)	61 (51.7%)	54 (45.8%)	46		45		1	
	(N)NNCC 82, No. 14	(N)NNCC 82, No. 6	No	62	56 (90.3%)	43 (69.4%)	42 (67.7%)	42 (67.7%)	37		37			
	(N)NNCC 82, No. 15	(N)NNCC 82, No. 6	No	67	64 (95.5%)	57 (85.1%)	57 (85.1%)	56 (83.6%)	51		50		1	
	Total		No	926	666 (71.9%)	528 (57.0%)	509 (55.0%)	482 (52.1%)	417	3 (0.7%)	409 (98.1%)	1 (0.2%)	4 (1.0%)	
1981	(N)NNCC 80, No. 1	(N)NNCC 80, No. 1	Cold	317	108 (34.1%)	65 (20.5%)	45 (14.2%)	29 (9.1%)	20	2	2	14	2	
1982	(N)NNCC 81, No. 2	(N)NNCC 81, No. 2	Cold	332	64 (19.3%)	34 (10.2%)	26 (7.8%)	17 (5.1%)	8			8		
	(N)NNCC 81, No. 3	(N)NNCC 81, No. 2	Cold	580	152 (26.2%)	32 (5.5%)	12 (2.1%)	9 (1.6%)	4			3		1
1983	(N)NNCC 81, No. 5	(N)NNCC 81, No. 3	Cold	353	29 (8.2%)	8 (2.3%)	7 (2.0%)	6 (1.7%)	4	3		1		
	(N)NNCC 81, No. 6	(N)NNCC 81, No. 3	Cold	352	62 (17.6%)	17 (4.8%)	15 (4.3%)	13 (3.7%)	11	10		1		

(N)NNCC 82, No. 9	(N)NNCC 82, No. 4	Cold	379	195 (51.5%)	96 (25.3%)	73 (19.3%)	40 (10.6%)	25	9	9	7		
(N)NNCC 82, No. 10	(N)NNCC 82, No. 4	Cold	455	132 (29.0%)	15 (3.3%)	9 (2.0%)	6 (1.3%)	6	5		1		
(N)NNCC 82, No. 12	(N)NNCC 82, No. 5	Cold	810	170 (21.0%)	45 (5.6%)	34 (4.2%)	24 (3.0%)	17	10		7		
(N)NNCC 82, No. 13	(N)NNCC 82, No. 5	Cold	814	285 (35.0%)	117 (14.4%)	69 (8.5%)	48 (5.9%)	16	11	3	2		
(N)NNCC 82, No. 14	(N)NNCC 82, No. 6	Cold	783	145 (18.5%)	85 (10.9%)	58 (7.4%)	42 (5.4%)	21	15		6		
(N)NNCC 82, No. 15	(N)NNCC 82, No. 6	Cold	428	35 (8.2%)	11 (2.6%)	8 (1.9%)	6 (1.4%)	5	2		3		
Total		Cold	5603	1377 (24.6%)	525 (9.4%)	356 (6.4%)	240 (4.3%)	137	67 (48.9%)	14 (10.2%)	53 (38.7%)	2 (1.5%)	1 (0.7%)

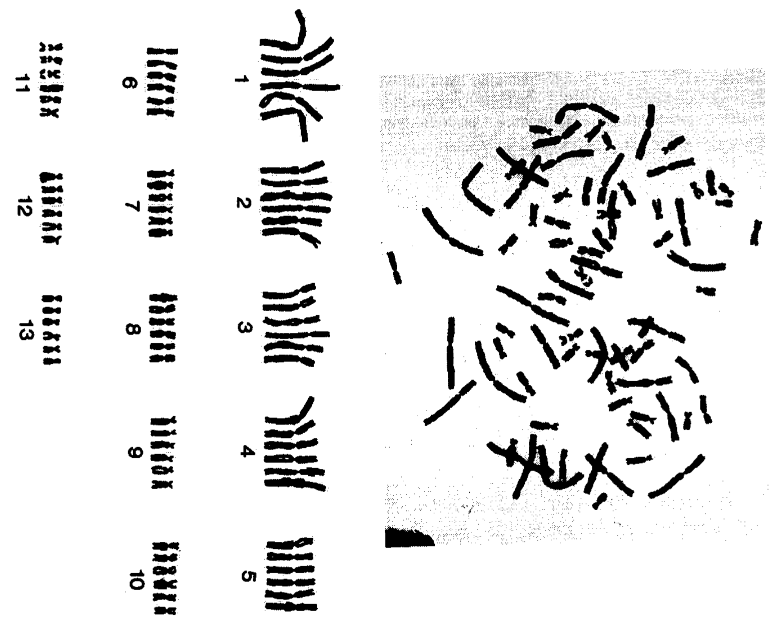

Fig. 6. Metaphase plate and the karyotype of an allohexaploid, (N)NNNCCC, between *Rana nigromaculata* and *R. plancyi chosenica*. ×600

Of the 240 feeding tadpoles obtained from the 11 experimental series, 137 developed normally and their chromosome numbers were counted. Of these tadpoles, 53 (38.7%) were hexaploids, (N)NNNCCC (Fig. 6), and 67 (48.9%) were diploids. The diploids were not produced from two female amphidiploids, (N)NNCC♀, Nos. 2 and 3, while they were produced from all the other nine female amphidiploids. In addition to hexaploids and diploids, there were 14 tetraploids, two 2n—6n mosaics and one aneuploid (2n+3) (Table 3).

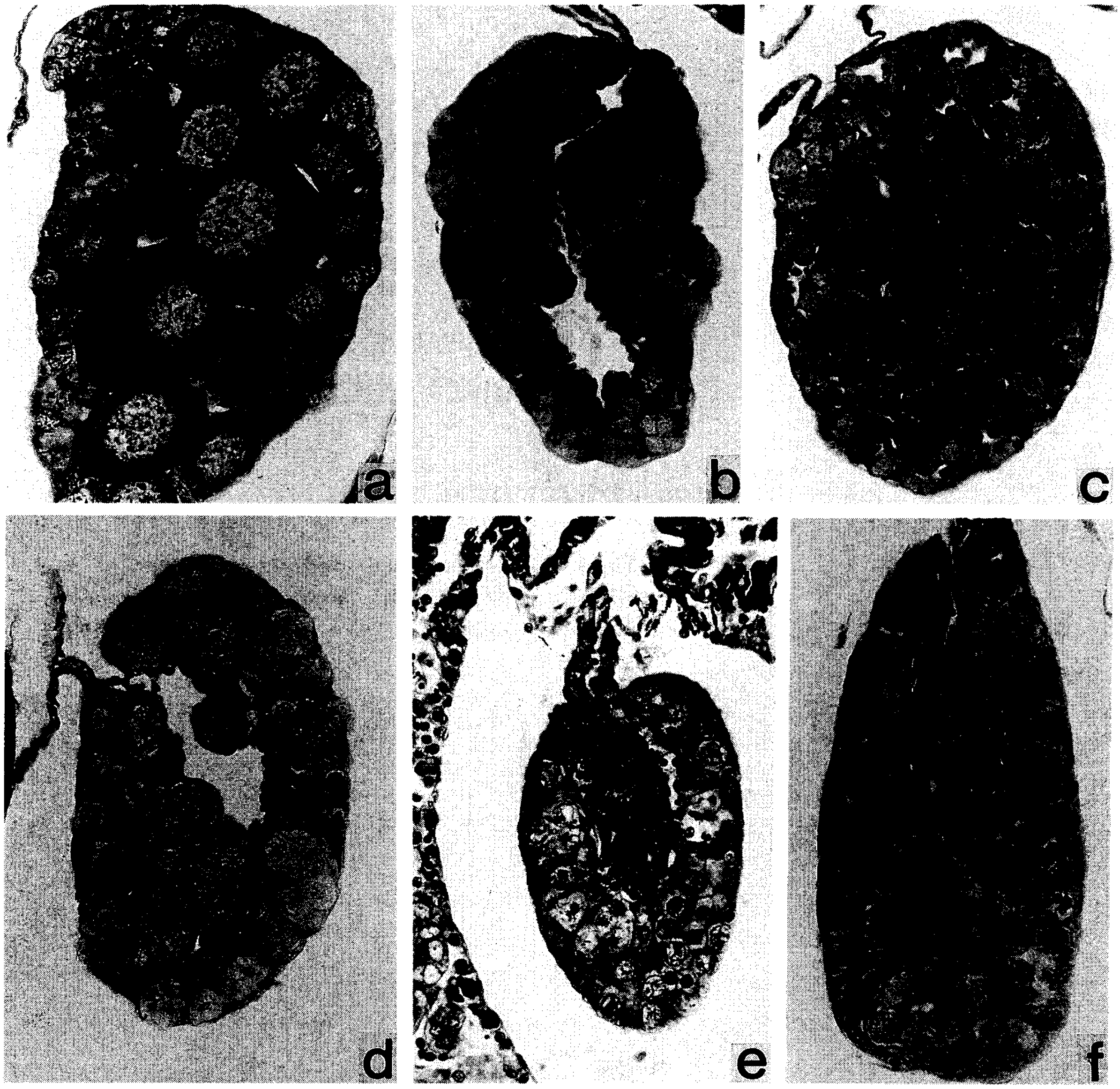

Fig. 7. Cross-sections of the gonads of the control young frogs which died within six months after metamorphosis. ×175

a. Normal ovary (♀N) of a female amphidiploid, (N)NNCC
b. Underdeveloped ovary (♀U) of a female allotriploid, (N)NCC
c. Normal testis (♂N) of a male allotriploid, (N)NCC
d. Gonad of a hermaphroditic (⚥) allotriploid, (N)NNC
e, f. Gonads of hermaphroditic (⚥) amphidiploids, (N)NNCC

IV. Metamorphosis and sex

1. Allopentaploids, (N)NNNCC

a. Allotriploids, (N)NNC, produced in the control series

In the eight control matings between eight female amphidiploids, (N)NNCC 80~82♀, Nos. 1~8, and four male diploid *Rana nigromaculata*, (N)NN 80, 81♂, Nos. 1~4, 418 of the 444 normally feeding allotriploid tadpoles completed metamorphosis. Of these metamorphosed frogs, 139 died or were preserved within six months after metamorphosis. The gonads of these frogs were sectioned to observe their inner structures (Fig. 7). It was found that 13 of them were approximately normal females (♀N) having auxocytes in the ovaries, 42 were underdeveloped female (♀U) whose ovaries had a few auxocytes, 25 were hermaphrodites (⚥) transforming from females to males, and 59 were males (♂N). Of 193 frogs which lived more than one year and attained maturity, 88 were females and 105 were males (Fig. 8). When the hermaphrodites were counted as males, there were 143 females and 189 (56.9%) males in total (Table 4).

Three pentaploids produced from the foregoing eight matings completed metamorphosis. All of them died within three months after metamorphosis. Two of them were males, while the remainder was a hermaphrodite transforming from a female to a male (Table 4).

b. Allopentaploids, (N)NNNCC, produced in the experimental series

In the eight experimental series, 100 of the 138 normally feeding allopentaploid tadpoles completed metamorphosis. As 40 of these frogs died within six months after metamorphosis, their gonads were sectioned to observe their inner structures (Fig. 9). It was found that 13 of them were underdeveloped females (♀U)which had a few auxocytes in their ovaries, seven were hermaphrodites (⚥) transforming from females to males and the remaining 20 were males (♂N). Among 52 allopentaploids, (N)NNNCC, which lived more than one year and attained sexual maturity, there were seven females and 45 males (Fig. 10). When the hermaphrodites were counted as males, there were 20 females and 72 (78.3%) males in the 92 allopentaploids in total (Table 4).

