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Reciprocal Hybrids and Backcrosses between *Rana nigromaculata* and *Rana plancyi chosenica*, with Special Reference to Allotriploids and Amphidiploids Appearing in their Offspring

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(With 20 Text-figures)

CONTENTS

INTRODUCTION

In Korea and China, there are two sympatric pond-frog species, *Rana nigromaculata* and *Rana plancyi*, as *Rana nigromaculata* and *Rana brevipoda* are found in Japan. *Rana nigromaculata* distributed in Korea and China are almost completely identical with Japanese *Rana nigromaculata* in every respect of morphology, physiology and ecology. While Korean *Rana plancyi chosenica* scarcely differ from Chinese *Rana plancyi plancyi* in appearance, these two subspecies remarkably differ from *Rana brevipoda* in various respects. Hybridization experiments between Chinese *Rana nigromaculata* and *Rana plancyi plancyi* have been reported by TING (1939, 1948), and those between Japanese *Rana nigromaculata* and *Rana brevipoda* have been repeatedly described since the reports by MORIYA (1951, 1954, 1960a, b) and KAWAMURA and NISHIOKA (1960). While *Rana nigromaculata* are scarcely isolated from *Rana brevipoda* by gametic isolation or hybrid inviability, these two species are almost completely isolated from each other by male hybrid sterility. In this respect, the relationship between *Rana nigromaculata* and *Rana plancyi chosenica* is nearly equal to that between *Rana nigromaculata* and *Rana brevipoda* (KAWAMURA and NISHIOKA, 1973, 1975, 1977).

KAWAMURA and NISHIOKA (1978) reported on the four lines of descendants derived from reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda* by repeated backcrossings with the two species. The four lines included two restoration lines derived from reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda* by backcrossings with the maternal species and the other two substitution lines derived from reciprocal hybrids by backcrossings with the paternal species. These four lines differed from one another in various respects. The frogs of the two restoration lines were generally much more fertile than those of the two substitution lines. In each line, males produced from female parents by backcrossing were distinctly inferior to their sisters in reproductive capacity, owing to poverty of normal spermatozoa in their testes. About one- or two-tenths of the females of reciprocal hybrids laid a considerable number of large eggs together with normal-sized eggs. It was found that all these large eggs and a few normal-sized ones developed into triploid tadpoles by fertilization with normal spermatozoa. However, the mating experiments in the four lines were performed without considering seriously the ploidy of male and female backcrosses. Thus, it is probable that triploid males and females were occasionally used in producing offspring.

In the present study, reciprocal hybrids and their backcrosses between *Rana nigromaculata* and *Rana plancyi chosenica* were produced in order to elucidate their sex, ploidy and reproductive capacity. The production of reciprocal hybrids between *Rana nigromaculata* and *Rana plancyi chosenica* have been preliminarily reported by KAWAMURA and NISHIOKA (1973, 1975, 1977, 1979).

MATERIALS AND METHODS

Ten male and eight female *Rana plancyi chosenica* OKADA were collected from Suwon, Korea. Nine male and 12 female *Rana nigromaculata* HALLOWELL were collected from the suburbs of Hiroshima. These males and females and their offspring were used as materials. Ovulation was accelerated by injecting pituitaries of *Rana catesbeiana* SHAW. Matings were always made by artificial fertilization.

Embryos and tadpoles at the early stage were reared at room temperature. When tadpoles became more than 30 mm in total length, they were removed outdoors and reared in concrete tanks, 90 cm × 60 cm × 24 cm in size. Tadpoles were fed on boiled spinach or chard. Metamorphosed frogs were removed indoors again and reared at less than 25°C. While they were fed on mosquitoes, flies and bagworms until about the middle of 1975, they were thereafter exclusively fed on crickets, *Gryllus bimaculatus* DE GEER.

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) and the bone marrow method (OMURA, 1967). The meiotic chromosomes of the spermatocytes were prepared according to the methods of OKUMOTO (1980) and SCHMID, OLERT and KLETT (1979).

Serum proteins, hemoglobin and enzymes extracted from skeletal muscles were analyzed by the method of starch-gel electrophoresis, as utilized by BREWER (1970), NISHIOKA, OHTANI and SUMIDA (1980) and NISHIOKA, UEDA and SUMIDA (1981).

The gonads of frogs were fixed in NAVASHIN's fluid, sectioned at 10~12 μ and stained with HEIDENHAIN's iron hematoxylin.

The following signs are used in the present paper.

C ..... A set of *Rana plancyi chosenica* chromosomes
N ..... A set of *Rana nigromaculata* chromosomes
(C)..... *Rana plancyi chosenica* cytoplasm
(N)..... *Rana nigromaculata* cytoplasm
(C)CC ..... Diploid *Rana plancyi chosenica*
(N)NN..... Diploid *Rana nigromaculata*
(C)CN ..... Diploid hybrid between a female *Rana plancyi chosenica* and a male *Rana nigromaculata*
(N)NC..... Diploid hybrid between a female *Rana nigromaculata* and a male *Rana plancyi chosenica*
(C)CCN ..... Allotriploid consisting of two *Rana plancyi chosenica* genomes and one *Rana nigromaculata* genome
(N)NNC .... Allotriploid consisting of two *Rana nigromaculata* genomes and one *Rana plancyi chosenica* genome
(N)NNCC... Amphidiploid between *Rana nigromaculata* and *Rana plancyi chosenica*

OBSERVATION

I. Hybrids between Rana plancyi chosenica and Rana nigromaculata

1. Developmental capacity

a. Controls

i) *Rana plancyi chosenica*, (C)CC

Matings were made between eight females and eight males in the four seasons of 1962, 1965, 1973 and 1976. The results are presented in Table 1. Of the respective total number of eggs, 64.6~97.9%, 653 (79.3%) of 823 eggs in total, cleaved normally, 48.7~95.9%, 565 (68.7%) eggs in total, hatched normally and 26.4~68.4%, 427 (51.9%) eggs in total, attained completion of metamorphosis. The number of metamorphosed frogs corresponded to 36.2~77.3%, 65.4% on the average, of the respective total number of normally cleaved eggs (Table 1).

ii) *Rana nigromaculata*, (N)NN

Matings were made between 12 females and nine males in the five seasons of

TABLE 1

Developmental capacity of the hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata* and the controls

Year	Parents		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	PMC
	Female	Male							
1962	(C)CC, Nos. 1~3	(C)CC, Nos. 1~3	193	189 (97.9%)	186 (96.4%)	185 (95.9%)	159 (82.4%)	132 (68.4%)	69.8
		(N)NN, Nos. 1~3	610	610 (100 %)	382 (62.6%)	370 (60.7%)	370 (60.7%)	370 (60.7%)	60.7
1965	(C)CC, Nos. 4, 5	(C)CC, Nos. 4, 5	261	211 (80.8%)	195 (74.7%)	183 (70.1%)	176 (67.4%)	163 (62.5%)	77.3
		(N)NN, Nos. 4, 5	246	215 (87.4%)	203 (82.5%)	199 (80.9%)	185 (75.2%)	184 (74.8%)	85.6
1973	(C)CC, No. 6	(C)CC, No. 6	174	127 (73.0%)	125 (71.8%)	102 (58.6%)	93 (53.4%)	46 (26.4%)	36.2
		(N)NN, No. 6	77	44 (57.1%)	42 (54.5%)	40 (51.9%)	40 (51.9%)	37 (48.1%)	84.1
1976	(C)CC, Nos. 7, 8	(C)CC, Nos. 9, 10	195	126 (64.6%)	110 (56.4%)	95 (48.7%)	90 (46.2%)	86 (44.1%)	68.3
		(N)NN, Nos. 8, 9	163	129 (79.1%)	125 (76.7%)	117 (71.8%)	115 (70.6%)	114 (69.9%)	88.4
Total	(C)CC (8)	(C)CC (8)	823	653 (79.3%)	616 (74.8%)	565 (68.7%)	518 (62.9%)	427 (51.9%)	65.4
		(N)NN (8)	1096	998 (91.1%)	752 (68.6%)	726 (66.2%)	710 (64.8%)	705 (64.3%)	70.6

PMC, Percentage of metamorphosed frogs to normally cleaved eggs

1962, 1965, 1973, 1975 and 1976. As presented in Table 2, 85.6~98.3% of the respective total number of eggs, 2190 (93.8%) of 2336 eggs in total, cleaved normally, 56.8~97.5%, 1962 (84.0%) eggs in total, hatched normally and 41.8~94.1%, 1820 (77.9%) eggs in total, attained completion of metamorphosis. The number of metamorphosed frogs corresponded to 48.8~97.3%, 83.1% on the average, of the respective total number of normally cleaved eggs (Table 2).

b. Experimental series

i) *Rana plancyi chosenica*♀ × *Rana nigromaculata*♂, (C)CN

Crossings were made between eight female *Rana plancyi chosenica* and eight male *Rana nigromaculata* in the four seasons of 1962, 1965, 1973 and 1976. As presented in Table 1, 57.1~100% of the respective total number of eggs, 998 (91.1%) of 1096 eggs in total, cleaved normally, 51.9~80.9%, 726 (66.2%) eggs in total, hatched normally and 48.1~74.8%, 705 (64.3%) eggs in total, attained completion of metamorphosis. The number of metamorphosed frogs corresponded to 60.7~88.4%, 70.6% on the average, of the respective total number of normally cleaved eggs (Table 1).

ii) *Rana nigromaculata*♀ × *Rana plancyi chosenica*♂, (N)NC

Crossings were made between 12 female *Rana nigromaculata* and seven male

TABLE 2

Developmental capacity of the hybrids between female *Rana nigromaculata* and *Rana plancyi chosenica* and the controls

Year	Parents		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	PMC
	Female	Male							
1962	(N)NN, Nos. 1, 2	(N)NN, Nos. 1~3	569	532 (93.5%)	524 (92.1%)	521 (91.6%)	517 (90.9%)	502 (88.2%)	94.4
1965	(N)NN, Nos. 3, 4	(N)NN, Nos, 4, 5	621	590 (95.0%)	584 (94.0%)	582 (93.7%)	580 (93.4%)	574 (92.4%)	97.3
		(C)CC, Nos. 4, 5	227	205 (90.3%)	195 (85.9%)	190 (83.7%)	187 (82.4%)	184 (81.1%)	89.8
1973	(N)NN, Nos. 5, 6	(N)NN, No. 6	119	117 (98.3%)	116 (97.5%)	116 (97.5%)	113 (95.0%)	112 (94.1%)	95.7
		(C)CC, No. 6	822	512 (62.3%)	298 (36.3%)	253 (30.8%)	200 (24.3%)	173 (21.0%)	33.8
1975	(N)NN, Nos. 7~9	(N)NN, No. 7	673	648 (96.3%)	557 (82.8%)	542 (80.5%)	490 (72.8%)	484 (71.9%)	74.7
		(C)CC, Nos. 7, 8	645	130 (20.2%)	122 (18.9%)	119 (18.4%)	115 (17.8%)	113 (17.5%)	86.9
1976	(N)NN, Nos. 10~12	(N)NN, Nos. 8, 9	354	303 (85.6%)	232 (65.5%)	201 (56.8%)	173 (48.9%)	148 (41.8%)	48.8
		(C)CC, Nos. 9, 10	708	425 (60.0%)	350 (49.4%)	309 (43.6%)	296 (41.8%)	274 (38.7%)	64.5
Total	(N)NN (12)	(N)NN (9)	2336	2190 (93.8%)	2013 (86.2%)	1962 (84.0%)	1873 (80.2%)	1820 (77.9%)	83.1
		(C)CC (7)	2402	1272 (53.0%)	965 (40.2%)	871 (36.3%)	798 (33.2%)	744 (31.0%)	58.5

PMC, Percentage of metamorphosed frogs to normally cleaved eggs

Rana plancyi chosenica in the four seasons of 1965, 1973, 1975 and 1976. As presented in Table 2, 20.2~90.3% of the respective total number of eggs, 1272 (53.0%) of 2402 eggs in total, cleaved normally, 18.4~83.7%, 871 (36.3%) eggs in total, hatched normally and 17.5~81.1%, 744 (31.0%) eggs in total, attained completion of metamorphosis. The number of metamorphosed frogs corresponded to 33.8~89.8%, 58.5% on the average, of the respective total number of normally cleaved eggs (Table 2).