2. Allopentaploids, (N)NNCCC

a. Allotriploids, (N)NCC, produced in the control series

Of the 123 normally feeding allotriploid tadpoles, (N)NCC, produced from the five control matings between five female amphidiploids, (N)NNCC 80~82♀, Nos. 1, 2, 9, 10 and 11, and five male diploid *Rana plancyi chosenica,* (C)CC 76,80♂, Nos. 1~5, 117 completed metamorphosis. As 36 of these frogs died within six months after metamorphosis, their gonads were sectioned to observe their inner structures (Fig. 7). It was found that three of them were normal females (♀N) having numerous auxocytes, 14 were underdeveloped females (♀U) whose ovaries contained a few auxocytes, five were hermaphrodites (⚥) transforming from females

TABLE 4

Sex of allotriploids, (N)NNC, and allopentaploids, (N)NNNCC, produced by matings of female amphidiploids, (N)NNCC, with male diploid *Rana nigromaculata* after no- or cold-treatment of eggs

Year	Parents		Treatment of eggs	Ploidy	No. of Tadpoles	No. of metamorphosed frogs	Sex of frogs observed within six months after metamorphosis					Sex of frogs, one or two years old			Sex of all frogs examined		
	Female	Male					Total	♀N	♀U	⚥	♂N	Total	♀	♂	Total	♀	♂(%)*
1981	(N)NNCC 80, No. 1	(N)NN 80, No. 1	No	3n	84	80	41	6	8	6	21	23	9	14	64	23	41(64.1)
1982	(N)NNCC 81, No. 2	(N)NN 80, No. 2	No	3n	43	41	13	1	5	3	4	17	7	10	30	13	17(56.7)
	(N)NNCC 81, No. 3	(N)NN 80, No. 2	No	3n	80	76	26	1	9	7	9	22	8	14	48	18	30(62.5)
				5n	1	1	1	0	0	1	0	—	—	—	1	0	1
	(N)NNCC 81, No. 4	(N)NN 80, No. 2	No	3n	140	135	50	4	17	6	23	59	26	33	109	47	62(56.9)
				5n	1	1	1	0	0	0	1	—	—	—	1	0	1
1983	(N)NNCC 81, No. 5	(N)NN 81, No. 3	No	3n	13	11	—	—	—	—	—	11	7	4	11	7	4(36.4)
	(N)NNCC 81, No. 6	(N)NN 81, No. 3	No	3n	29	26	3	0	1	2	0	20	8	12	23	9	14(60.9)
	(N)NNCC 82, No. 7	(N)NN 81, No. 4	No	3n	26	23	5	1	1	1	2	18	8	10	23	10	13(56.5)
				5n	1	1	1	0	0	0	1	—	—	—	1	0	1
	(N)NNCC 82, No. 8	(N)NN 81, No. 4	No	3n	29	26	1	0	1	0	0	23	15	8	24	16	8(33.3)
	Total		No	3n	444	418	139	13	42	25	59	193	88	105	332	143	189(56.9)
				5n	3	3	3	0	0	1	2	—	—	—	3	0	3
1981	(N)NNCC 80, No. 1	(N)NN 80, No. 1	Cold	5n	21	19	10	0	5	2	3	9	0	9	19	5	14(73.7)
1982	(N)NNCC 81, No. 2	(N)NN 80, No. 2	Cold	5n	31	39	17	0	6	4	7	22	5	17	39	11	28(71.8)
	(N)NNCC 81, No. 3	(N)NN 80, No. 2	Cold	5n	16												
	(N)NNCC 81, No. 4	(N)NN 80, No. 2	Cold	5n	27												
1983	(N)NNCC 81, No. 5	(N)NN 81, No. 3	Cold	5n	10	10	4	0	0	0	4	3	0	3	7	0	7
	(N)NNCC 81, No. 6	(N)NN 81, No. 3	Cold	5n	13	12	2	0	0	0	2	7	1	6	9	1	8(88.9)
	(N)NNCC 82, No. 7	(N)NN 81, No. 4	Cold	5n	2	2	—	—	—	—	—	2	0	2	2	0	2
	(N)NNCC 82, No. 8	(N)NN 81, No. 4	Cold	5n	18	18	7	0	2	1	4	9	1	8	16	3	13(81.3)
	Total		Cold	5n	138	100	40	0	13	7	20	52	7	45	92	20	72(78.3)

♀N, Females with normal ovaries ♀U, Females with underdeveloped ovaries ⚥, Hermaphrodites ♂N, Males with normal testes *, Including hermaphrodites

to males and 14 were males (♂N). Of 76 allotriploids which lived more than one year after metamorphosis and attained sexual maturity, 34 were females and 42 were males (Fig. 8). When the hermaphrodites were counted as males, 51 of the 112 allotriploids in total were females and 61 (54.5%) were males (Table 5).

Of the three allopentaploids produced in 1981 from a female amphidiploid (N)NNCC 80♀, No. 1, two completed metamorphosis, and died shortly after metamorphosis. One of them was a hermaphrodite transforming from a female to a male, while the other was a male (Table 5).

b. Allopentaploids, (N)NNCCC, produced in the experimental series

In the five experimental series, 59 of the 63 normally feeding allopentaploid tadpoles completed metamorphosis. As 19 of them died within six months after

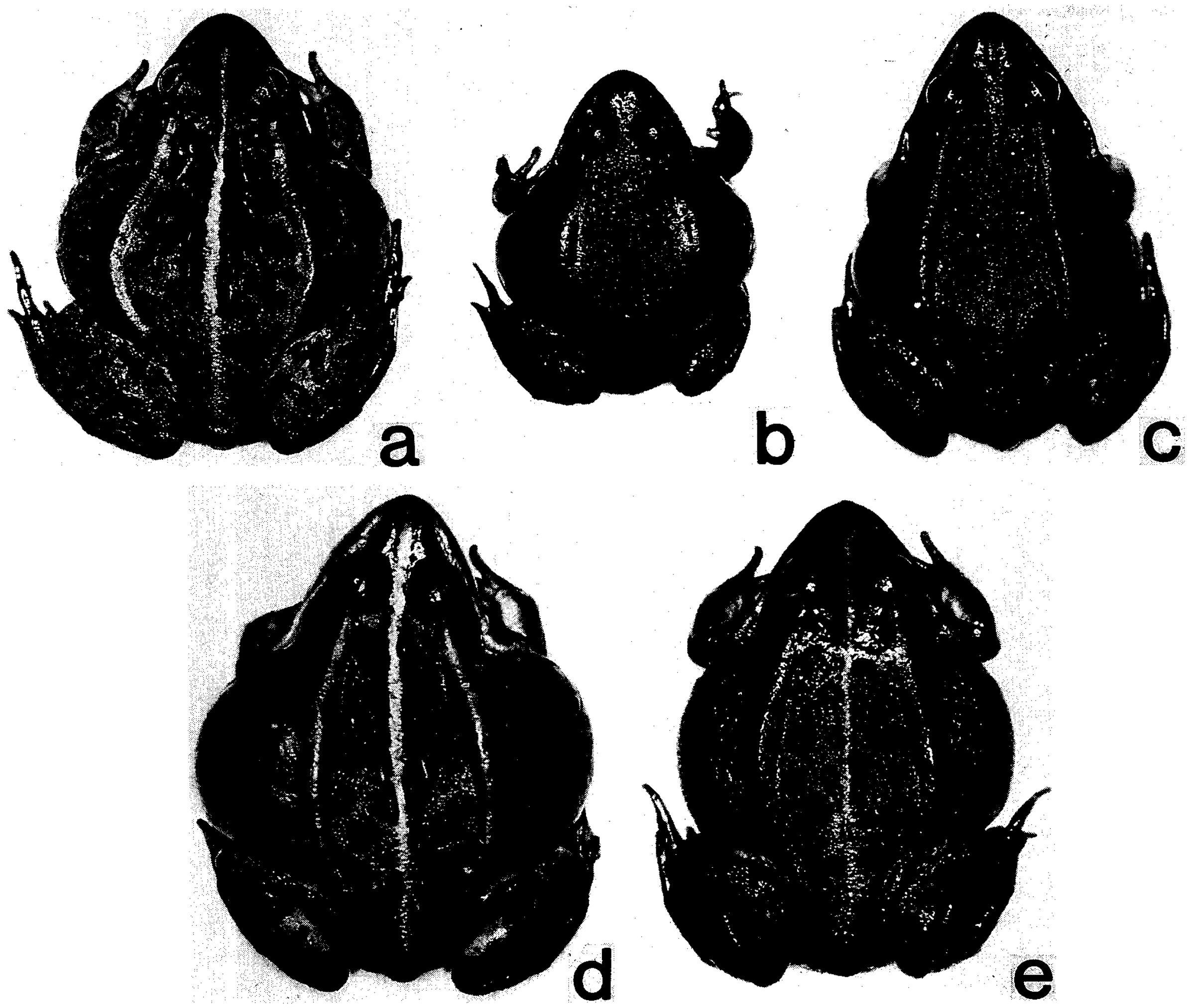

Fig. 8. One-year-old amphidiploid and allotriploids between *Rana nigromaculata* and *R. plancyi chosenica*, and the controls. ×0.8

a. Female diploid *R. nigromaculata*, (N)NN
b. Female diploid *R. plancyi chosenica*, (C)CC
c. Male amphidiploid, (N)NNCC
d. Female allotriploid, (N)NNC
e. Female allotriploid, (N)NCC

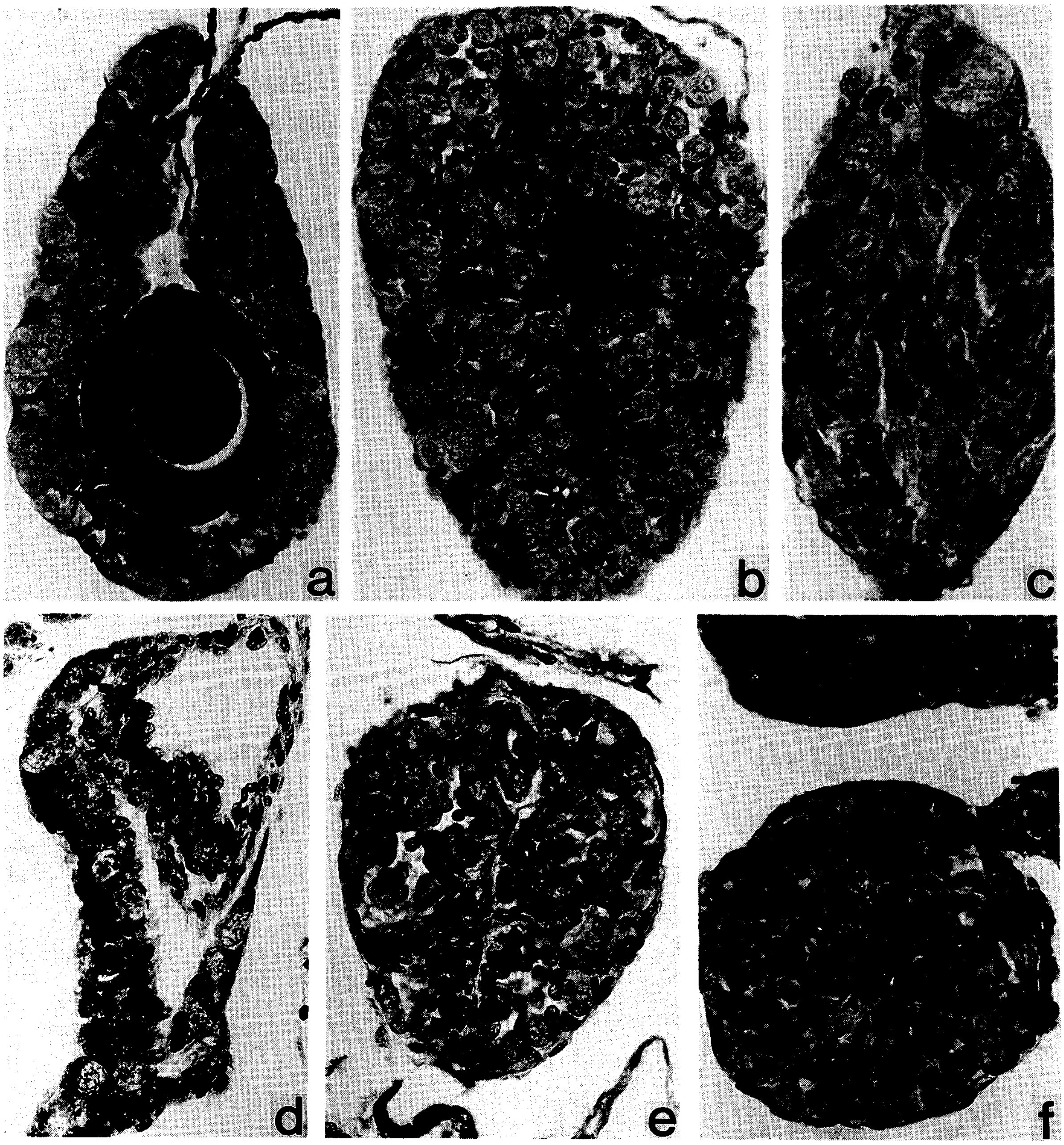

Fig. 9. Cross-sections of the gonads of allopentaploids and allohexaploids which died within six months after metamorphosis. ×175

a. Underdeveloped ovaries (♀U) of a female allopentaploid, (N)NNNCC
b. Normal testis (♂N) of a male allopentaploid, (N)NNCCC
c. Gonad of a hermaphroditic (⚥) allohexaploid, (N)NNNCCC
d. Underdeveloped ovary (♀U) of a female allohexaploid, (N)NNNCCC
e, f. Normal testes (♂N) of male allohexaploids, (N)NNNCCC

metamorphosis, their gonads were sectioned to observe their inner structures (Fig. 9). It was found that four were underdeveloped females (♀N) whose ovaries contained a few auxocytes, five were hermaphrodites (⚥) transforming from females to males and ten were males (♂N). Among 37 allopentaploids, (N)NNCCC, which lived more than one year and attained sexual maturity, there

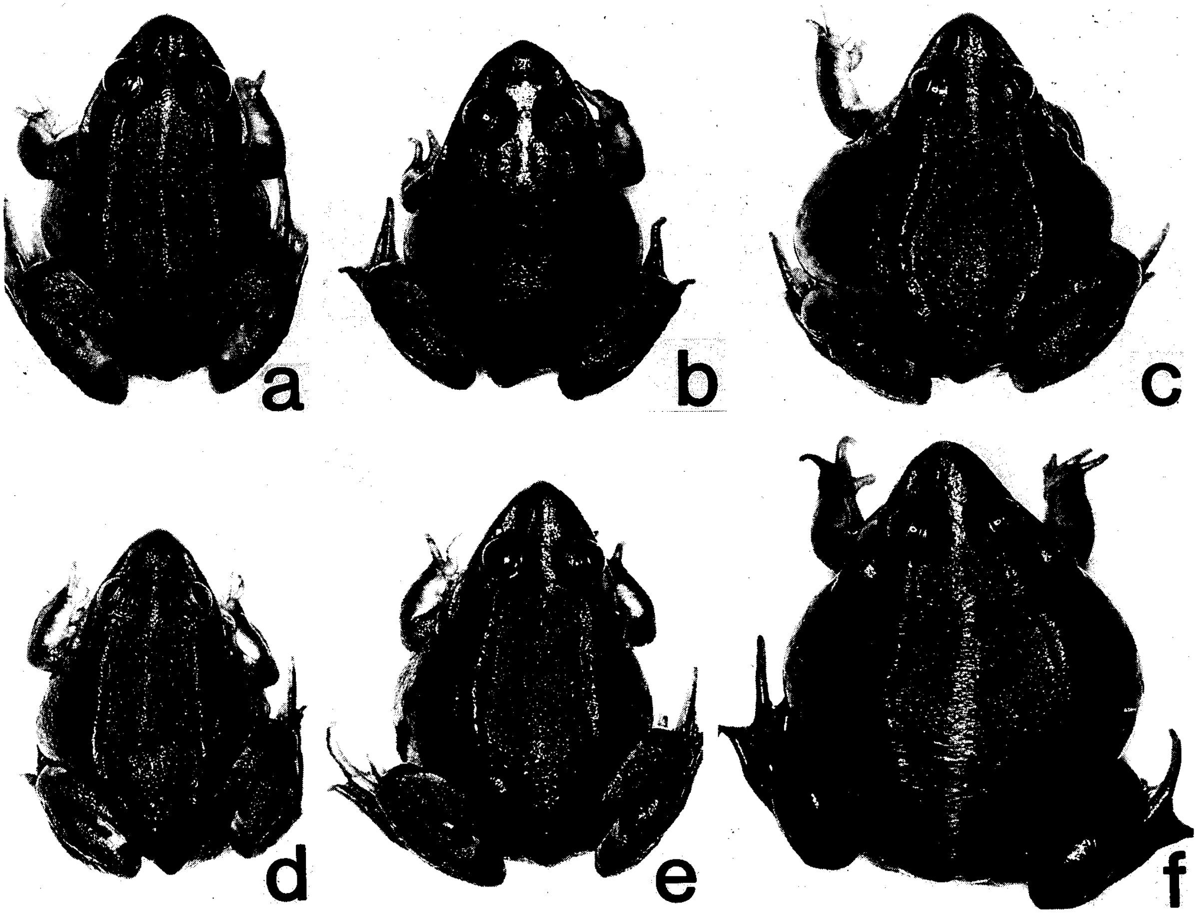

Fig. 10. One-year-old male allopentaploids, (N)NNNCC, between *Rana nigromaculata* and *R. plancyi chosenica.* ×0.8

a, c, d, e. Allopentaploids produced from (N)NNCC 81♀, No. 6×(N)NN 81♂, No. 3 by cold-treatment of the eggs

b, f. Allopentaploids produced from (N)NNCC 81♀, No. 5×(N)NN 81♂, No. 3 by cold-treatment of the eggs

were 11 females and 26 males (Fig. 11). When the hermaphrodites were counted as males, 15 of the 56 pentaploids in total were females and 41 (73.2%) were males (Table 5; Fig. 11).

3. Allohexaploids, (N)NNNCCC

a. Amphidiploids, (N)NNCC, produced in the control series

Of the 409 normally feeding amphidiploid tadpoles produced in 1981~1983 from the 11 control matings between 11 female amphidiploids, (N)NNCC, and six male amphidiploids, (N)NNCC, 393 completed metamorphosis. As 95 of them died or were preserved within six months after metamorphosis, their gonads were sectioned to observe the inner structures (Fig. 7). It was found that 32 were normal females (♀N) having numerous auxocytes, 10 were underdeveloped females (♀U) having a few auxocytes, six were hermaphrodites (⚥) transforming from females to males, and 47 were males (♂N). In the 263 amphidiploids which

TABLE 5

Sex of allotriploids, (N)NCC, and allopentaploids, (N)NNCCC, produced by matings of female amphidiploids, (N)NNCC, with male diploid *Rana plancyi chosenica* after no- or cold-treatment of eggs

Year	Parents		Treatment of eggs	Ploidy	No. of Tadpoles	No. of metamorphosed frogs	Sex of frogs observed within six months after metamorphosis					Sex of frogs, one or two years old			Sex of all frogs examined		
	Female	Male					Total	♀N	♀U	⚥	♂N	Total	♀	♂	Total	♀	♂ (%)*
1981	(N)NNCC 80, No. 1	(C)CC 76, No. 1	No	3n	49	49	29	3	11	3	12	20	9	11	49	23	26(53.1)
				5n	3	2	2	0	0	1	1	—	—	—	2	0	2
1982	(N)NNCC 81, No. 2	(C)CC 80, No. 2	No	3n	24	24	—	—	—	—	—	21	11	10	21	11	10(47.6)
1983	(N)NNCC 82, No. 9	(C)CC 80, No. 3	No	3n	24	21	—	—	—	—	—	19	7	12	19	7	12(63.2)
	(N)NNCC 82, No. 10	(C)CC 80, No. 4	No	3n	17	16	4	0	2	1	1	12	6	6	16	8	8(50.0)
	(N)NNCC 82, No. 11	(C)CC 80, No. 5	No	3n	9	7	3	0	1	1	1	4	1	3	7	2	5(71.4)
	Total		No	3n	123	117	36	3	14	5	14	76	34	42	112	51	61(54.5)
				5n	3	2	2	0	0	1	1	—	—	—	2	0	2
1981	(N)NNCC 80, No. 1	(C)CC 76, No. 1	Cold	5n	11	9	6	0	0	2	4	3	0	3	9	0	9
1982	(N)NNCC 81, No. 2	(C)CC 80, No. 2	Cold	5n	9	8	6	0	2	3	1	2	0	2	8	2	6(75.0)
1983	(N)NNCC 82, No. 9	(C)CC 80, No. 3	Cold	5n	34	34	7	0	2	0	5	24	8	16	31	10	21(67.7)
	(N)NNCC 82, No. 10	(C)CC 80, No. 4	Cold	5n	7	6	—	—	—	—	—	6	3	3	6	3	3(50.0)
	(N)NNCC 82, No. 11	(C)CC 80, No. 5	Cold	5n	2	2	—	—	—	—	—	2	0	2	2	0	2
	Total		Cold	5n	63	59	19	0	4	5	10	37	11	26	56	15	41(73.2)

♀N, Females with normal ovaries ♀U, Females with underdeveloped ovaries ⚥, Hermaphrodites ♂N, Males with normal testes *, Including hermaphrodites