2. Sex

a. Hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata*, (C)CN

In the four seasons of 1962, 1965, 1973 and 1976, 189 of the 427 metamorphosed frogs produced from the control matings between female and male *Rana plancyi chosenica* attained sexual maturity. Of these mature frogs, 97 were females and 92 (48.7%) were males.

In the experimental series of the same seasons, 383 of the 705 metamorphosed hybrids produced from the same females as those used in the control matings by crossing with male *Rana nigromaculata* attained sexual maturity. Of these mature hybrids, 197 were females and 186 (48.6%) were males (Table 3).

TABLE 3

Sex of mature hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata* and the controls

Year	Parents		No. of metamorphosed frogs	No. of mature frogs		
	Female	Male		Total	Female	Male (%)
1962	(C)CC, Nos. 1~3	(C)CC, Nos. 1~3	132	63	31	32 (50.8)
		(N)NN, Nos, 1~3	370	158	78	80 (50.6)
1965	(C)CC, Nos. 4, 5	(C)CC, Nos. 4, 5	163	52	29	23 (44.2)
		(N)NN, Nos. 4, 5	184	115	60	55 (47.8)
1973	(C)CC, No. 6	(C)CC, No. 6	46	44	24	20 (45.5)
		(N)NN, No. 6	37	34	18	16 (47.1)
1976	(C)CC, Nos. 7, 8	(C)CC, Nos. 9, 10	86	30	13	17 (56.7)
		(N)NN, Nos. 8, 9	114	76	41	35 (46.1)
Total	(C)CC (8)	(C)CC (8)	427	189	97	92 (48.7)
		(N)NN (8)	705	383	197	186 (48.6)

b. Hybrids between female *Rana nigromaculata* and male *Rana plancyi chosenica*, (N)NC

In the five seasons of 1962, 1965, 1973, 1975 and 1976, only 164 of the 1820 metamorphosed frogs produced from the control matings between female and male *Rana nigromaculata* were reared until sexual maturity. Of these mature frogs, 81 were females and 83 (50.6%) were males.

In the experimental series of the four seasons, 318 of the 744 metamorphosed hybrids produced from the same females as those used in the control matings by

TABLE 4

Sex of mature hybrids between female *Rana nigromaculata* and male *Rana plancyi chosenica* and the controls

Year	Parents		No. of metamorphosed frogs	No. of mature frogs		
	Female	Male		Total	Female	Male (%)
1962	(N)NN, Nos. 1, 2	(N)NN, Nos. 1~3	502	46	24	22 (47.8)
1965	(N)NN, Nos. 3, 4	(N)NN, Nos. 4, 5	574	31	14	17 (54.8)
		(C)CC, Nos. 4, 5	184	154	79	75 (48.7)
1973	(N)NN, Nos. 5, 6	(N)NN, No. 6	112	24	12	12 (50.0)
		(C)CC, No. 6	173	65	31	34 (52.3)
1975	(N)NN, Nos. 7~9	(N)NN, No. 7	484	36	16	20 (55.6)
		(C)CC, Nos. 7, 8	113	40	19	21 (52.5)
1976	(N)NN, Nos. 10~12	(N)NN, Nos. 8, 9	148	27	15	12 (44.4)
		(C)CC, Nos. 9, 10	274	59	29	30 (50.8)
Total	(N)NN (12)	(N)NN (9)	1820	164	81	83 (50.6)
		(C)CC (7)	744	318	158	160 (50.3)

crossing with male *Rana plancyi chosenica* attained sexual maturity. Of these mature hybrids, 158 were females and 160 (50.3%) were males (Table 4).

3. Characters of hybrids

a. External characters

Reciprocal hybrids between *Rana nigromaculata* and *Rana plancyi chosenica* were intermediate between the two species in external characters. *Rana nigromaculata* are somewhat slender in body shape, while *Rana plancyi chosenica* are dumpy. The dermal tubercles on the back of *Rana nigromaculata* remarkably differ from those of *Rana plancyi chosenica* in shape and number. They are rod-shaped and numerous in *Rana nigromaculata*, while they are dot-like and few in *Rana plancyi chosenica*. The black spots on the dorsal surface of the body of *Rana nigromaculata* distinctly differ from those of *Rana plancyi chosenica* in shape, size and number. They are elongated oval, larger and more numerous in *Rana nigromaculata*, while they are circular, smaller and fewer in *Rana plancyi chosenica*. In some of the latter, the dorsal black spots are scarcely found or vague in outline. The dorsolateral folds of *Rana nigromaculata* are narrow in width and somewhat steep in elevation, while those of *Rana plancyi chosenica* are wide and gentle in elevation. The hind limbs of *Rana nigromaculata* are longer than those of *Rana plancyi chosenica*. While male *Rana nigromaculata* reveal a distinct nuptial color during the breeding season, male *Rana plancyi chosenica* show no distinguishable nuptial color. Reciprocal hybrids were intermediate between the two species in the above respects.

However, the pale dorsomedian stripe of *Rana nigromaculata* which is a dominant character appeared distinctly in reciprocal hybrids, while *Rana plancyi chosenica* have no dorsomedian stripe (Figs. 1, 2).

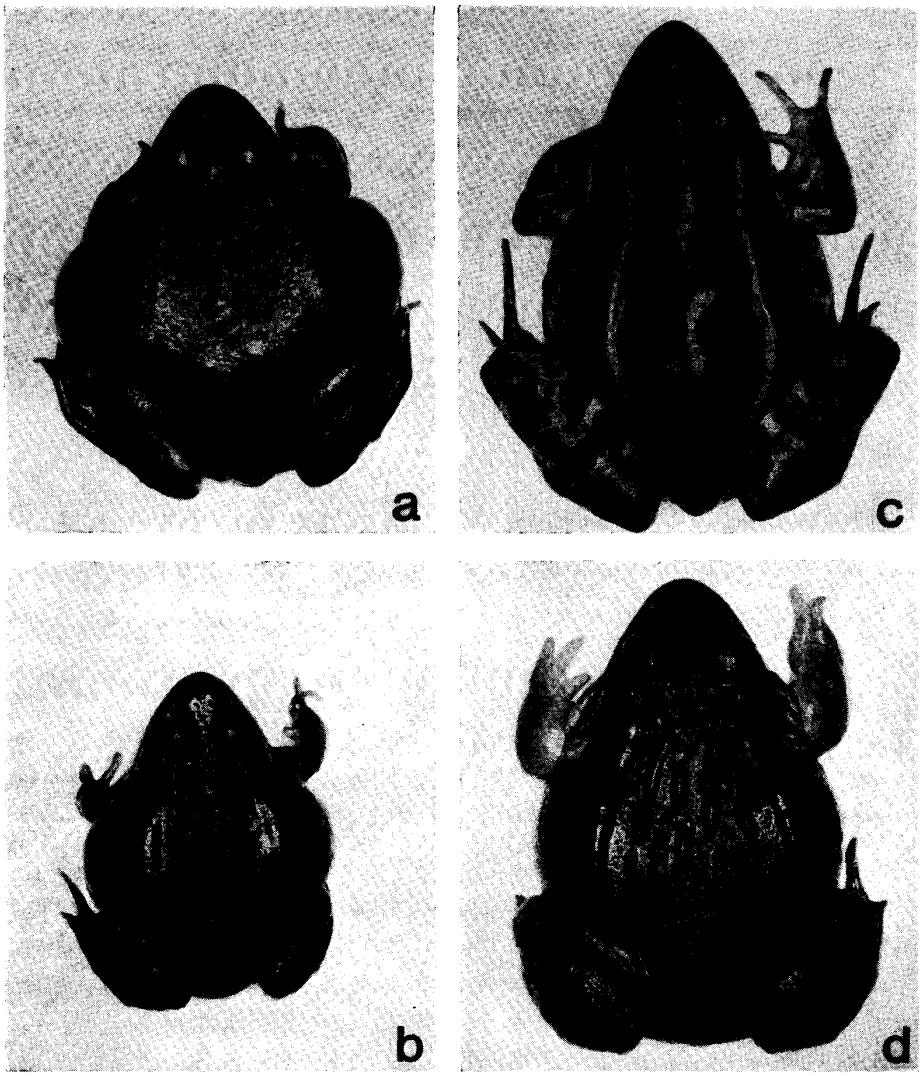


Fig. 1. Two-year-old *Rana plancyi chosenica* and *Rana nigromaculata*. ×0.7

a, b. Female and male *Rana plancyi chosenica*

c, d. Female and male *Rana nigromaculata*

b. Inner structure of testes

The testes of each of 17 mature male hybrids including six 3-year-old males, (C)CN 62♂, Nos. 1～6, produced in 1962 from a mating, (C)CC♀, No. 1×(N)NN♂, No. 1, six 6-year-old male hybrids, (C)CN 65♂, Nos. 7～12, produced in 1965 from a mating, (C)CC♀, No. 4×(N)NN♂, No. 4, and five 6-year-old male hybrids, (N)NC 65♂, Nos. 1～5, produced in 1965 from a mating, (N)NN♀, No. 3×(C)CC♂, No. 4, were used in examining their inner structure as well as making mating experiments (cf. Tables 5 and 6).

One of the testes of each male hybrid was sectioned and microscopically observed. It was found that the testes of five male hybrids, (C)CN 62♂, Nos. 2, 3 and 6 and (C)CN 65♂, Nos. 9 and 10, had small bundles of large spermatozoa contained here and there in the seminiferous tubules. These large spermatozoa mostly appeared normal in shape. Besides, normally and abnormally shaped

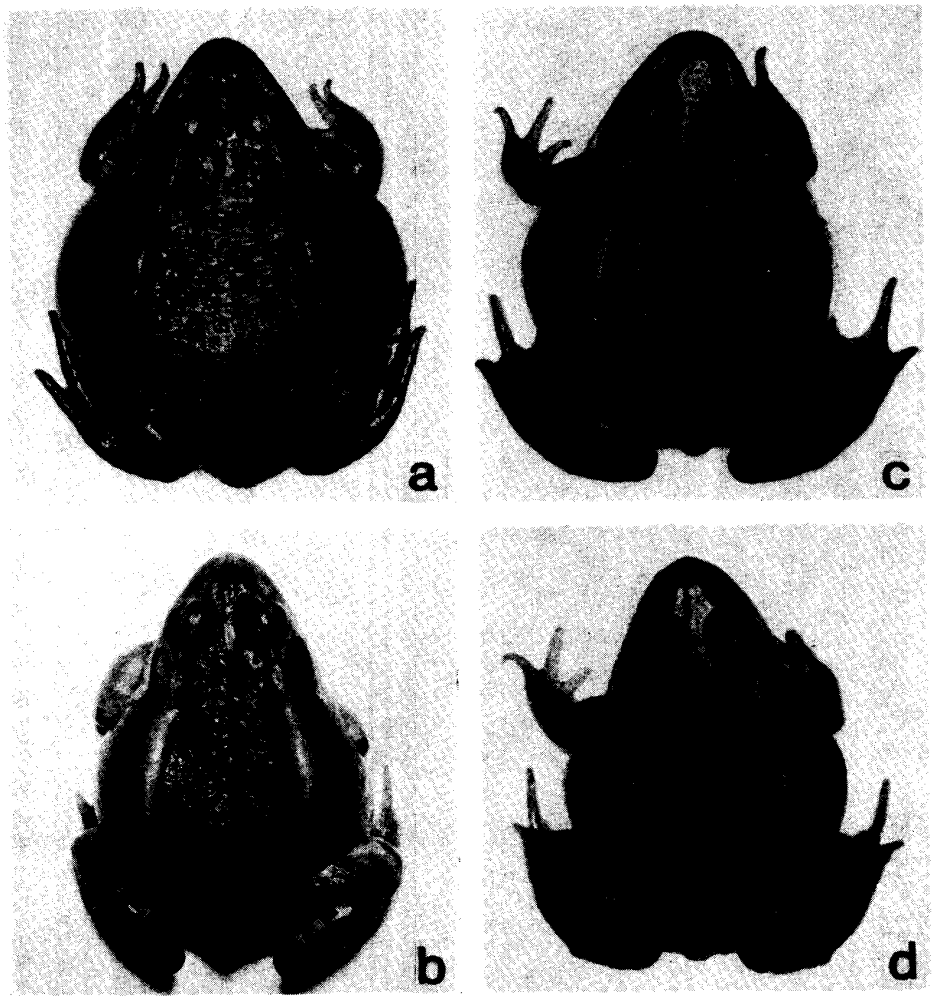


Fig. 2. Two-year-old reciprocal diploid hybrids between *Rana plancyi chosenica* and *Rana nigromaculata*. ×0.7

a, b. Female and male (C)CN hybrids produced from (C)CC ♀, No. 4 × (N)NN ♂, No. 4

c, d. Female and male (N)NC hybrids produced from (N)NN ♀, No. 3 × (C)CC ♂, No. 4

spermatozoa of various sizes and pycnotic nuclei were sparsely distributed in the wide central parts of the seminiferous tubules. Many first spermatocytes and spermatogonia were found in the peripheral parts (Fig. 3a, b). The testes of seven other male hybrids, (C)CN 62♂, Nos. 1, 4 and 5 and (C)CN 65♂, Nos. 7, 8, 11 and 12, had no bundle of large spermatozoa. However, a few normally shaped, large and small spermatozoa and pycnotic nuclei were found in the wide central parts. In the peripheral parts, there were many first spermatocytes and spermatogonia.