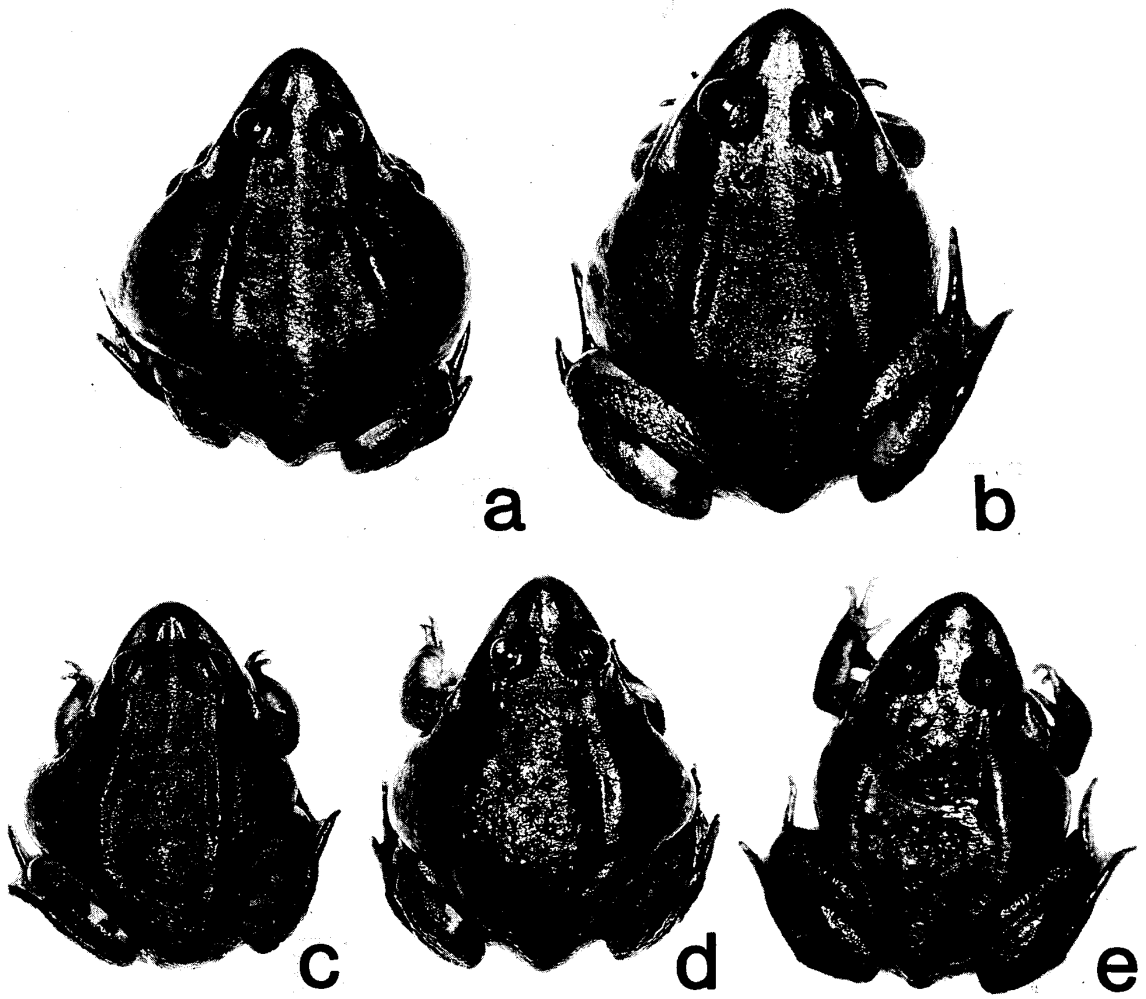

Fig. 11. One-year-old allopentaploids and allohexaploid between *Rana nigromaculata* and *R. plancyi chosenica*.

- a. Female allopentaploid, (N)NNCCC, produced from (N)NNCC 82♀, No. 9×(C)CC 80♂, No. 3 by cold-treatment of the egg ×1
- b. Female allopentaploid, (N)NNCCC, produced from (N)NNCC 82♀, No. 10×(C)CC 80♂, No. 4 by cold-treatment of the egg ×1
- c, d. Male allopentaploids, (N)NNCCC, produced from (N)NNCC 82♀, No. 9×(C)CC 80♂, No. 3 by cold-treatment of the eggs ×1
- e. Male allohexaploid, (N)NNNCCC, produced from (N)NNCC 80♀, No. 1×(N)NNCC 80♂, No. 1 by cold-treatment of the egg ×1.3

lived more than one year and attained sexual maturity, there were 108 females and 155 males (Fig. 8). When the hermaphrodites were counted as males, 150 of the 358 amphidiploids in total were females and 208 (58.1%) were males (Table 6). Although an allohexaploid tadpole normally completed metamorphosis, this frog died at the stage about two months after metamorphosis. It was an underdeveloped female(♀u) (Table 6).

b. Allohexaploids, (N)NNNCCC, produced in the experimental series

Of the 53 feeding allohexaploid tadpoles in the 11 experimental series, 41

TABLE 6

Sex of amphidiploids, (N)NNCC, and allohexaploids, (N)NNNCCC, produced by matings between female and male amphidiploids, (N)NNCC, obtained from *Rana nigromaculata* and *Rana plancyi chosenica* after no- or cold-treatment of eggs

Year	Parents		Treatment of eggs	Ploidy	No. of Tadpoles	No. of metamorphosed frogs	Sex of frogs observed within six months after metamorphosis					Sex of frogs, one or two years old			Sex of all frogs examined		
	Female	Male					Total	♀N	♀U	⚥	♂N	Total	♀	♂	Total	♀	♂(%)*
1981	(N)NNCC 80, No. 1	(N)NNCC 80, No. 1	No	4n	79	74	36	11	3	3	19	38	16	22	74	30	44(59.5)
				6n	1	1	1	0	1	0	0	—	—	—	1	1	0
1982	(N)NNCC 81, No. 2	(N)NNCC 81, No. 2	No	4n	34	32	1	0	1	0	0	30	11	19	31	12	19(61.3)
	(N)NNCC 81, No. 3	(N)NNCC 81, No. 2	No	4n	36	33	4	2	1	0	1	27	8	19	31	11	20(64.5)
1983	(N)NNCC 81, No. 5	(N)NNCC 81, No. 3	No	4n	24	21	4	2	0	0	2	17	7	10	21	9	12(57.1)
	(N)NNCC 81, No. 6	(N)NNCC 81, No. 3	No	4n	37	37	4	1	0	0	3	26	12	14	30	13	17(56.7)
	(N)NNCC 82, No. 9	(N)NNCC 82, No. 4	No	4n	13	13	—	—	—	—	—	9	2	7	9	2	7(77.8)
	(N)NNCC 82, No. 10	(N)NNCC 82, No. 4	No	4n	29	29	5	2	0	0	3	21	7	14	26	9	17(65.4)
	(N)NNCC 82, No. 12	(N)NNCC 82, No. 5	No	4n	25	25	6	2	1	2	1	19	7	12	25	10	15(60.0)
	(N)NNCC 82, No. 13	(N)NNCC 82, No. 5	No	4n	45	45	5	0	1	0	4	31	17	14	36	18	18(50.0)
	(N)NNCC 82, No. 14	(N)NNCC 82, No. 6	No	4n	37	35	7	2	1	1	3	28	11	17	35	14	21(60.0)
	(N)NNCC 82, No. 15	(N)NNCC 82, No. 6	No	4n	50	49	23	10	2	0	11	17	10	7	40	22	18(45.0)
	Total		No	4n	409	393	95	32	10	6	47	263	108	155	358	150	208(58.1)
				6n	1	1	1	0	1	0	0	—	—	—	1	1	0
1981	(N)NNCC 80, No. 1	(N)NNCC 80, No. 1	Cold	6n	14	9	5	0	2	1	2	4	1	3	9	3	6(66.7)
1982	(N)NNCC 81, No. 2	(N)NNCC 81, No. 2	Cold	6n	8	5	2	0	1	1	0	3	1	2	5	2	3(60.0)
	(N)NNCC 81, No. 3	(N)NNCC 81, No. 2	Cold	6n	3	2	1	0	0	0	1	1	1	0	2	1	1(50.0)
1983	(N)NNCC 81, No. 5	(N)NNCC 81, No. 3	Cold	6n	1	1	1	0	1	0	0	—	—	—	1	1	0
	(N)NNCC 81, No. 6	(N)NNCC 81, No. 3	Cold	6n	1	1	1	0	0	0	1	—	—	—	1	0	1
	(N)NNCC 82, No. 9	(N)NNCC 82, No. 4	Cold	6n	7	7	1	0	1	0	0	6	1	5	7	2	5(71.4)
	(N)NNCC 82, No. 10	(N)NNCC 82, No. 4	Cold	6n	1	1	1	0	0	0	1	—	—	—	1	0	1
	(N)NNCC 82, No. 12	(N)NNCC 82, No. 5	Cold	6n	7	7	2	0	1	0	1	5	1	4	7	2	5(71.4)
	(N)NNCC 82, No. 13	(N)NNCC 82, No. 5	Cold	6n	2	1	1	0	0	0	1	—	—	—	1	0	1
	(N)NNCC 82, No. 14	(N)NNCC 82, No. 6	Cold	6n	6	4	2	0	0	0	2	2	0	2	4	0	4
	(N)NNCC 82, No. 15	(N)NNCC 82, No. 6	Cold	6n	3	3	2	0	0	0	2	1	0	1	3	0	3
	Total		Cold	6n	53	41	19	0	6	2	11	22	5	17	41	11	30(73.2)

♀N, Females with normal ovaries ♀U, Females with underdeveloped ovaries ⚥, Hermaphrodites ♂N, Males with normal testes *, Including hermaphrodites

completed metamorphosis. As 19 of them died within six months after metamorphosis, their gonads were sectioned to observe the inner structures (Fig. 9). It was found that there were no normal females with well-developed ovaries. Six allohexaploids were underdeveloped females (♀U) whose ovaries scarcely contained auxocytes. Two others were hermaphrodites (⚥) transforming from females to males. The remaining 11 were males (♂N). Among 22 allohexaploids which lived more than one year and attained sexual maturity, there were five females and 17 males (Fig. 11). When the hermaphrodites were counted as males, 11 of the 41 allohexaploids in total were females and the other 30 (73.2%) were males (Table 6).

V. Reproductive capacity of male allopentaploids

Of the allopentaploids produced in 1981～1983, 45 of (N)NNNCC and 26 of (N)NNCCC, 71 in total, were clearly identified as males by the secondary sexual characters at the age of one or two years (Tables 4 and 5). As six of (N)NNNCC and two of (N)NNCCC of these male allopentaploids produced in 1981 and 1982 had comparatively large testes, sperm suspension was made from these testes to inseminate the eggs of female diploid *Rana nigromaculata* (N)NN. However, no normally cleaved eggs were obtained.

The external characters of the testes in the remaining male allopentaploids were observed after removing them from the bodies. In many cases, the right and left testes differed from each other in size and the smaller ones were often rudimentary. The testes of 14 male allopentaploids including 12 of (N)NNNCC and two of (N)NNCCC were almost degenerated, even if they lived more than one year. These 14 allopentaploids were less than 3 cm in body length.

1. Inner structure of testes

a. Allotriploids

When the testes of several male allotriploids, (N)NNC or (N)NCC, obtained by matings between eight female amphidiploids, (N)NNCC♀, Nos. 1～8, and male diploid *Rana nigromaculata*, (N)NN, or *Rana plancyi chosenica*, (C)CC, were crushed, the sperm suspension contained no normal spermatozoa, while abnormal spermatozoa of various sizes and pycnotic nuclei were abundantly or sparsely found. When the testes of five males of (N)NNC and five males of (N)NCC were sectioned to observe the inner structures, abnormal spermatozoa and pycnotic nuclei were sparsely distributed in the seminiferous tubules. Along the inner walls of the seminiferous tubules, there were numerous first spermatocytes and spermatogonia as previously reported by Nishioka (1983) and Nishioka and Okumoto (1983).

b. Allopentaploids

When sperm suspension was prepared by crushing the testes of several pentaploids, (N)NNNCC or (N)NNCCC, there were no normal spermatozoa, while

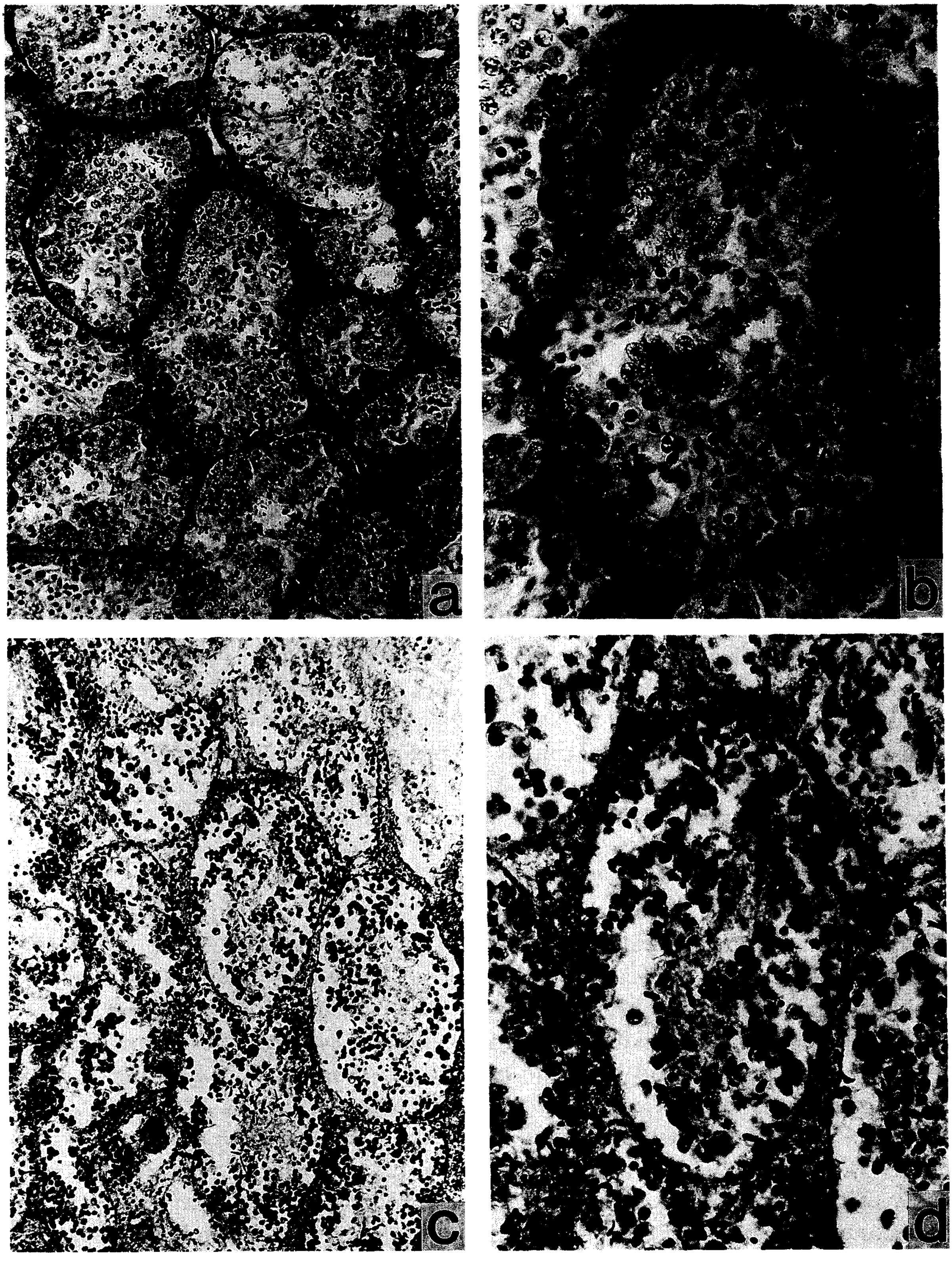

Fig. 12. Cross-sections of the testes of male allopentaploids between *Rana nigromaculata* and *R. plancyi chosenica*.

a.	Male allopentaploid, (N)NNNCC	×115
b.	Ditto	×230
c.	Male allopentaploid, (N)NNCCC	×115
d.	Ditto	×230

abnormal spermatozoa and pycnotic nuclei were abundantly or sparsely found. It was assumed that these abnormal spermatozoa had no fertilizing ability. The inner structures of the testes of 11 mature male allopentaploids including six (N)NNNCC and five (N)NNCCC were examined by making sections. It was found that all the male allopentaploids had no normal spermatozoa. In the seminiferous tubules, there were some abnormal spermatozoa which were irregular in size and shape, and pycnotic nuclei were sparsely or abundantly distributed. Along the walls of the seminiferous tubules, the first spermatocytes and spermatogonia were most abundantly found, while the second spermatocytes were very scarce (Fig. 12).

2. Meioses

At the first meiotic division of the two kinds of male allopentaploids, (N)NNNCC and (N)NNCCC, univalent, bivalent, trivalent, tetravalent and pentavalent chromosomes were formed (Figs. 14~16). These kinds of chromosomes greatly differed in frequency from one another. In all the meiotic divisions, bivalents were most abundantly found. Next to the bivalents, univalents and trivalents were fairly numerous. These three kinds of chromosomes were observed in all the first meiotic figures. In contrast to them, tetravalents and pentavalents were very scarce. Some of the first meiotic figures contained these two kinds of chromosomes, but the others did not.

a. Allopentaploids, (N)NNNCC

In 50 first meiotic figures of allopentaploids, (N)NNNCC, 1617 chromosomes were examined in detail. Of these chromosomes, 855 (52.9%) were bivalents which were most numerous, 398 (24.6%) were univalents and 335 (20.7%) were trivalents. The pentavalents and tetravalents were 21 (1.3%) and 8 (0.5%) in number, respectively (Table 7).

Of the 50 first meiotic figures, 27 contained only univalents, bivalents and trivalents. In addition to them, one pentavalent, two pentavalents and one tetravalent were found in 13, two and three meiotic figures, respectively. In each of the remaining five meiotic figures, a tetravalent and a pentavalent were contained in addition to the univalents, bivalents and trivalents (Table 7).

b. Allopentaploids, (N)NNCCC

In 50 first meiotic figures of allopentaploids, (N)NNCCC, 1590 chromosomes were examined in detail. Of these chromosomes, 823 (51.8%) were bivalents which were most abundant, 383 (24.1%) were univalents and 341 (21.4%) were trivalents. The pentavalents and tetravalents were 26 (1.6%) and 17 (1.1%) in number, respectively (Table 7).

Of the 50 first meiotic figures, 21 contained univalents, bivalents and trivalents. In addition to these three kinds of chromosomes, one pentavalent, two pentavalents, one tetravalent, one tetravalent and one pentavalent, two tetravalents and one pentavalent, and one tetravalent and three pentavalents were contained in 11,

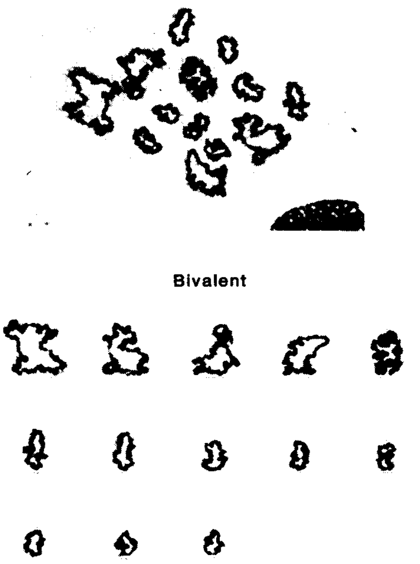

Fig. 13. Spread of a spermatoyte at the first meiosis and the chromosome complement containing 13 bivalents in a male diploid *Rana nigromaculata*. ×650

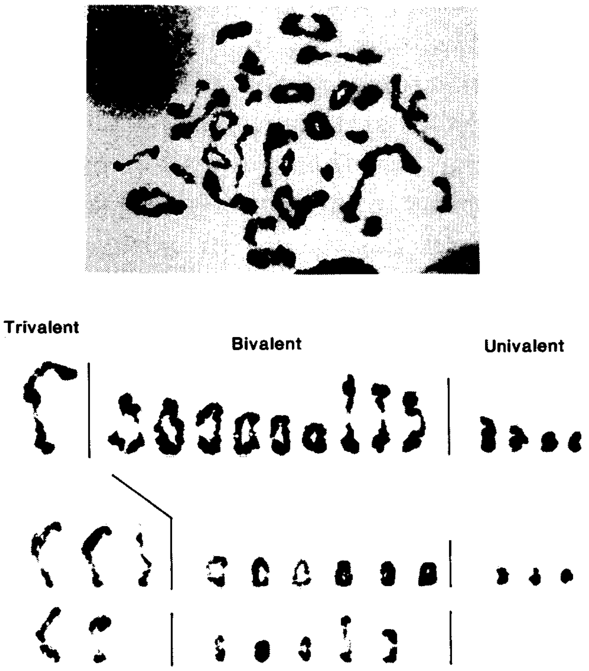

Fig. 14. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 6 trivalents, 20 bivalents and 7 univalents in a male allopentaploid, (N)NNNCC. ×650

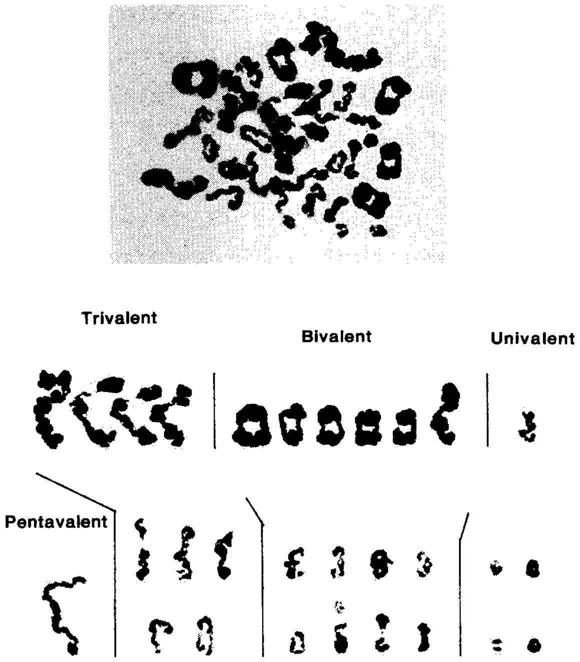

Fig. 15. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 1 pentavalent, 9 trivalents, 14 bivalents and 5 univalents in a male allopentaploid, (N)NNNCC. ×650

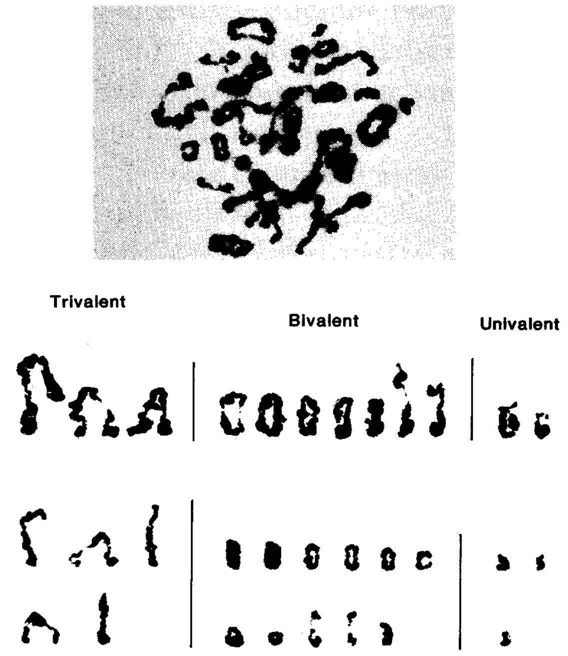

Fig. 16. Spread of a spermatocyte at the first meiosis and the chromosome compement containing 8 trivalents, 18 bivalents and 5 univalents in a male allopentaploid, (N)NNCCC. ×650

TABLE 7

Number of meiotic chromosomes in the testes of two kinds of male allopentaploids, (N)NNNCC and (N)NNCCC, obtained from *Rana nigromaculata* and *Rana plancyi chosenica*

Kinds	No. of meioses	Number of chromosomes					
		Total	Pentavalent	Tetravalent	Trivalent	Bivalent	Univalent
(N)NNNCC	50	1617	21 (1.3%)	8 (0.5%)	335 (20.7%)	855 (52.9%)	398 (24.6%)
(N)NNCCC	50	1590	26 (1.6%)	17 (1.1%)	341 (21.4%)	823 (51.8%)	383 (24.1%)
Total	100	3207	47 (1.5%)	25 (0.8%)	676 (21.1%)	1678 (52.3%)	781 (24.4%)

two, seven, seven, one and one meiotic figures, respectively (Table 7).