Of the remaining five male hybrids, (N)NC 65♂, Nos. 1~5, a single male (No. 5) had small bundles of normally shaped, large spermatozoa sporadically in the seminiferous tubules. In the testes of the other four male hybrids, a few normally shaped, large and small spermatozoa were sparsely distributed together with abnormally shaped spermatozoa of various sizes and pycnotic nuclei. In the peripheral parts of seminiferous tubules, there were many first spermatocytes and spermatogonia (Fig. 3c, d).

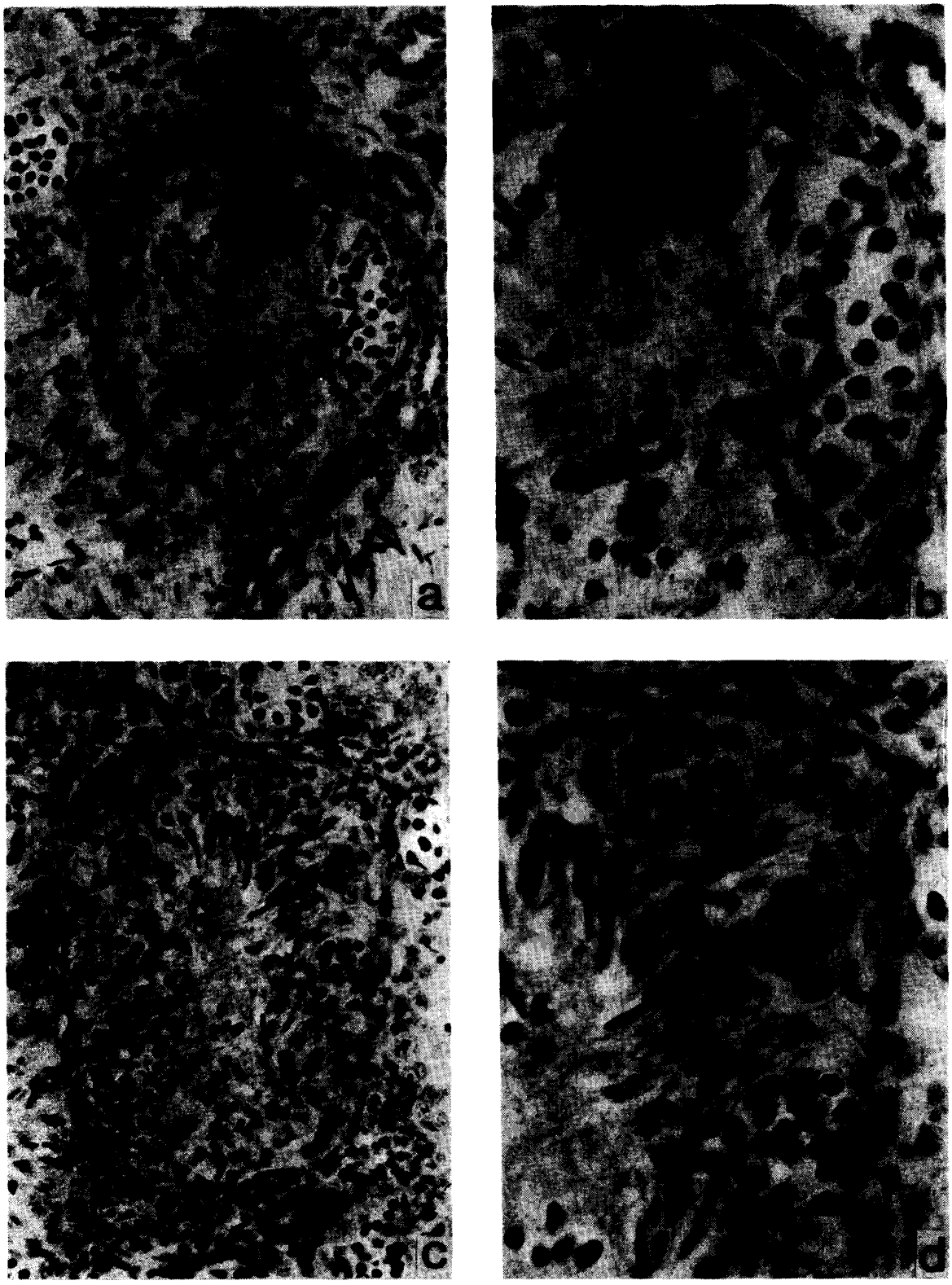


Fig. 3. Cross-sections of the testes of reciprocal diploid male hybrids between *Rana plancyi chosenica* and *Rana nigromaculata*.

a. (C)CN hybrid produced from (C)CC♀, No. 4×(N)NN♂, No. 4 ×260
b. Ditto ×520
c. (N)NC hybrid produced from (N)NN♀, No. 3×(C)CC♂, No. 4 ×260
d. Ditto ×520

II. Backcrossing of male hybrids

1. Reproductive capacity

a. Male hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata*

In the breeding season of 1965, six 3-year-old male hybrids, (C)CN 62♂, Nos. 1~6, produced in 1962 from a cross, (C)CC♀, No. 1 × (N)NN♂, No. 1, were backcrossed with two female *Rana nigromaculata*, (N)NN 62♀, Nos. 1 and 2, produced in 1962 from (N)NN♀, No. 1 × (N)NN♂, No. 1. These two females were mated with their two brothers, (N)NN 62♂, Nos. 1 and 2, as the control matings. Of 183 control eggs, 175 (95.6%) cleaved normally, 162 (88.5%)

TABLE 5

Developmental capacity of the backcrosses of male hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata* with female *Rana nigromaculata* and the controls

Year	Parents		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						
1965	(N)NN 62, Nos. 1, 2	(N)NN 62, Nos. 1, 2	183	175 (95.6%)	169 (92.3%)	162 (88.5%)	157 (85.8%)	148 (80.9%)
		(C)CN 62, No. 1	164	6 (3.7%)	3 (1.8%)	2 (1.2%)	1 (0.6%)	1 (0.6%)
		(C)CN 62, No. 2	151	57 (37.7%)	55 (36.4%)	43 (28.5%)	41 (27.2%)	40 (26.5%)
		(C)CN 62, No. 3	188	62 (33.0%)	46 (24.5%)	40 (21.3%)	40 (21.3%)	38 (20.2%)
		(C)CN 62, No. 4	240	8 (3.3%)	6 (2.5%)	5 (2.1%)	2 (0.8%)	1 (0.4%)
		(C)CN 62, No. 5	216	17 (7.9%)	16 (7.4%)	13 (6.0%)	11 (5.1%)	3 (1.4%)
		(C)CN 62, No. 6	319	129 (40.4%)	113 (35.4%)	99 (31.0%)	96 (30.1%)	76 (23.8%)
1971	(N)NN 65, Nos. 1, 2	(N)NN 65, Nos. 1, 2	134	121 (90.3%)	118 (88.1%)	114 (85.1%)	111 (82.8%)	107 (79.9%)
		(C)CN 65, No. 7	103	3 (2.9%)	2 (1.9%)	0	0	0
		(C)CN 65, No. 8	126	5 (4.0%)	4 (3.2%)	3 (2.4%)	1 (0.8%)	0
		(C)CN 65, No. 9	134	36 (26.9%)	27 (20.1%)	21 (15.7%)	21 (15.7%)	19 (14.2%)
		(C)CN 65, No. 10	155	56 (36.1%)	54 (34.8%)	54 (34.8%)	52 (33.5%)	47 (30.3%)
		(C)CN 65, No. 11	146	9 (6.2%)	5 (3.4%)	4 (2.7%)	2 (1.4%)	2 (1.4%)
		(C)CN 65, No. 12	127	4 (3.1%)	2 (1.6%)	1 (0.8%)	0	0
Total	(N)NN (4)	(C)CN (12)	2069	392 (18.9%)	333 (16.1%)	285 (13.8%)	267 (12.9%)	227 (11.0%)

hatched normally, 157 (85.8%) became normally feeding tadpoles and 148 (80.9%) became metamorphosed frogs. All the male hybrids were distinctly inferior to the controls in reproductive capacity and were divided into two groups in degree of inferiority (Table 5). Of 164~240 eggs of females backcrossed with three male hybrids Nos. 1, 4 and 5, only 3.3~7.9%, 31 (5.0%) eggs in total, cleaved normally, 1.2~6.0%, 20 (3.2%) eggs in total, hatched normally, 0.6~5.1%, 14 (2.3%) eggs in total, became normally feeding tadpoles and 0.4~1.4%, five (0.8%) eggs in total, attained completion of metamorphosis. In contrast, of 151~319 eggs of females backcrossed with the other three male hybrids Nos. 2, 3 and 6, 33.0~40.4%, 248 (37.7%) eggs in total, cleaved normally, 21.3~31.0%, 182 (27.7%) eggs in total, hatched normally, 21.3~30.1%, 177 (26.9%) eggs in total, became normally feeding tadpoles and 20.2~26.5%, 154 (23.4%) eggs in total, attained completion of metamorphosis.

In the breeding season of 1971, six 6-year-old male hybrids, (C)CN 65♂, Nos. 7~12, produced in 1965 from a cross, (C)CC♀, No. 4×(N)NN♂, No. 4, were backcrossed with two female *Rana nigromaculata*, (N)NN 65♀, Nos. 1 and 2, produced in 1965 from a mating, (N)NN♀, No. 4×(N)NN♂, No. 4. The same females were also mated with two of their brothers as the control matings. Of 134 control eggs, 121 (90.3%) cleaved normally, 114 (85.1%) hatched normally, 111 (82.8%) became normally feeding tadpoles and 107 (79.9%) attained completion of metamorphosis. All the male hybrids were distinctly inferior to the control *Rana nigromaculata* in reproductive capacity and were divided into two groups in degree of the inferiority (Table 5). Of 103~146 eggs backcrossed with male hybrids Nos. 7, 8, 11 and 12, only 2.9~6.2%, 21 (4.2%) eggs in total, cleaved normally, 0~2.7%, eight (1.6%) eggs in total, hatched normally, 0~1.4%, three (0.6%) eggs in total, became normally feeding tadpoles and 0~1.4%, two (0.4%) eggs attained completion of metamorphosis. In contrast, of 134 and 155 eggs backcrossed with male hybrids Nos. 9 and 10, 36 (26.9%) and 56 (36.1%) cleaved normally, 21 (15.7%) and 54 (34.8%) hatched normally, 21 (15.7%) and 52 (33.5%) became normally feeding tadpoles, and 19 (14.2%) and 47 (30.3%) attained completion of metamorphosis, respectively.

In sum total, 2069 eggs obtained from four female *Rana nigromaculata* were inseminated in 1965 and 1971 with sperm of 12 male (C)CN hybrids. It was found that 392 (18.9%) eggs cleaved normally, 285 (13.8%) hatched normally, 267 (12.9%) became normally feeding tadpoles and 227 (11.0%) attained completion of metamorphosis.

b. Male hybrids between female *Rana nigromaculata* and male *Rana plancyi chosenica*

In the breeding season of 1971, five 6-year-old male hybrids, (N)NC 65♂, Nos. 1~5, produced in 1965 from a cross, (N)NN♀ No. 3×(C)CC♂, No. 4, were backcrossed with two female *Rana nigromaculata*, (N)NN 65♀, Nos. 3 and 4, produced in 1965 from a control mating, (N)NN♀, No. 4×(N)NN♂, No. 4 (Table 6). The two females were also mated with one, (N)NN 65♂, No. 3, of

TABLE 6

Developmental capacity of the backcrosses of male hybrids between a female *Rana nigromaculata* and a male *Rana plancyi chosenica* with female *Rana nigromaculata* and the controls

Year	Parents		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						
1971	(N)NN 65, Nos. 3, 4	(N)NN 65, No. 3	519	475 (91.5%)	456 (87.9%)	452 (87.1%)	441 (85.0%)	423 (81.5%)
		(N)NC 65, No. 1	365	7 (1.9%)	5 (1.4%)	5 (1.4%)	4 (1.1%)	2 (0.5%)
		(N)NC 65, No. 2	411	19 (4.6%)	15 (3.6%)	13 (3.2%)	9 (2.2%)	7 (1.7%)
		(N)NC 65, No. 3	523	40 (7.6%)	38 (7.3%)	38 (7.3%)	36 (6.9%)	32 (6.1%)
		(N)NC 65, No. 4	377	24 (6.4%)	22 (5.8%)	20 (5.3%)	19 (5.0%)	19 (5.0%)
		(N)NC 65, No. 5	462	134 (29.0%)	126 (27.3%)	125 (27.1%)	119 (25.8%)	107 (23.2%)
Total	(N)NN (2)	(N)NC (5)	2138	224 (10.5%)	206 (9.6%)	201 (9.4%)	187 (8.7%)	167 (7.8%)

their brothers as the control matings. Of 519 control eggs, 475 (91.5%) cleaved normally, 452 (87.1%) hatched normally, 441 (85.0%) became normally feeding tadpoles, and 423 (81.5%) attained completion of metamorphosis. In contrast, all the five male hybrids were remarkably inferior to the control males in reproductive capacity. In degree of the inferiority, four of the five males were clearly distinguishable from the remainder. Of 365～523 eggs backcrossed with sperm of male hybrids, (N)NC 65♂, Nos. 1～4, only 1.9～7.6%, 90 (5.4%) eggs in total, cleaved normally, 1.4～7.3%, 76 (4.5%) eggs in total, hatched normally, 1.1～6.9%, 68 (4.1%) eggs in total, became normally feeding tadpoles and 0.5～6.1%, 60 (3.6%) eggs in total, attained completion of metamorphosis, while of 462 eggs backcrossed with sperm of a male hybrid, (N)NC 65♂, No. 5, 134 (29.0%) cleaved normally, 125 (27.1%) hatched normally, 119 (25.8%) became normally feeding tadpoles and 107 (23.2%) attained completion of metamorphosis.

2. Chromosomes

a. Backcrosses from (N)NN♀ × (C)CN♂

The chromosomes of the normally feeding tadpoles produced in 1965 from male (C)CN hybrids by backcrossing with female *Rana nigromaculata* as well as from the control matings were examined in the tail-tips by the squash method with water-pretreatment. The results showed that of 157 control tadpoles produced by matings, (N)NN♀, Nos. 1 and 2 × (N)NN♂, Nos. 1 and 2, 48 of 50 whose chromosomes were examined were diploids and the other two were triploids. Of 177 normally feeding tadpoles produced in 1965 from three male hybrids, (C)CN 62♂, Nos. 2, 3 and 6, by backcrossing with two female *Rana nigromaculata*, (N)NN 62♀, Nos. 1 and 2, 51 were diploid, two were 27 (2n+1), one was 30 (2n+4), one was 38 (3n−1) in chromosome number and the remaining 122 were

triploids. On the other hand, of 14 normally feeding tadpoles produced from backcrossings of the three male hybrids, (C)CN 62♂, Nos. 1, 4 and 5, four were diploid, 9 were triploid and the remainder was 28 (2n+2) in chromosome number.

Twenty of 111 normally feeding tadpoles produced in 1971 from the control matings, (N)NN 65♀, Nos. 1 and 2×(N)NN 65♂, Nos. 1 and 2, were all diploids. In contrast, of 73 normally feeding tadpoles produced in 1971 from two male hybrids, (C)CN 65♂, Nos. 9 and 10, by backcrossing with two female *Rana nigromaculata*, (N)NN 65♀, Nos. 1 and 2, 27 were diploid, 43 were triploid, one was 27 (2n+1), one was 28 (2n+2) and the remainder was 38 (3n−1) in chromosome number. On the other hand, of three normally feeding tadpoles produced from backcrossings of four male hybrids, (C)CN 65♂, Nos. 7, 8, 11 and 12, with two female *Rana nigromaculata*, (N)NN 65♀, Nos. 1 and 2, two were triploids and the other was a diploid.

In sum total, of 267 feeding tadpoles produced in 1965 and 1971 from 12 male hybrids, (C)CN♂, Nos. 1~12, by backcrossing with female *Rana nigromaculata*, 176 (65.9%) were triploid, 83 (31.1%) were diploid, three (1.1%) were 27 (2n+1), two (0.7%) were 28 (2n+2), one (0.4%) was 30 (2n+4) and the remaining two (0.7%) were 38 (3n−1) in chromosome number.

b. Backcrosses from (N)NN♀×(N)NC♂

Twenty of 441 feeding tadpoles produced in 1971 from the control matings, (N)NN 65♀, Nos. 3 and 4×(N)NN 65♂, No. 3, were all diploids, when their chromosomes were examined in the tail-tips by the squash method with water-pretreatment.

The chromosomes of the normally feeding tadpoles produced from male (N)NC hybrids by backcrossing with female *Rana nigromaculata* were also examined in the tail-tips by the squash method with water-pretreatment. The results showed that of 68 normally feeding tadpoles produced from four male hybrids, (N)NC 65♂, Nos. 1~4, 20 were diploid, 46 were triploid and the remaining two were 27 (2n+1) in chromosome number. Of 119 normally feeding tadpoles produced from a male hybrid, (N)NC 65♂, No. 5, 43 were diploid, 72 were triploid, two were 27 (2n+1), one was 29 (2n+3) and the remainder was 34 (3n−5) in chromosome number.

In sum total, of the 187 normally feeding tadpoles produced from five male hybrids, (N)NC 65♂, Nos. 1~5, by backcrossing with two female *Rana nigromaculata*, 118 (63.1%) were triploid, 63 (33.7%) were diploid, four (2.1%) were 27 (2n+1), one (0.5%) was 34 (3n−5) and the remainder (0.5%) was 29 (2n+3) in chromosome number.

3. Metamorphosis, sex and external characters

a. Backcrosses from (N)NN♀×(C)CN♂

A total of 267 feeding tadpoles produced in 1965 and 1971 from backcrossing of 12 male diploid hybrids, (C)CN♂, Nos. 1~12, with four female *Rana nigromaculata* were continuously reared. As described above, 83 of them were diploids

and 176 were triploids. Of the diploid tadpoles, 69 including 9 of 10 derived from male hybrid (C)CN♂, No. 2, 15 from (C)CN♂, No. 3, one from (C)CN♂, No. 4, 19 of 26 from (C)CN♂, No. 6, five from (C)CN♂, No. 9 and 20 of 22 from (C)CN♂, No. 10, metamorphosed normally.

Of the triploid tadpoles, 158 including one derived from male hybrid (C)CN♂, No. 1, 31 from (C)CN♂, No. 2, 23 of 24 from (C)CN♂, No. 3, three of eight from (C)CN♂, No. 5, 57 of 67 from (C)CN♂, No. 6, 14 from (C)CN♂, No. 9, 27 of 29 from (C)CN♂, No. 10 and two from (C)CN♂, No. 11, metamorphosed normally.

Eight aneuploid tadpoles including one, one, three, two and one derived from male hybrids, (C)CN♂, Nos. 3, 4, 6, 9 and 10, respectively, all died before metamorphosis.

Of the 69 metamorphosed diploid backcrosses, 26 died within three months after metamorphosis. Twelve of them were females and the other 14 were males. Of 36 others which lived more than six months after metamorphosis, 15 were females and 21 were males. In total, 27 of the 62 metamorphosed diploid backcrosses were females and 35 (56.5%) were males. The sex of the remaining seven metamorphosed diploid backcrosses was unknown owing to postmortem changes.

Of the 158 metamorphosed triploid backcrosses, 63 died within three months after metamorphosis. Twenty-one of them were females and the other 42 were males. Eighty triploid backcrosses lived more than six months after metamorphosis. Of these frogs, 37 were females and 43 were males. In total, 58 of the 143 metamorphosed triploid backcrosses were females and the other 85 (59.4%) were males. The sex of the remaining 15 metamorphosed triploid backcrosses was unknown owing to postmortem changes.

External characters were observed in 36 diploid and 80 triploid backcrosses which lived more than six months after metamorphosis. The diploid backcrosses were variable in external character from those closely resembling *Rana nigromaculata*, to those being very similar to diploid (C)CN hybrids. In contrast, 80 triploid backcrosses were almost constant and intermediate in external character between *Rana nigromaculata* and (C)CN hybrids. Thus, the triploid backcrosses were considered to be (N)NNC allotriploids.

b. Backcrosses from (N)NN♀ × (N)NC♂

A total of 187 feeding tadpoles produced in 1971 from backcrossings of five diploid male hybrids, (N)NC♂, Nos. 1～5, with two female *Rana nigromaculata* were continuously reared. As described above, 63 of them were diploids and 118 others were triploids. Of the diploid tadpoles, 57 including one derived from male hybrid (N)NC♂, No. 1, two of three from (N)NC♂, No. 2, eight of 10 from (N)NC♂, No. 3, six from (N)NC♂, No. 4, 40 of 43 from (N)NC♂, No. 5, metamorphosed normally.

Of the triploid tadpoles, 110 including one of two derived from male hybrid (N)NC♂, No. 1, five of six from (N)NC♂, No. 2, 24 of 25 from (N)NC♂, No. 3,

13 from (N)NC♂, No. 4 and 67 of 72 from (N)NC♂, No. 5, metamorphosed normally. One, one and four aneuploid tadpoles derived from male hybrids (N)NC♂, Nos. 1, 3 and 5, respectively, died before metamorphosis.

Of the 57 metamorphosed diploid backcrosses, 19 died within three months after metamorphosis. Eight of them were females and the other 11 were males. Thirty-two others lived for more than six months. Of these diploids, 13 were females and 19 were males. In total, 21 of 51 metamorphosed diploid backcrosses were females and 30 (58.8%) were males. The sex of the remaining six was unknown owing to postmortem changes.

Of the 110 metamorphosed triploid backcrosses, 57 died or were killed within three months after metamorphosis. Twenty-six of them were females and the other 31 were males. Forty-four others lived for more than six months. Of these triploids, 18 were females and 26 were males. In total, 44 of 101 metamorphosed backcrosses were females and the other 57 (56.4%) were males. The sex of the remaining nine metamorphosed triploid backcrosses was unknown owing to postmortem changes.

External characters were observed in the 32 diploid and 44 triploid backcrosses which lived for more than six months after metamorphosis. The diploid backcrosses were variable in external character from those closely resembling *Rana nigromaculata* to those being very similar to diploid (N)NC hybrids. In contrast, the triploid backcrosses were uniform and intermediate between *Rana nigromaculata* and (N)NC hybrids in external character. This finding seems to indicate that the triploid backcrosses were (N)NNC allotriploids.