VI. Reproductive capacity of female allopentaploids

1. Production of offspring

Seven female allopentaploids, (N)NNNCC, produced by (N)NNCC♀ × (N)NN♂ lived more than one year and attained sexual maturity (Table 4). Five of them were those obtained from three female amphidiploids, (N)NNCC 81♀, Nos. 2~4, and had poorly-developed ovaries. The other two were those obtained from two female amphidiploids, (N)NNCC 81♀, No. 6 and (N)NNCC 82♀, No. 8, and had ovaries which contained many mature eggs. On the other hand, 11 female allopentaploids, (N)NNCCC, produced from (N)NNCC♀ × (C)CC♂ lived more than one year and attained sexual maturity (Table 5). Two of them were those obtained from two female amphidiploids, (N)NNCC 82♀, Nos. 9 and 10, and had poorly-developed ovaries. The other nine were those obtained also from the same two female amphidiploids, and had ovaries which contained many mature eggs.

Of the 11 female allopentaploids which contained many mature eggs in their ovaries, the two allopentaploids, (N)NNNCC, were 58.0 mm and 62.0 mm in body length, while the nine allopentaploids, (N)NNCCC, were 50.0~58.0 mm in body length. These two kinds of female allopentaploids were injected with bullfrog pituitaries to accelerate their ovulation. It was found that only two of the 11 female allopentaploids laid 191 and 180 eggs of various sizes (Fig. 17a). The two female allopentaploids were those produced from a female amphidiploid, (N)NNCC 82♀, No. 10, mated with a male *Rana plancyi chosenica,* (C)CC 80♂, No. 4, by refrigeration of the eggs after insemination. Thus, their chromosomes were constituted of two genomes of *Rana nigromaculata* and three genomes of *Rana plancyi chosenica* (Fig. 5). These two female allopentaploids, (N)NNCCC 83♀, No. 1 and No. 2 were 52.0 mm and 57.5 mm in body length, respectively.

When their eggs were inseminated with sperm of a male diploid *Rana nigromaculata,* (N)NN 82♂, No. 1, 122 (63.9%) of the 191 eggs and 55 (30.6%) of the 180 eggs, 177 (47.7%) of the 371 eggs in total, cleaved normally. Thereafter, two and

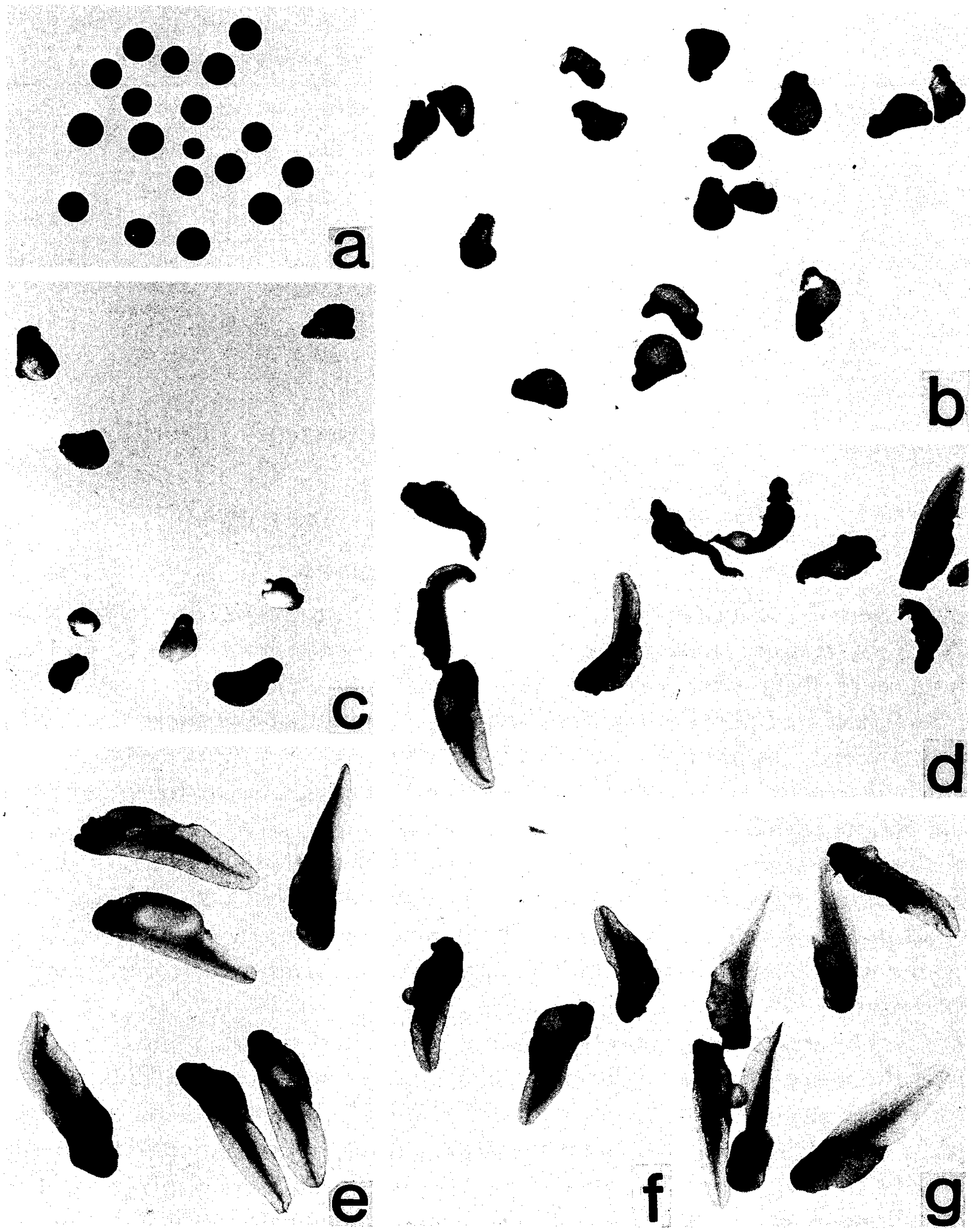

Fig. 17. Abnormal embryos and tadpoles found in the offspring of two female allopentaploids, (N)NNCCC, Nos. 1 and 2, and their eggs. ×2.3

a. Eggs of a female allopentaploid, (N)NNCCC, No. 1
b. Abnormal 2-day-old embryos produced from (N)NNCCC 83♀, No. 1×(N)NN 82♂, No. 1
c. Abnormal 2-day-old embryos produced from (N)NNCCC 83♀, No. 2×(N)NN 82♂, No. 1
d. Abnormal 4-day-old tadpoles produced from (N)NNCCC 83♀, No. 1×(N)NN 82♂, No. 1
e. Abnormal 6-day-old tadpoles produced from (N)NNCCC 83♀, No. 2×(N)NN 82♂, No. 1
f. Abnormal 4-day-old tadpoles produced from (N)NNCCC 83♀, No. 1×(N)NN 82♂, No. 1
g. Abnormal 6-day-old tadpoles produced from (N)NNCCC 83♀, No. 2×(N)NN 82♂, No. 1

five of the normally cleaved eggs produced from the two female allopentaploids, Nos. 1 and 2, stopped their development during the blastula stage, while 103 (53.9%) and 48 (26.7%) eggs, 151 (40.7%) eggs in total, became normal gastrulae. The other eggs became abnormal gastrulae, having protrusions or wrinkles on the animal surfaces, and died. At the neurula stage, 10 and nine embryos, 19 in total, became abnormal and died, while 93 (48.7%) and 39 (21.7%), 132 (35.6%) in total, became normal neurulae. At the tail-bud stage, 21 and two became edematous or dwarfish, while 72 (37.7%) and 37 (20.6%), 109 (29.4%) in total, became normal embryos. At the hatching stage, 13 and 3, 16 in total, became abnormal embryos which were edematous, underdeveloped or had curved bodies, while 59 (30.9%) and 34 (18.9%), 93 (25.1%) in total, became normally hatched tadpoles. Thereafter, 24 and 14 tadpoles, 38 in total, became edematous and underdeveloped or showed various abnormalities and could not take food, while 35 (18.3%) and 20 (11.1%), 55 (14.8%) in total, became normally feeding tadpoles. Eventually, 12 (6.3%) and nine (5.0%), 21 (5.7%) in total, attained the stage of metamorphosis (Table 8).

TABLE 8

Reproductive capacity of female allopentaploids consisting of two *Rana nigromaculata* and three *Rana plancyi chosenica* genomes

Parents		No. of eggs	No. of cleavages		No. of gastrulae		No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of feeding tadpoles		No. of metamorphosed frogs
Female	Male		Nor.	Ab.	Nor.	Ab.	Nor.	Ab.	Nor.	Ab.	Nor.	Ab.	Nor.	Ab.	
(N)NNCCC 83, No. 1	(N)NN 82, No. 1	191	122 (63.9%)	24	103 (53.9%)	17	93 (48.7%)	10	72 (37.7%)	21	59 (30.9%)	13	35 (18.3%)	24	12 (6.3%)
(N)NNCCC 83. No. 2	(N)NN 82, No. 1	180	55 (30.6%)	88	48 (26.7%)	2	39 (21.7%)	9	37 (20.6%)	2	34 (18.9%)	3	20 (11.1%)	14	9 (5.0%)
Total		371	177 (47.7%)	112	151 (40.7%)	19	132 (35.6%)	19	109 (29.4%)	23	93 (25.1%)	16	55 (14.8%)	38	21 (5.7%)

2. Chromosome numbers of offspring

The chromosomes of the offspring of the two female allopentaploids, (N)NNCCC 83♀, Nos. 1 and 2, mated with a male diploid *Rana nigromaculata*, (N)NN 82♂, No. 1, were examined in abnormal embryos and tadpoles as well as in normally feeding tadpoles.

a. Offspring produced from (N)NNCCC 83♀, No. 1×(N)NN 82♂, No. 1

i) Abnormal embryos and tadpoles (Fig. 17b, d, f)

The chromosomes of abnormal embryos and tadpoles, including 10 neurulae, 21 tail-bud embryos, 13 hatched tadpoles and 24 feeding tadpoles, 68 individuals in total, were examined and clarified in 59 of them. It was found that one was a haploid having 13 chromosomes, one was a hypodiploid (2n−2) having 24 chromosomes, four were hypotriploids (3n−α), including one having 34 chromosomes and three having 38 chromosomes. Two were triploids having 39 chromosomes, and 19 were hypertriploids ($3n+\alpha$), including one having 40 chromosomes, four having 41 chromosomes, three having 42 chromosomes, three having 43 chromosomes, seven having 44 chromosomes and one having 45 chromosomes. Ten were hypotetraploids ($4n-\alpha$), including three having 46 chromosomes, two having 47 chromosomes, one having 48 chromosomes, one having 49 chromosomes, one having 50 chromosomes and two having 51 chromosomes. Four were tetraploids ($4n=52$), one of which contained a ring chromosome, three were hypertetraploids ($4n+\alpha$), having 56, 57 and 58 chromosomes, respectively, and one was a hypopentaploid having 60 chromosomes. Two were pentaploids, one was a hexaploid ($6n=78$), seven were mosaics of n—3n, 2n—3n, 3n—4n, 3n—6n, 5n—6n, 26–41 chromosomes and 34–47 chromosomes, respectively, two were n—5n mosaics, and two were 30–43 chromosome mosaics (Table 9).