III. Backcrossing of female hybrids

1. Reproductive capacity

a. Female hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata*

Five 3-year-old female hybrids, (C)CN 62♀, Nos. 1～5, produced in 1962 from a cross, (C)CC♀, No. 1×(N)NN♂, No. 1, five 6-year-old female hybrids, (C)CN 65♀, Nos. 6～10, produced in 1965 from a cross, (C)CC♀, No. 4×(N)NN♂, No. 4, six 2-year-old female hybrids, (C)CN 73♀, Nos. 11～16, produced in 1973 from a cross (C)CC♀, No. 6×(N)NN♂, No. 6, and four 4-year-old female hybrids, (C)CN 76♀, Nos. 17～20, produced in 1976 from a cross, (C)CC♀, No. 7×(N)NN♂, No. 8, laid eggs after injection of frog pituitaries in the breeding seasons of 1965, 1971, 1975 and 1980, respectively. The number and size of the eggs laid by each of these 20 female hybrids in total are presented in Table 7. Fourteen female hybrids, Nos. 1～12, 14 and 15, laid almost normal-sized eggs, although only one, two, three or four large eggs were usually found among the eggs of each female. The other six females, Nos. 13, 16～20, laid fairly numerous large eggs together with abundant normal-sized ones. The large eggs of each female were 61～393 in number which corre-

TABLE 7

Size of the eggs of female hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata*

Year	Kinds	Individual no.	Age (years)	Body length (mm)	No. of total eggs	Normal-sized eggs		Large eggs		
						Number	Diameter (mm)	Number	Diameter (mm)	(%)
1965	(C)CC♀, No. 1×	1	3	57.5	667	665	1.67±0.01	2	2.0	0.3
	(N)NN♂, No. 1	2	3	59.0	683	680	1.80±0.01	3	2.2	0.4
		3	3	57.0	524	523	1.72±0.01	1	2.2	0.2
		4	3	58.0	631	631	1.64±0.01	0		
		5	3	56.5	213	122	1.63±0.01	1	2.0	0.5
1971	(C)CC♀, No. 4×	6	6	59.0	124	122	1.70±0.01	2	2.1	1.6
	(N)NN♂, No. 4	7	6	62.5	635	633	1.59±0.01	2	2.0	0.3
		8	6	64.0	621	618	1.68±0.01	3	2.2	0.5
		9	6	63.0	524	520	1.69±0.01	4	2.1	0.8
		10	6	63.5	291	292	1.69±0.01	0		
1975	(C)CC♀, No. 6×	11	2	52.5	862	858	1.67±0.01	4	2.1	0.5
	(N)NN♂, No. 6	12	2	50.0	94	94	1.72±0.01	0		
		13	2	51.0	465	396	1.68±0.01	69	2.20±0.01	14.8
		14	2	56.0	1103	1101	1.72±0.01	2	2.1	0.2
		15	2	57.5	795	792	1.66±0.01	3	2.0	0.4
		16	2	57.0	1080	938	1.76±0.01	142	2.20±0.01	13.1
1980	(C)CC♀, No. 7×	17	4	60.0	1415	1303	1.73±0.01	112	2.21±0.01	7.9
	(N)NN♂, No. 8	18	4	61.5	989	596	1.75±0.01	393	2.18±0.01	39.7
		19	4	59.5	850	789	1.69±0.01	61	2.20±0.02	7.2
		20	4	63.0	1335	1212	1.64±0.01	123	2.12±0.02	9.2

sponded to 7.2~39.7% of the respective total number of eggs (Table 7).

The 20 female hybrids, (C)CN♀, Nos. 1~20, were backcrossed with male *Rana nigromaculata* or *Rana plancyi chosenica*. The results are presented in Table 8.

i) Normal-sized eggs inseminated with (N)NN sperm

A total of 5628 normal-sized eggs obtained from 17 female hybrids, (C)CN♀, Nos. 1~10, 14~20, were inseminated with sperm of four male *Rana nigromaculata*. Of the respective number of eggs of each female, 25.7~100%, 4270 (75.9%) eggs in total, cleaved normally, 25.0~93.9%, 3977 (70.7%) eggs in total, hatched normally, 24.5~87.4%, 3342 (59.4%) eggs in total, became normally feeding tadpoles and 23.8~67.7%, 2676 (47.5%) eggs in total, attained completion of metamorphosis (Table 8).

ii) Large eggs inseminated with (N)NN sperm

A total of 739 large eggs obtained from five females, (C)CN♀, Nos. 16~20 were inseminated with sperm of two male *Rana nigromaculata*. Of the respective number of eggs of each female, 55.8~97.5%, 588 (79.6%) eggs in total, cleaved normally, 32.7~81.0%, 463 (62.7%) eggs in total, hatched normally, 30.8~64.5%, 351 (47.5%) eggs in total, became normally feeding tadpoles and 17.3~48.8%, 306 (41.4%) eggs in total, attained completion of metamorphosis (Table 8).

iii) Normal-sized eggs inseminated with (C)CC sperm

A total of 1718 normal-sized eggs obtained from six female hybrids, (C)CN♀, Nos. 11~16, were inseminated with sperm of two male *Rana plancyi chosenica*. Of the respective number of eggs of each female, 14.3~91.2%, 1050 (61.1%) eggs in total, cleaved normally, 14.3~76.5%, 908 (52.9%) eggs in total, hatched normally, 14.3~68.1%, 847 (49.3%) eggs in total, became normally feeding tadpoles and 14.3~65.4%, 800 (46.6%) eggs in total attained completion of metamorphosis (Table 8).

TABLE 8

Developmental capacity of the backcrosses of female hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata*

Year	Parents		Egg size	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							
1965	(C)CN 62, No. 1	(N)NN 62, No. 3	Normal	133	92 (69.2%)	92 (69.2%)	90 (67.7%)	90 (67.7%)	90 (67.7%)
	(C)CN 62, No. 2	(N)NN 62, No. 3	Normal	137	127 (92.7%)	124 (90.5%)	122 (89.1%)	116 (84.7%)	67 (48.9%)
	(C)CN 62, No. 3	(N)NN 62, No. 3	Normal	108	88 (81.5%)	86 (79.6%)	83 (76.9%)	82 (75.9%)	67 (62.0%)
	(C)CN 62, No. 4	(N)NN 62, No. 3	Normal	139	116 (83.5%)	113 (81.3%)	107 (77.0%)	98 (70.5%)	90 (64.7%)
	(C)CN 62, No. 5	(N)NN 62, No. 3	Normal	145	107 (73.8%)	97 (66.9%)	96 (66.2%)	96 (66.2%)	87 (60.0%)
1971	(C)CN 65, No. 6	(N)NN 65, No. 3	Normal	72	60 (83.3%)	41 (56.9%)	41 (56.9%)	35 (48.6%)	31 (43.1%)
	(C)CN 65, No. 7	(N)NN 65, No. 3	Normal	105	93 (88.6%)	89 (84.8%)	83 (79.0%)	44 (41.9%)	27 (25.7%)
	(C)CN 65, No. 8	(N)NN 65, No. 3	Normal	124	95 (76.6%)	90 (72.6%)	77 (62.1%)	69 (55.6%)	49 (39.5%)
	(C)CN 65, No. 9	(N)NN 65, No. 3	Normal	127	127 (100 %)	122 (96.1%)	112 (88.2%)	111 (87.4%)	42 (33.1%)
	(C)CN 65, No. 10	(N)NN 65, No. 3	Normal	163	159 (97.5%)	155 (95.1%)	153 (93.9%)	142 (87.1%)	68 (41.7%)
1975	(C)CN 73, No. 11	(C)CC 73, No. 1	Normal	282	58 (20.6%)	57 (20.2%)	57 (20.2%)	52 (18.4%)	46 (16.3%)
	(C)CN 73, No. 12	(C)CC 73, No. 1	Normal	49	7 (14.3%)	7 (14.3%)	7 (14.3%)	7 (14.3%)	7 (14.3%)
	(C)CN 73, No. 13	(C)CC 73, No. 1	Normal	191	153 (80.1%)	146 (76.4%)	131 (68.6%)	130 (68.1%)	125 (65.4%)
			Large	63	52 (82.5%)	50 (79.4%)	46 (73.0%)	40 (63.5%)	37 (58.7%)
	(C)CN 73, No. 14	(N)NN 73, No. 1	Normal	760	195 (25.7%)	192 (25.3%)	190 (25.0%)	186 (24.5%)	181 (23.8%)
		(C)CC 73, No. 2	Normal	341	311 (91.2%)	300 (88.0%)	261 (76.5%)	228 (66.9%)	214 (62.8%)
	(C)CN 73, No. 15	(N)NN 73, No. 1	Normal	363	213 (58.7%)	196 (54.0%)	195 (53.7%)	193 (53.2%)	180 (49.6%)
		(C)CC 73, No. 2	Normal	431	305 (70.8%)	300 (69.6%)	275 (63.8%)	256 (59.4%)	242 (56.1%)
	(C)CN 73, No. 16	(N)NN 73, No. 1	Normal	503	314 (62.4%)	294 (58.4%)	285 (56.7%)	256 (50.9%)	213 (42.3%)
			Large	73	51 (69.9%)	48 (65.8%)	32 (43.8%)	26 (35.6%)	19 (26.0%)
		(C)CC 73, No. 2	Normal	424	216 (50.9%)	181 (42.7%)	177 (41.7%)	174 (41.0%)	166 (39.2%)
			Large	65	42 (64.6%)	40 (61.5%)	27 (41.5%)	21 (32.3%)	15 (23.1%)
1980	(C)CN 76, No. 17	(N)NN 76, No. 1	Normal	778	715 (91.9%)	691 (88.8%)	684 (87.9%)	525 (67.5%)	412 (53.0%)
			Large	112	78 (69.6%)	52 (46.4%)	45 (40.2%)	35 (31.3%)	35 (31.3%)
	(C)CN 76, No. 18	(N)NN 76, No. 1	Normal	556	472 (84.9%)	466 (83.8%)	447 (80.4%)	334 (60.1%)	285 (51.3%)
			Large	381	312 (81.9%)	285 (74.8%)	271 (71.1%)	196 (51.4%)	186 (48.8%)
	(C)CN 76, No. 19	(N)NN 76, No. 1	Normal	311	278 (89.4%)	261 (83.9%)	254 (81.7%)	232 (74.6%)	167 (53.7%)
			Large	52	29 (55.8%)	17 (32.7%)	17 (32.7%)	16 (30.8%)	9 (17.3%)
	(C)CN 76, No. 20	(N)NN 76, No. 1	Normal	1104	1019 (92.3%)	971 (88.0%)	958 (86.8%)	733 (66.4%)	620 (56.2%)
			Large	121	118 (97.5%)	104 (86.0%)	98 (81.0%)	78 (64.5%)	57 (47.1%)

(Continued)

Total	(C)CN (17)	(N)NN (4)	Normal	5628	4270 (75.9%)	4080 (72.5%)	3977 (70.7%)	3342 (59.4%)	2676 (47.5%)
	(C)CN (5)	(N)NN (2)	Large	739	588 (79.6%)	506 (68.5%)	463 (62.7%)	351 (47.5%)	306 (41.4%)
	(C)CN (6)	(C)CC (2)	Normal	1718	1050 (61.1%)	991 (57.7%)	908 (52.9%)	847 (49.3%)	800 (46.6%)
	(C)CN (2)	(C)CC (2)	Large	128	94 (73.4%)	90 (70.3%)	73 (57.0%)	61 (47.7%)	52 (40.6%)

iv) Large eggs inseminated with (C)CC sperm

A total of 128 large eggs obtained from two females, (C)CN♀, Nos. 13 and 16, were inseminated with sperm of two male *Rana plancyi chosenica*. Of the respective numbers of eggs of the two females, 82.5% and 64.6%, 94 (73.4%) eggs in total, cleaved normally, 73.0% and 41.5%, 73 (57.0%) eggs in total, hatched normally, 63.5% and 32.3%, 61 (47.7%) eggs in total, became normally feeding tadpoles and 58.7% and 23.1%, 52 (40.6%) eggs in total, attained completion of metamorphosis (Table 8).

b. Female hybrids between female *Rana nigromaculata* and male *Rana plancyi chosenica*

Six 6-year-old female hybrids, (N)NC 65♀, Nos. 1～6, produced in 1965 from a cross, (N)NN♀, No. 3×(C)CC♂, No. 4, two 2-year-old female hybrids, (N)NC 73♀, Nos. 7 and 8, produced in 1973 from a cross (N)NN♀, No. 6×(C)CC♂, No. 6, four 5-year-old female hybrids, (N)NC 75♀, Nos. 9～12, produced in 1975 from a cross, (N)NN♀, No. 7×(C)CC♂, No. 7 and four 4-year-old female hybrids, (N)NC 76♀, Nos. 13～16 in 1976 from a cross, (N)NN♀, No. 10×(C)CC♂, No. 10, normally laid eggs after injection of frog pituitaries in the breeding seasons of 1971, 1975, 1980 and 1980, respectively. The number and size of the eggs laid by each of these 16 females are presented in Table 9.

The eggs laid in 1971 by each of the six females, (N)NC 65♀, Nos. 1～6, were

TABLE 9

Size of the eggs of female hybrids between female *Rana nigromaculata* and male *Rana plancyi chosenica*

Year	Kinds	Individual no.	Age (years)	Body length (mm)	No. of total eggs	Normal-sized eggs		Large eggs		(%)
						Number	Diameter (mm)	Number	Diameter (mm)	
1971	(N)NN♀, No. 3× (C)CC♂, No. 4	1	6	59.5	429	427	1.62±0.01	2	2.1	0.5
		2	6	64.5	652	652	1.71±0.01	0		
		3	6	63.0	1183	1180	1.68±0.01	3	2.1	0.3
		4	6	62.0	677	672	1.73±0.01	5	2.2	0.7
		5	6	64.5	1032	1029	1.70±0.01	3	2.1	0.3
		6	6	63.5	1241	1232	1.66±0.01	9	2.0	0.7
1975	(N)NN♀, No. 6× (C)CC♂, No. 6	7	2	57.0	893	892	1.64±0.01	1	2.0	0.1
		8	2	60.0	1047	833	1.69±0.01	214	2.15±0.01	20.4
1980	(N)NN♀, No. 7× (C)CC♂, No. 7	9	5	63.5	527	521	1.74±0.02	6	2.21	1.1
		10	5	64.0	374	366	1.76±0.02	8	2.24	2.1
		11	5	59.5	564	562	1.70±0.02	2	2.10	0.4
		12	5	61.0	609	604	1.72±0.02	5	2.17	0.8
	(N)NN♀, No. 10× (C)CC♂, No. 10	13	4	60.0	625	582	1.67±0.01	43	2.14±0.02	6.9
		14	4	59.5	576	462	1.74±0.01	114	2.19±0.02	19.8
		15	4	61.0	856	823	1.71±0.01	33	2.20±0.02	3.9
		16	4	58.0	475	422	1.69±0.01	53	2.25±0.02	11.2

almost uniform in size, except that 0.3~0.7% of the respective number of eggs were large eggs in five of the females. The eggs laid in 1975 by one of the two females, (N)NC 73♀, No. 7 and those laid in 1980 by four of the eight females, (N)NC 75♀, Nos. 9~12, were also almost uniform in size. In these five females, only 0.1~2.1% of the respective number of eggs were large ones. In contrast, the remaining five females, (N)NC 73♀, No. 8 and (N)NC 75♀, Nos. 13~16, laid considerably many large eggs together with abundant normal-sized ones. In these females, 3.9~20.4% of the respective number of eggs were large ones.

A total of 6256 eggs obtained from the above 16 female hybrids, (N)NC♀, Nos. 1~16, were inseminated with sperm of male *Rana nigromaculata* or *Rana plancyi chosenica*. The results of these backcrossing are presented in Table 10.

i) Normal-sized eggs inseminated with (N)NN sperm

A total of 4969 normal-sized eggs obtained from the 16 female hybrids, (N)NC♀, Nos. 1~16, were inseminated with sperm of four male *Rana nigromaculata*. Of the respective number of each female, 43.2~97.6%, 4081 (82.3%) eggs in total, cleaved normally, 35.6~85.7%, 3089 (62.2%) eggs in total, hatched normally, 28.4~82.3%, 2694 (54.2%) eggs in total, became normally feeding tadpoles and 28.1~73.8%, 2514 (50.6%) eggs in total, attained completion of metamorphosis (Table 10).

ii) Large eggs inseminated with (N)NN sperm

A total of 328 large eggs obtained from 9 female hybrids, (N)NC♀, Nos. 8~16, were inseminated with sperm of three male *Rana nigromaculata*. Of the respective

TABLE 10

Developmental capacity of the backcrosses of female hybrids between female *Rana nigromaculata* and male *Rena plancyi chosenica*

Year	Parents		Egg size	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							
1971	(N)NC 65, No. 1	(N)NN 65, No. 4	Normal	413	359 (86.9%)	322 (78.0%)	270 (65.4%)	234 (56.7%)	215 (52.1%)
	(N)NC 65, No. 2	(N)NN 65, No. 4	Normal	204	174 (85.3%)	156 (76.5%)	123 (60.3%)	116 (56.9%)	112 (54.9%)
	(N)NC 65, No. 3	(N)NN 65, No. 4	Normal	236	102 (43.2%)	95 (40.3%)	93 (39.4%)	92 (39.0%)	79 (33.5%)
	(N)NC 65, No. 4	(N)NN 65, No. 4	Normal	232	213 (91.8%)	197 (84.9%)	184 (79.3%)	177 (76.3%)	165 (71.1%)
	(N)NC 65, No. 5	(N)NN 65, No. 4	Normal	227	181 (79.7%)	172 (75.8%)	168 (74.0%)	143 (63.0%)	126 (55.5%)
	(N)NC 65, No. 6	(N)NN 65, No. 4	Normal	250	132 (52.8%)	128 (51.2%)	104 (41.6%)	94 (37.6%)	87 (34.8%)
1975	(N)NC 73, No. 7	(N)NN 73, No. 2	Normal	428	289 (67.5%)	248 (57.9%)	236 (55.1%)	228 (53.3%)	212 (49.5%)
		(C)CC 73, No. 2	Normal	464	398 (85.8%)	334 (72.0%)	324 (69.8%)	315 (67.9%)	298 (64.2%)
	(N)NC 73, No. 8	(N)NN 73, No. 2	Normal	413	403 (97.6%)	381 (92.3%)	354 (85.7%)	340 (82.3%)	305 (73.8%)
			Large	106	93 (87.7%)	80 (75.5%)	73 (68.9%)	67 (63.2%)	61 (57.5%)
		(C)CC 73, No. 2	Normal	400	238 (59.5%)	184 (46.0%)	184 (46.0%)	182 (45.5%)	182 (45.5%)
			Large	95	71 (74.7%)	54 (56.8%)	47 (49.5%)	36 (37.9%)	33 (34.7%)

(Continued)

1980	(N)NC 75, No. 9	(N)NN 76, No. 2	Normal	320	312 (97.5%)	132 (41.3%)	114 (35.6%)	91 (28.4%)	90 (28.1%)
			Large	6	6 (100 %)	3 (50.0%)	3 (50.0%)	3 (50.0%)	3 (50.0%)
	(N)NC 75, No. 10	(N)NN 76, No. 2	Normal	182	171 (94.0%)	99 (54.4%)	87 (47.8%)	79 (43.4%)	75 (41.2%)
			Large	4	4 (100 %)	3 (75.0%)	2 (50.0%)	2 (50.0%)	2 (50.0%)
	(N)NC 75, No. 11	(N)NN 76, No. 2	Normal	189	163 (86.2%)	151 (79.9%)	103 (54.5%)	97 (51.3%)	97 (51.3%)
			Large	2	2 (100 %)	2 (100 %)	2 (100 %)	2 (100 %)	2 (100 %)
	(N)NC 75, No. 12	(N)NN 76, No. 2	Normal	302	273 (90.4%)	259 (85.8%)	216 (71.5%)	198 (65.6%)	192 (63.6%)
			Large	5	4 (80.0%)	4 (80.0%)	4 (80.0%)	4 (80.0%)	3 (60.0%)
	(N)NC 76, No. 13	(N)NN 76, No. 3	Normal	550	400 (72.7%)	382 (69.5%)	374 (68.0%)	198 (36.0%)	186 (33.8%)
			Large	43	39 (90.7%)	36 (83.7%)	26 (60.5%)	22 (51.2%)	22 (51.2%)
	(N)NC 76, No. 14	(N)NN 76, No. 3	Normal	396	385 (97.2%)	361 (91.2%)	252 (63.6%)	224 (56.6%)	207 (52.3%)
			Large	95	93 (97.9%)	87 (91.6%)	85 (89.5%)	68 (71.6%)	60 (63.2%)
	(N)NC 76, No. 15	(N)NN 76, No. 3	Normal	431	364 (84.5%)	344 (79.8%)	266 (61.7%)	253 (58.7%)	241 (55.9%)
			Large	16	11 (68.8%)	5 (31.3%)	4 (25.0%)	3 (18.8%)	3 (18.3%)
	(N)NC 76, No. 16	(N)NN 76, No. 3	Normal	196	167 (85.2%)	161 (82.1%)	145 (74.0%)	130 (66.3%)	125 (63.8%)
			Large	51	38 (74.5%)	30 (58.8%)	30 (58.8%)	27 (52.9%)	21 (41.2%)
Total	(N)NC (16)	(N)NN (4)	Normal	4969	4088 (82.3%)	3588 (72.2%)	3089 (62.2%)	2694 (54.2%)	2514 (50.6%)
	(N)NC (9)	(N)NN (3)	Large	328	290 (88.4%)	250 (76.2%)	229 (69.8%)	198 (60.4%)	177 (54.0%)
	(N)NC (2)	(C)CC (1)	Normal	864	636 (73.6%)	518 (60.0%)	508 (58.8%)	497 (57.5%)	480 (55.6%)
	(N)NC (1)	(C)CC (1)	Large	95	71 (74.7%)	54 (56.8%)	47 (49.5%)	36 (37.9%)	33 (34.7%)

number of eggs of each female, 68.8～100%, 290 (88.4%) eggs in total, cleaved normally, 25.0～100%, 229 (69.8%) eggs in total, hatched normally, 18.8～100 %, 198 (60.4%) eggs in total, became normally feeding tadpoles and 18.3～100%, 177 (54.0%) eggs in total, attained completion of metamorphosis (Table 10).

iii) Normal-sized eggs inseminated with (C)CC sperm

A total of 864 normal-sized eggs obtained from two female hybrids, (N)NC 73♀, Nos. 7 and 8, were inseminated with sperm of a male *Rana plancyi chosenica*. Of the respective numbers of eggs of the two females, 85.8% and 59.5%, 636 (73.6%) eggs in total, cleaved normally, 69.8% and 46.0%, 508 (58.8%) eggs in total, hatched normally, 67.9% and 45.5%, 497 (57.5%) eggs in total, became normally feeding tadpoles and 64.2% and 45.5%, 480 (55.6%) eggs in total, attained completion of metamorphosis (Table 10).

iv) Large eggs inseminated with (C)CC sperm

Of 95 large eggs obtained from a female hybrid, (N)NC 73♀, No. 8, 71 (74.7%) cleaved normally after inseminating with sperm of a male *Rana plancyi chosenica*. Thereafter, 47 (49.5%) hatched normally, 36 (37.9%) became normally feeding tadpoles and 33 (34.7%) attained the completion of metamorphosis (Table 10).