TABLE 9

Chromosome numbers of the offspring of female allopentaploids, (N)NNCCC, mated with male diploid *Rana nigromaculata*

Parents		Offspring	Number of embryos and tadpoles													
Female	Male		Total	Number of chromosomes												Mosaics
				13 (n)	20~25 (2n−)	33~38 (3n−)	39 (3n)	40~45 (3n+)	46~51 (4n−)	52 (4n)	53~58 (4n+)	59~64 (5n−)	65 (5n)	72~77 (6n−)	78 (6n)	
(N)NNCCC 83, No. 1	(N)NN 82, No. 1	Abnormal	59	1	1	4	2	19	10	4	3	1	2	0	1	11
		Normal	30	0	0	0	2	16	10	1	0	0	0	0	1	0
(N)NNCCC 83, No. 2	(N)NN 82, No. 1	Abnormal	21	0	0	3	0	7	4	0	0	0	2	1	0	4
		Normal	18	0	0	0	0	10	5	0	0	1	0	0	2	0
(N)NNCCC	(N)NN	Abnormal	80	1	1	7	2	26	14	4	3	1	4	1	1	15
		Normal	48	0	0	0	2	26	15	1	0	1	0	0	3	0
Total embryos and tadpoles			128	1	1	7	4	52	29	5	3	2	4	1	4	15

ii) Normally feeding tadpoles

Chromosomes were examined in 35 normally feeding tadpoles produced from the female allopentaploid, (N)NNCCC♀, No. 1. In each tadpole, five to 10 or more mitotic figures were observed in the tail-tips regenerated one to three times. The chromosomes were clarified in 30 tadpoles. It was found that two were triploids ($3n=39$) and 16 were hypertriploids ($3n+\alpha$) including one having 40 chromosomes, three having 41 chromosomes, four having 42 chromosomes, three having 43 chromosomes, three having 44 chromosomes and two having 45 chromosomes. Ten were hypotetraploids ($4n-\alpha$), including four having 46 chromosomes, two having 47 chromosomes, three having 48 chromosomes and one having 51 chromosomes. One was a tetraploid ($4n=52$), and the remainder was a hexaploid ($6n=78$) (Table 9).

Of these 30 tadpoles whose chromosome numbers were clarified, 12 attained metamorphosis, while the other 18 died without attaining metamorphosis. Of the former 12 tadpoles, eight extruded both forelegs, three extruded one foreleg, and the remainder did not extrude the forelegs. Of the eight tadpoles which extruded both forelegs, two died at the stage of small vestiges of the tails. They were 42 in chromosome number. Another tadpole completed metamorphosis and died after 24 days without taking food. This tadpole had 43 chromosomes. The other five tadpoles died before disappearance of their tails, although shrinkage of the tails had begun. Each of these five tadpoles had 45, 46, 47, 51 or 52 chromosomes.

Two of the three tadpoles which extruded one foreleg died when their tails almost completely disappeared. These two tadpoles were 42 in chromosome number. The remainder had 44 chromosomes. The only tadpole which did not extrude the forelegs had 43 chromosomes.

The remaining 18 tadpoles which had normal hind legs and died without attaining metamorphosis were 40~78 in chromosome number.

b. (N)NNCCC 83♀, No. 2×(N)NN 82♂, No. 1

i) Abnormal embryos and tadpoles (Fig. 17c, e, g)

The chromosomes of 28 abnormal individuals including nine neurulae, two tailbud embryos, three hatched tadpoles and 14 feeding tadpoles were examined and clarified in 21 of them. It was found that three were hypotriploids ($3n-\alpha$) having 33, 34 or 36 chromosomes, and seven were hypertriploids, including three with 41 chromosomes and four having 42, 43, 44 and 45 chromosomes, respectively. Four were hypotetraploids, two of which had 46 chromosomes and each of the other two had 48 or 49 chromosomes. Two were pentaploids ($5n=65$) and one was a hypohexaploid having 75 chromosomes. There were four mosaics, including a 3n—4n mosaic, a 4n—5n mosaic and two 35–48 chromosome mosaics (Table 9).

ii) Normally feeding tadpoles

Chromosomes were examined in 20 normally feeding tadpoles. In each tadpole, five to 10 or more mitotic figures were observed in the tail-tips regenerated one to three times. The chromosome numbers were clarified in 18 of the 20 tadpoles. Ten tadpoles were hypertriploids ($3n+\alpha$) including one having 40 chromosomes, one having 41 chromosomes, two having 42 chromosomes, three having 43 chromosomes, two having 44 chromosomes and one having 45 chromosomes. Five tadpoles were hypotetraploids ($4n-\alpha$), including three having 46, 47 and 49 chromosomes, respectively, and two having 51 chromosomes. One was a hypopentaploid having 64 chromosomes and two were hexaploids having 78 chromosomes (Table 9).

Nine of the 18 tadpoles attained the stage of metamorphosis. All these tadpoles had normal hind legs. Seven of them extruded both forelegs and climbed out of water. They had 42, 44, 46, 47, 51, 64 and 78 chromosomes, respectively. One of them having 44 chromosomes died with a small vestige of the tail, while the other six died before disappearance of their tails, although the tails had somewhat shrunk in some individuals. Another tadpole extruded one foreleg, while the other in which the tail began to shrink did not extrude the forelegs. These two tadpoles had 44 and 43 chromosomes, respectively. The remaining nine tadpoles died without attaining metamorphosis. They were 40~78 in chromosome number.

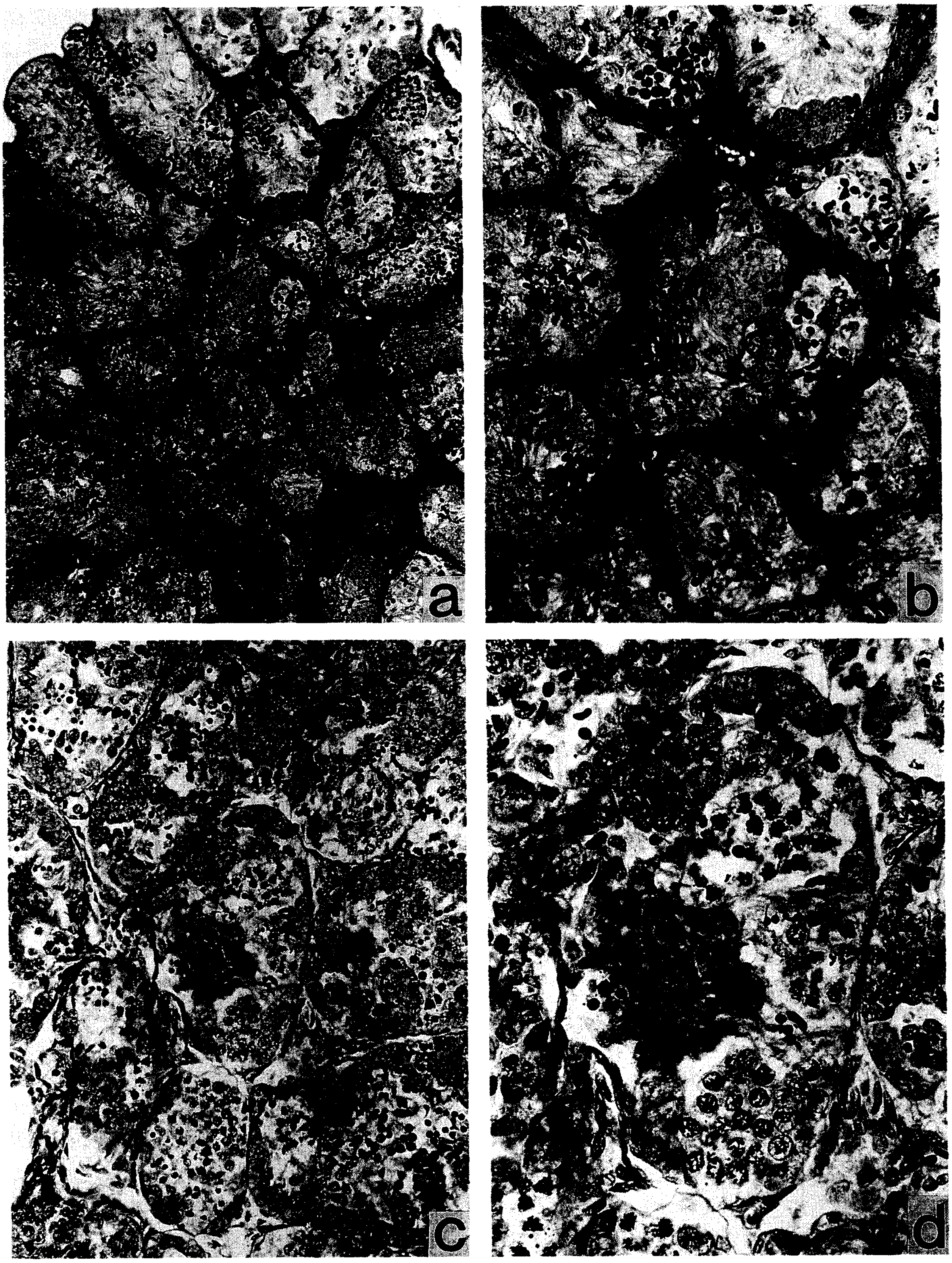

Fig. 18. Cross-sections of the testes of mature male allohexaploids, (N)NNNCCC, between *Rana nigromaculata* and *R. plancyi chosenica*.

a. Male allohexaploid produced from (N)NNCC 82♀, No. 9×(N)NNCC 82♂, No. 4 by cold-treatment of the egg ×115

b. Ditto ×230

c. Male allohexaploid produced from (N)NNCC 82♀, No. 12×(N)NNCC 82♂, No. 5 by cold-treatment of the egg ×115

d. Ditto ×230

VII. Reproductive capacity of allohexaploids

As described above, there were five females and 17 males among 22 allohexaploids which attained sexual maturity. When one testis was removed from each of three males and crushed to examine the presence of normal spermatozoa, it was found that there were only a few abnormal spermatozoa of various sizes among huge or smaller pycnotic nuclei. It was determined that these abnormal spermatozoa had no fertilizing ability. When the inner structures of the testes of five other males were examined by making sections, there were no normal spermatozoa, and they were very similar to those of male allopentaploids (Figs. 18, 19). The testes of the remaining nine male allohexaploids could not be examined, owing to postmortem changes, as these males died in summer.