2. Chromosomes

a. Backcrosses from (C)CN♀ × (N)NN♂ or (C)CC♂

The chromosomes of 1017 feeding tadpoles produced in 1971, 1975 and 1980 from 9 female hybrids, (C)CN♀, Nos. 6, 8, 14~20, by backcrossing with three male *Rana nigromaculata* or a male *Rana plancyi chosenica* were examined in the tail-tips by the squash method with water-pretreatment. The results are presented in Table 11. It was found that 674 (66.3%) were diploid, 322 (31.7%) were triploid, six (0.6%) were 27 (2n+1), two (0.2%) were 28 (2n+2), two (0.2%) were 34 (3n−5), two (0.2%) were 37 (3n−2), seven (0.7%) were 38 (3n−1), one (0.1%) was 40 (3n+1) and the remainder (0.1%) was 41 (3n+2) in chromosome number.

i) Normal-sized eggs inseminated with (N)NN sperm

Of 531 feeding tadpoles produced from normal-sized eggs of nine female hybrids, (C)CN♀ Nos. 6, 8, 14~20, by backcrossing with three male *Rana nigromaculata*, 482 were diploid, 31 were triploid, five were 27 (2n+1), two were 28 (2n+2), one was 34 (3n−5), two were 37 (3n−2), six were 38 (3n−1), one was 40 (3n+1) and the remainder was 41 (3n+2) in chromosome number.

TABLE 11

Chromosome number of the backcrosses of female hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata*

Year	Parents		Egg size	Number of tadpoles									
				Ana-lyzed	Number of chromosomes								
	Female	Male			26 (2n)	27	28	34	37	38	39 (3n)	40	41
1971	(C)CN 65, No. 6	(N)NN 65, No. 3	Normal	35	32	1					2		
	(C)CN 65, No. 8	(N)NN 65, No. 3	Normal	69	62	1	1			1	3	1	
1975	(C)CN 73, No. 14	(N)NN 73, No. 1	Normal	50	46						4		
		(C)CC 73, No. 2	Normal	60	51						9		
	(C)CN 73, No. 15	(N)NN 73, No. 1	Normal	50	47						3		
		(C)CC 73, No. 2	Normal	55	51						4		
	(C)CN 73, No. 16	(N)NN 73, No. 1	Normal	101	94	1	1		1	1	3		
			Large	26	0					1	25		
		(C)CC 73, No. 2	Normal	95	90	1					4		
			Large	21	0			1			20		
1980	(C)CN 76, No. 17	(N)NN 76, No. 1	Normal	50	46					1	3		
			Large	35	0						35		
	(C)CN 76, No. 18	(N)NN 76, No. 1	Normal	60	52	1			1	1	5		
			Large	100	0						100		
	(C)CN 76, No. 19	(N)NN 76, No. 1	Normal	56	48	1				1	6		
			Large	16	0						16		
	(C)CN 76, No. 20	(N)NN 76, No. 1	Normal	60	55			1		1	2		1
			Large	78	0						78		

ii) Large eggs inseminated with (N)NN sperm

Of 255 feeding tadpoles produced from large eggs of five female hybrids, (C)CN♀ Nos. 16~20, by backcrossing with two male *Rana nigromaculata*, 254 were triploid and the remainder was 38 (3n−1) in chromosome number.

iii) Normal-sized eggs inseminated with (C)CC sperm

Of 210 feeding tadpoles produced from normal-sized eggs of three female hybrids, (C)CN 73♀, Nos. 14~16, by backcrossing with a male *Rana plancyi chosenica*, 192 were diploid, 17 were triploid and the remainder was 27 (2n+1) in chromosome number.

iv) Large eggs inseminated with (C)CC sperm

Of 21 feeding tadpoles produced from large eggs of a female hybrid, (C)CN 73♀, No. 16, by backcrossing with a male *Rana plancyi chosenica*, 20 were triploid and the remainder was 34 (3n−5) in chromosome number.

b. Backcrosses from (N)NC♀ × (N)NN♂ or (C)CC♂

The chromosomes of 1312 feeding tadpoles produced in 1971, 1975 and 1980 from 12 female hybrids, (N)NC♀, Nos. 4, 5, 7~16, by backcrossing with four male *Rana nigromaculata* or a male *Rana plancyi chosenica* were examined in the tail-tips by the squash method with water-pretreatment. The results are presented in Table 12. It was found that 1013 (77.2%) were diploid, 271 (20.7%) were triploid, 9 (0.7%) were 27 (2n+1), four (0.3%) were 28 (2n+2), one (0.1%) was 29 (2n+3), one (0.1%) was 34 (3n−5), one (0.1%) was 36 (3n−3), two (0.2%)

TABLE 12

Chromosome number of the backcrosses of female hybrids between female *Rana nigromaculata* and male *Rana plancyi chosenica*

Year	Parents		Egg size	Number of tadpoles												
				Ana-lyzed	Number of chromosomes											
	Female	Male			26 (2n)	27	28	29	34	35	36	37	38	39 (3n)	41	42
1971	(N)NC 65, No. 4	(N)NN 65, No. 4	Normal	105	103	1								1		
	(N)NC 65, No. 5	(N)NN 65, No. 4	Normal	94	91	1							1	1		
1975	(N)NC 73, No. 7	(N)NN 73, No. 2	Normal	93	90	1								2		
		(C)CC 73, No. 2	Normal	99	95	1	1						1	1		
	(N)NC 73, No. 8	(N)NN 73, No. 2	Normal	125	105	3	1		1			1	2	11	1	
			Large	67	0									67		
		(C)CC 73, No. 2	Normal	142	126	1	1	1			1	1	2	8		1
			Large	33	0									33		
1980	(N)NC 75, No. 9	(N)NN 76, No. 2	Normal	91	85									6		
			Large	3	0									3		
	(N)NC 75, No. 10	(N)NN 76, No. 2	Normal	79	67	1							1	10		
			Large	2	0									2		
	(N)NC 75, No. 11	(N)NN 76, No. 2	Normal	97	97									0		
			Large	2	0									2		
	(N)NC 75, No. 12	(N)NN 76, No. 2	Normal	50	47		1							2		
			Large	3	0									3		
	(N)NC 76, No. 13	(N)NN 76, No. 3	Normal	50	48									2		
			Large	22	0									22		
	(N)NC 76, No. 14	(N)NN 76, No. 3	Normal	20	19								1	0		
			Large	65	0									65		
	(N)NC 76, No. 15	(N)NN 76, No. 3	Normal	20	20									0		
			Large	3	0									3		
	(N)NC 76, No. 16	(N)NN 76, No. 3	Normal	20	20									0		
			Large	27	0									27		

were 37 (3n−2), eight (0.6%) were 38 (3n−1), one (0.1%) was 41 (3n+2) and the remainder (0.1%) was 42 (3n+3) in chromosome number.

i) Normal-sized eggs inseminated with (N)NN sperm

Of 844 feeding tadpoles produced from normal-sized eggs of 12 female hybrids, (N)NC♀, Nos. 4, 5, 7~16, by backcrossing with four male *Rana nigromaculata*, 792 were diploid, 35 were triploid, seven were 27 (2n+1), two were 28 (2n+2), one was 34 (3n−5), one was 37 (3n−2), five were 38 (3n−1) and the remainder was 41 (3n+2) in chromosome number.

ii) Large eggs inseminated with (N)NN sperm

A total of 194 feeding tadpoles produced from large eggs of 9 female hybrids, (N)NC♀, Nos. 8~16, by backcrossing with three male *Rana nigromaculata*, were all triploids.

iii) Normal-sized eggs inseminated with (C)CC sperm

Of 241 feeding tadpoles produced from normal-sized eggs of two female hybrids, (N)NC 73♀, Nos. 7 and 8, backcrossed with a male *Rana plancyi chosenica*, 221 were diploid, 9 were triploid, two were 27 (2n+1), two were 28 (2n+2), one was 29 (2n+3), one was 36 (3n−3), one was 37 (3n−2), three were 38 (3n−1) and the remainder was 42 (3n+3) in chromosome number.

iv) Large eggs inseminated with (C)CC sperm

Thirty-three feeding tadpoles produced from large eggs of a female hybrid, (N)NC 73♀, No. 8, backcrossed with a male *Rana plancyi chosenica* were all triploids.

3. Sex and external characters

a. Backcrosses of female hybrids, (C)CN

As stated above, of 1017 feeding tadpoles produced in 1971, 1975 and 1980 from 9 female hybrids, (C)CN♀, Nos. 6, 8, 14~20, by backcrossing with three male *Rana nigromaculata* or a male *Rana plancyi chosenica*, 674 were diploids, while 322 others were triploids (Table 11). Of these feeding tadpoles, all the diploids and 274 triploids raised from large eggs were continuously reared, while 48 triploids raised from normal-sized eggs were abandoned. Of the 948 tadpoles including diploids and triploids, 858 completed metamorphosis, and eventually, 656 attained sexual maturity. Of these mature backcrosses, 332 were females and 324 (49.4%) were males (Table 13).

i) Diploids raised from normal-sized eggs inseminated with (N)NN sperm

Of 482 diploid tadpoles raised from normal-sized eggs of 9 female hybrids, (C)CN♀, Nos. 6, 8, 14~20, 451 completed metamorphosis, and 341 attained sexual maturity. Of these mature diploids, 176 were females and 165 (48.4%) were males.

The mature diploid backcrosses were variable in external character from those closely resembling *Rana nigromaculata* to those being very similar to diploid (C)CN hybrids. However, the pale dorsomedian stripe was distinctly observable in all the diploid backcrosses.

ii) Triploids raised from large eggs inseminated with (N)NN sperm

TABLE 13

Sex of the mature backcrosses of female hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata*

Year	Parents		Egg size	Ploidy	No. of metamorphosed frogs	No. of mature frogs		
	Female	Male				Total	Female	Male (%)
1971	(C)CN 65, No. 6	(N)NN 65, No. 3	Normal	2n	31	27	12	15 (55.6)
	(C)CN 65, No. 8	(N)NN 65, No. 3	Normal	2n	49	36	19	17 (47.2)
1975	(C)CN 73, No. 14	(N)NN 73, No. 1	Normal	2n	44	36	18	18 (50.0)
		(C)CC 73, No. 2	Normal	2n	47	28	15	13 (46.4)
	(C)CN 73, No. 15	(N)NN 73, No. 1	Normal	2n	45	27	13	14 (51.9)
		(C)CC 73, No. 2	Normal	2n	42	28	12	16 (57.1)
	(C)CN 73, No. 16	(N)NN 73, No. 1	Normal	2n	89	44	24	20 (45.5)
			Large	3n	19	19	12	7 (36.8)
		(C)CC 73, No. 2	Normal	2n	83	69	33	36 (52.2)
			Large	3n	15	8	3	5 (62.5)
1980	(C)CN 76, No. 17	(N)NN 76, No. 1	Normal	2n	46	40	21	19 (47.5)
			Large	3n	35	35	18	17 (48.6)
	(C)CN 76, No. 18	(N)NN 76, No. 1	Normal	2n	48	45	27	18 (40.0)
			Large	3n	100	72	37	35 (48.6)
	(C)CN 76, No. 19	(N)NN 76, No. 1	Normal	2n	46	41	21	20 (48.8)
			Large	3n	9	6	3	3 (50.0)
	(C)CN 76, No. 20	(N)NN 76, No. 1	Normal	2n	53	45	21	24 (53.3)
			Large	3n	57	50	23	27 (54.0)
Total	(C)CN (9)	(N)NN (3)	Normal	2n	451	341	176	165 (48.4)
	(C)CN (5)	(N)NN (2)	Large	3n	220	182	93	89 (48.9)
	(C)CN (3)	(C)CC (1)	Normal	2n	172	125	60	65 (52.0)
	(C)CN (1)	(C)CC (1)	Large	3n	15	8	3	5 (62.5)

Of 254 triploid tadpoles raised from large eggs of five female hybrids, (C)CN♀, Nos. 16～20, 220 completed metamorphosis, and 182 attained sexual maturity. Of these mature triploids, 93 were females and 89 (48.9%) were males.

In contrast to the diploid backcrosses, the mature triploid backcrosses were uniform in external character and intermediate between *Rana nigromaculata* and diploid (C)CN hybrids. All the triploid backcrosses had a distinct dorsomedian stripe. As they did not differ in external character from (N)NNC allotriploids produced from eggs of female *Rana nigromaculata* by refrigeration after inseminating with sperm of *Rana plancyi chosenica*, they were also considered to be (C)CNN allotriploids.

iii) Diploids raised from normal-sized eggs inseminated with (C)CC sperm

Of 192 diploid tadpoles raised from normal-sized eggs of three female hybrids, (C)CN 73♀, Nos. 14～16, 172 completed metamorphosis, and 125 attained sexual maturity. Of these mature diploids, 60 were females and 65 (52.0%) were males.