One of the five female allohexaploids, (N)NNNCCC, had several large eggs in the ovaries (Fig. 19), while these eggs did not develop by insemination. The other four females had degenerated ovaries, in which no mature ova were found. It was probable that both males and females of this kind of allohexaploids were quite sterile.

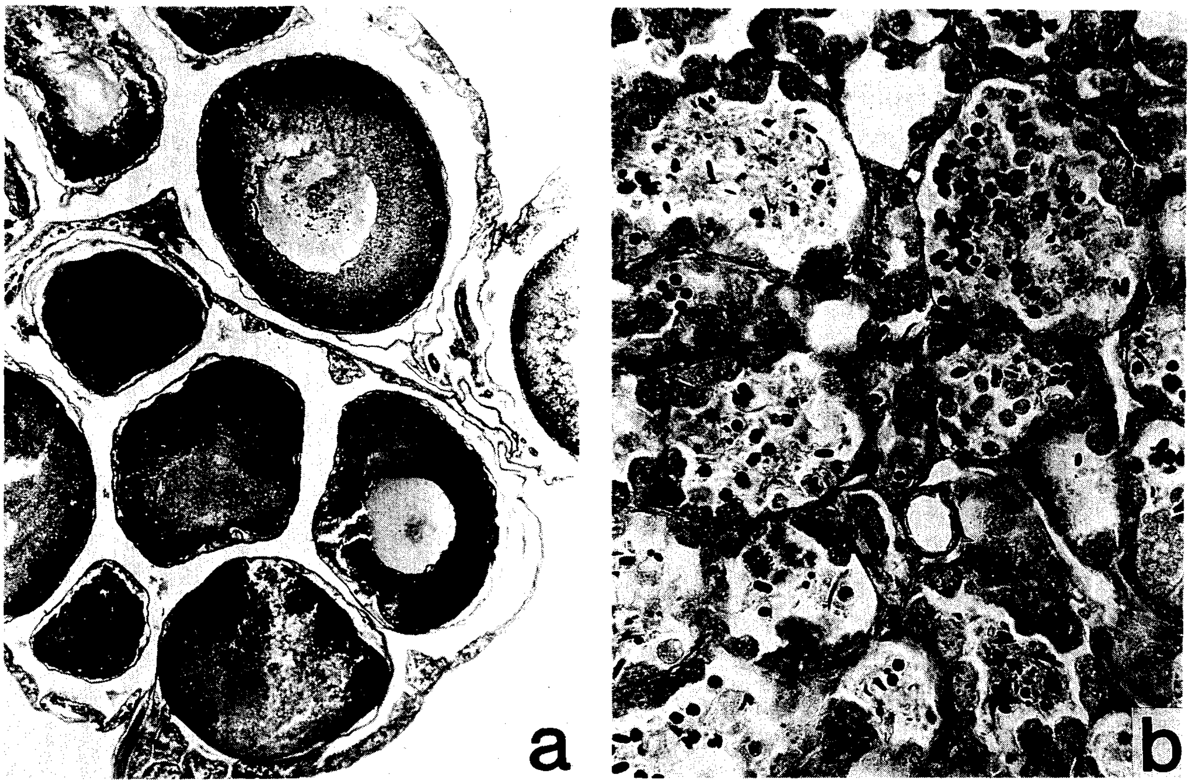

Fig. 19. Cross-sections of the gonads of mature allohexaploids, (N)NNNCCC, between *Rana nigromaculata* and *R. plancyi chosenica*.

a. Female allohexaploid produced from (N)NNCC 82♀, No:9×(N)NNCC 82♂, No. 4 by cold-treatment of the egg ×45

b. Male allohexaploid produced from (N)NNCC 82♀, No. 12×(N)NNCC 82♂, No. 5 by cold-treatment of the egg ×230

DISCUSSION

In amphibians, it seems to be very interesting to produce higher degrees than tetraploidy in artificial polyploids where they can almost normally live and reproduce, as there are frogs or toads which are considered to be natural hexaploid and octaploid species in Africa and South America. KOBEL, PASQUIER, FISCHBERG and GLOOR (1980) reported that the *Xenopus ruwenzoriensis* distributed in West Uganda had 108 chromosomes. This number corresponds to hexaploidy as compared with those of seven species of *Xenopus* observed by TYMOWSKA and FISCHBERG (1973) and those of two species of *Xenopus* observed by TYMOWSKA (1973) and FISCHBERG and KOBEL (1978). These nine species of *Xenopus* had 36 chromosomes in diploid number. Three other species of *Xenopus* were tetraploids as they had 72 chromosomes. BEÇAK, BEÇAK and RABELLOW (1967) reported that *Ceratophrys dorsata* collected from Brazil had 104 chromosomes and was an octaploid species as compared with *C. calcarata* from Colombia which was 26 in diploid chromosome number (BEÇAK, 1969). *Ceratophrys ornata* collected from Argentina was octaploid species, having 104 chromosomes, while there was the same species having 26 chromosomes in Argentina (BARRIO and DE CHIERI, 1970). SAEZ and BRUM (1960) reported that there was *C. ornata* having 88, 92, 98 or 108 chromosomes in South America. There were no natural pentaploid species anywhere.

On the basis of the actual existence of natural hexaploids and octaploids, fertile hexaploids and octaploids may be produced by artificial means some day. Up to the present, there have been many reports on artificial production of polyploids which were of higher degrees than tetraploidy in amphibians. FANKHAUSER (1945) reported that there were 10 spontaneous pentaploid larvae including seven *Notophthalmus viridescens*, one *Cynops pyrrhogaster* and two axolotls. Of a total of eight pentaploid larvae of *Notophthalmus viridescens* including an experimentally produced one, three were raised to metamorphosis. However, they were greatly reduced in growth and remained longer in the larval stage than the controls. All the pentaploid *Notophthalmus viridescens* and *Cynops pyrrhogaster* showed S-shaped curvature of the body axis. In axolotls, FANKHAUSER and HUMPHREY (1950) discovered three pentaploids and two hexaploids among the larvae produced from matings between triploid females and diploid males. HUMPHREY and FANKHAUSER (1956) and FANKHAUSER and HUMPHREY (1959) reported that 0.14% and 0.17%, respectively, of the larvae produced from diploid parents were pentaploids. A single hexaploid was found among the abundant larvae. All the pentaploids were females which were considered to be WWZZZ in sex chromosome constitution. HUMPHREY and FANKHAUSER (1956) stated that the pentaploids were retarded in growth and usually died before attaining the age of two years. The ovaries contained very few or no mature ova. SANADA (1952) obtained a single larva of pentaploid *Cynops pyrrhogaster* by cold-treatment of a fertilized egg. This was somewhat larger and began metamorphosis a week later as compared with the diploid controls.

SUBTELNY (1966) discovered that 12% of the tadpoles obtained from a female

Rana pipiens were pentaploids. Bogart (1972) found that there were many cross combinations of *Bufo* species which produced triploids or pentaploids, or diploids and pentaploids. Nishioka and Okumoto (1983) obtained six allohexaploid tadpoles from matings between a female and two male amphidiploids, (N)NNCC, produced from *Rana nigromaculata,* (N)NN, and *R. plancyi chosenica,* (C)CC, while eight allopentaploid tadpoles were produced from a mating between the above female amphidiploid and a diploid male *R. nigromaculata* or *R. plancyi chosenica.* These hexaploids and pentaploids were metamorphosed normally and became males. All of them lived more than one year and revealed thumb-pads. Nishioka and Ohtani (1983) obtained two kinds of amphitriploids, (B)BBNNCC and (B)BBNNFF, from large eggs of allotriploids, (B)BNC, among *R. brevipoda, R. nigromaculata* and *R. plancyi chosenica* and another kind of allotriploids, (B)BNF, among *R. brevipoda, R. nigromaculata* and *R. plancyi fukienensis,* by the method of diploid gynogenesis. Nishioka, Kondo and Ohtani (1983) also obtained many amphitriploids, (B)BBFFNN, by refrigeration of the eggs of female amphidiploids, (B)BBFF, between *R. brevipoda* and *R. plancyi fukienensis* after fertilization with sperm of male autotetraploids, (N)NNNN, of *R. nigromaculata.* Nishioka and Ueda (1983) reported in *Hyla japonica* that 7.1% of the tadpoles produced from matings between female and male tetraploids were hexaploids and that 17.0% of those produced from matings between the above female tetraploids and male diploids were pentaploids. While only three of the hexaploid tadpoles were metamorphosed and died without taking food, 40 pentaploid tadpoles were metamorphosed and 10 of them attained sexual maturity. The pentaploid tree-frogs resembled diploids closely in external characters, and had somewhat normal testes, whose seminiferous tubules were roughly filled with coarse bundles of normally or abnormally shaped spermatozoa surrounded with many pycnotic nuclei.

In the present study, allopentaploids and allohexaploids between *Rana nigromaculata,* (N)NN, and *R. plancyi chosenica,* (C)CC, were produced. The allopentaploids, (N)NNNCC or (N)NNCCC, were obtained from eggs of female amphidiploids, (N)NNCC, by refrigeration of the eggs to suppress the extrusion of the second polar body after insemination with sperm of male *R. nigromaculata* or *R. plancyi chosenica.* The allohexaploids, (N)NNNCCC, were produced from eggs of female amphidiploids, (N)NNCC, by refrigeration of the eggs after insemination with sperm of male amphidiploids, (N)NNCC. Among 52 allopentaploids, (N)NNNCC, which lived more than one year and attained sexual maturity, there were seven females and 45 males. On the other hand, there were 11 females and 26 males among 37 allopentaploids, (N)NNCCC, which lived more than one year and attained sexual maturity. Among 22 allohexaploids which lived more than one year and attained sexual maturity, there were five females and 17 males.

A total of 71 allopentaploids including 45 (N)NNNCC and 26 (N)NNCCC was identified as males by the secondary sexual characters at the age of one or two years. While the eggs of female diploid *Rana nigromaculata* were inseminated with sperm suspension of eight allopentaploids including six (N)NNNCC and two (N)NNCCC, no normally cleaved eggs were obtained. The testes of 11 male allopentaploids including six (N)NNNCC and five (N)NNCCC had no normal spermatozoa, when they were examined by sectioning. A total of 18 female allopentaploids including seven (N)NNNCC and 11 (N)NNCCC lived more than one year and attained sexual maturity. Two of the former and nine of the latter pentaploids had ovaries which contained many mature eggs. When these 11 pentaploids were injected with bullfrog pituitaries, only two, (N)NNCCC 83♀, Nos. 1 and 2, laid 191 and 180 eggs of various sizes, respectively. When these eggs were inseminated with sperm of a male diploid *Rana nigromaculata*, 122 (63.9%) and 55 (30.6%) cleaved normally, respectively. However, most of them became abnormal and died at the embryonic and tadpole stages. Eventually, 12 (6.3%) and nine (5.0%) tadpoles, respectively, attained metamorphosis. The chromosomes of 27 tadpoles which had normal hind legs and died without attaining metamorphosis were 40~78 in number. There were five females and 17 males among 22 allohexaploids which attained sexual maturity. While only one of the females had several large eggs in the ovaries, eight males examined had no normal spermatozoa. As all the other females and males died before long, it was found that the allopentaploids and allohexaploids are quite infertile, although they can attain sexual maturity.

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