The mature diploid backcrosses were not uniform in external character.

While 66 of them had a distinct dorsomedian stripe, 59 had none. Regardless of existence of the dorsomedian stripe, they were variable in external character from those closely resembling *Rana plancyi chosenica* to those being very similar to diploid (C)CN hybrids.

iv) Triploids raised from large eggs inseminated with (C)CC sperm

Of 20 triploid tadpoles raised from large eggs of a female hybrid, (C)CN 73♀, No. 16, 15 completed metamorphosis, and eight attained sexual maturity. Of these mature triploids, three were females and five (62.5%) were males.

The mature triploid backcrosses were uniform in external character and intermediate between *Rana plancyi chosenica* and diploid (C)CN hybrids. All of them had a dorsomedian stripe. They were very similar in external character to (C)CCN allotriploids produced from eggs of *Rana plancyi chosenica* by refrigeration after inseminating with sperm of male *Rana nigromaculata*. Thus, they were considered to be (C)CCN allotriploids.

b. Backcrosses of female (N)NC hybrids

Of 1312 feeding tadpoles produced in 1971, 1975 and 1980 from 12 female hybrids, (N)NC♀, Nos. 4, 5, 7~16, by backcrossing with four male *Rana nigromaculata* or a *Rana plancyi chosenica*, 1013 were diploids, while 271 others were triploids (Table 12). Of a total of 1284 tadpoles including diploids and triploids, 1210 completed metamorphosis, and 936 attained sexual maturity. Of these mature backcrosses, 461 were females and 475 (50.7%) were males (Table 14).

i) Diploids raised from normal-sized eggs inseminated with (N)NN sperm

Of 792 diploid tadpoles raised from normal-sized eggs of 12 female hybrids, (N)NC♀, Nos. 4, 5, 7~16, 780 completed metamorphosis, and 580 attained sexual maturity. Of these mature diploid backcrosses, 281 were females and 299 (51.6%) were males.

The mature diploid backcrosses were variable in external character from those being very similar to *Rana nigromaculata* to those being like diploid (N)NC hybrids.

ii) Triploids raised from large eggs inseminated with (N)NN sperm

Of 194 triploid tadpoles raised from large eggs of nine female hybrids, (N)NC♀, Nos. 8~16, 177 completed metamorphosis, and 143 attained sexual maturity. Of these mature triploid backcrosses, 73 were females and 70 (49.0%) were males.

The mature triploid backcrosses were uniform in external character and intermediate between *Rana nigromaculata* and diploid (N)NC hybrids. They did not differ in external character from (N)NNC allotriploids obtained from eggs of *Rana nigromaculata* by refrigeration after inseminating with sperm of *Rana plancyi chosenica*. Thus, they were considered to be (N)NNC allotriploids.

iii) Diploids raised from normal-sized eggs inseminated with (C)CC sperm

Of 221 diploid tadpoles raised from normal-sized eggs of two female hybrids, (N)NC 73♀, Nos. 7 and 8, 220 completed metamorphosis, and 185 attained sexual maturity. Of these mature diploid backcrosses, 92 were females and 93 (50.3%) were males.

The mature diploid backcrosses were variable in external character from those

TABLE 14

Sex of the mature backcrosses of female hybrids between female *Rana nigromaculata* and male *Rana plancyi chosenica*

Year	Parents		Egg size	Ploidy	No. of metamorphosed frogs	No. of mature frogs		
	Female	Male				Total	Female	Male (%)
1971	(N)NC 65, No. 4	(N)NN 65, No. 4	Normal	2n	97	57	29	28 (49.1)
	(N)NC 65, No. 5	(N)NN 65, No. 4	Normal	2n	87	66	31	35 (53.0)
1975	(N)NC 73, No. 7	(N)NN 73, No. 2	Normal	2n	90	49	26	23 (46.9)
		(C)CC 73, No. 2	Normal	2n	94	82	40	42 (51.2)
	(N)NC 73, No. 8	(N)NN 73, No. 2	Normal	2n	105	91	44	47 (51.6)
			Large	3n	61	45	21	24 (53.3)
		(C)CC 73, No. 2	Normal	2n	126	103	52	51 (49.5)
			Large	3n	33	28	15	13 (46.4)
1980	(N)NC 76, No. 9	(N)NN 76, No. 2	Normal	2n	83	54	28	26 (48.1)
			Large	3n	3	2	1	1
	(N)NC 76, No. 10	(N)NN 76, No. 2	Normal	2n	67	47	22	25 (53.2)
			Large	3n	2	2	1	1
	(N)NC 76, No. 11	(N)NN 76, No. 2	Normal	2n	97	92	41	51 (55.4)
			Large	3n	2	1	1	0
	(N)NC 76, No. 12	(N)NN 76, No. 2	Normal	2n	47	32	17	15 (46.9)
			Large	3n	3	3	1	2
	(N)NC 76, No. 13	(N)NN 76, No. 3	Normal	2n	48	43	21	22 (51.2)
			Large	3n	22	20	10	10 (50.0)
	(N)NC 76, No. 14	(N)NN 76, No. 3	Normal	2n	19	14	6	8 (57.1)
			Large	3n	60	53	28	25 (47.2)
	(N)NC 76, No. 15	(N)NN 76, No. 3	Normal	2n	20	18	8	10 (55.6)
			Large	3n	3	2	1	1
	(N)NC 76, No. 16	(N)NN 76, No. 3	Normal	2n	20	17	8	9 (52.9)
			Large	3n	21	15	9	6 (40.0)
Total	(N)NC (12)	(N)NN (4)	Normal	2n	780	580	281	299 (51.6)
	(N)NC (9)	(N)NN (3)	Large	3n	177	143	73	70 (49.0)
	(N)NC (2)	(C)CC (1)	Normal	2n	220	185	92	93 (50.3)
	(N)NC (1)	(C)CC (1)	Large	3n	33	28	15	13 (46.4)

closely resembling *Rana plancyi chosenica* to those being very similar to diploid (N)NC hybrids. While 92 had a distinct dorsomedian stripe, the other 93 had none.

iv) Triploids raised from large eggs inseminated with (C)CC sperm

A total of 33 triploid tadpoles raised from large eggs of a female hybrid, (N)NC 73♀, No. 8, all completed metamorphosis, and 28 attained sexual maturity. Of these mature triploid backcrosses, 15 were females and 13 (46.4%) were males.

The mature triploid backcrosses were uniform in external character and intermediate between *Rana plancyi chosenica* and diploid (N)NC hybrids. All of them had a dorsomedian stripe. As they did not differ in external character from (N)NCC allotriploids produced from matings between female (N)NNCC

amphidiploids and male *Rana plancyi chosenica*, they were also considered to be (N)NCC allotriploids.

IV. Reproductive capacity of male backcrosses from female hybrids

1. Diploid backcrosses

a. Backcrosses from (C)CN♀ × (N)NN♂

Three 4-year-old diploid male backcrosses, Nos. 1~3, produced in 1971 from a female hybrid, (C)CN 65♀, No. 6 by mating with a male *Rana nigromaculata*, (N)NN 65♂, No. 3, and three others, Nos. 4~6, produced in 1971 from a female hybrid, (C)CN 65♀, No. 8 by mating with the same male, (N)NN 65♂, No. 3, were mated in 1975 with two female *Rana nigromaculata*, (N)NN 73♀, Nos. 1 and 2 (Table 15). It was found that 44.3~83.4% of the respective number of eggs, 914 (61.7%) of 1482 eggs in total, cleaved normally, 38.0~81.6%, 864 (58.3%) in total, hatched normally, 33.5~81.6%, 816 (55.1%) became normally feeding tadpoles, being 20~30 mm in total length, and 31.4~56.7%, 640 (43.2%) in total, attained completion of metamorphosis. The metamorphosed frogs corresponded to 55.0~93.1%, 70.0% on the average, of the normally cleaved eggs.

In the control matings between the above two females, (N)NN 73♀, Nos. 1 and 2, and a male *Rana nigromaculata*, (N)NN 73♂, No. 3, 113 (93.4%) of 121 eggs cleaved normally, 107 (88.4%) hatched normally, 105 (86.8%) became

TABLE 15

Developmental capacity of the offspring of diploid males of backcrosses, (C)CN♀ × (N)NN♂, by mating with female *Rana nigromaculata* and the controls

Year	Parents		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	PMC
	Female	Male							
1975	(N)NN 73, Nos. 1, 2	(N)NN 73, No. 3	121	113 (93.4%)	108 (89.3%)	107 (88.4%)	105 (86.8%)	101 (83.5%)	89.4
	(N)NN 73, Nos. 1, 2	[(C)CN 65♀, No. 6 × (N)NN 65♂, No. 3]							
		71(2n), No. 1	277	231 (83.4%)	227 (81.9%)	226 (81.6%)	226 (81.6%)	127 (45.8%)	55.0
		71(2n), No. 2	179	130 (72.6%)	125 (69.8%)	122 (68.2%)	121 (67.6%)	84 (46.9%)	64.6
		71(2n), No. 3	215	111 (51.6%)	105 (48.8%)	104 (48.4%)	102 (47.4%)	73 (34.0%)	65.8
	(N)NN 73, Nos. 1, 2	[(C)CN 65♀, No. 8 × (N)NN 65♂, No. 3]							
		71(2n), No. 4	193	121 (62.7%)	115 (59.6%)	114 (59.1%)	92 (47.7%)	90 (46.6%)	74.4
		71(2n), No. 5	334	148 (44.3%)	141 (42.2%)	127 (38.0%)	112 (33.5%)	105 (31.4%)	70.9
		71(2n), No. 6	284	173 (60.9%)	172 (60.6%)	171 (60.2%)	163 (57.4%)	161 (56.7%)	93.1
Total	(N)NN (2)	71(2n), Nos. 1~6	1482	914 (61.7%)	885 (59.7%)	864 (58.3%)	816 (55.1%)	640 (43.2%)	70.0

PMC, Percentage of metamorphosed frogs to normally cleaved eggs

normally feeding tadpoles and 101 (83.5%) attained completion of metamorphosis. The metamorphosed frogs corresponded to 89.4% of the normally cleaved eggs.

b. Backcrosses from (N)NC♀ × (N)NN♂

Three 4-year-old diploid male backcrosses, Nos. 1~3, produced in 1971 from a female hybrid, (N)NC 65♀, No. 4, by mating with a male *Rana nigromaculata*, (N)NN 65♂, No. 4, and three others, Nos. 4~6, produced from a female hybrid, (N)NC 65♀, No. 5, by mating with the same male, (N)NN 65♂, No. 4, were mated in 1975 with a female *Rana nigromaculata*, (N)NN 73♀, No. 3 (Table 16). It was found that 61.4~88.0% of the respective number of eggs, 702 (73.3%) of 958 eggs in total, cleaved normally, 38.6~81.4%, 635 (66.3%) in total, hatched normally, 36.4~79.6%, 565 (59.0%) in total, became normally feeding tadpoles, being 20~30 mm in total length, and 31.5~69.2%, 482 (50.3%) in total, attained completion of metamorphosis. The metamorphosed frogs corresponded to 35.8~82.3%, 68.7% on the average, of the normally cleaved eggs.

In the control mating between the above female *Rana nigromaculata*, (N)NN 73♀, No. 3 and a male *Rana nigromaculata* (N)NN 73♂, No. 4, 124 (84.9%) of 146 eggs cleaved normally, 119 (81.5%) hatched normally, 110 (75.3%) became normally feeding tadpoles and 97 (66.4%) attained completion of metamorphosis. These metamorphosed frogs corresponded to 78.2% of the normally cleaved eggs.

TABLE 16

Developmental capacity of the offspring of diploid males of backcrosses, (N)NC♀ × (N)NN♂, by mating with female *Rana nigromaculata* and the controls

Year	Parents		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	PMC
	Female	Male							
1975	(N)NN 73, No. 3	(N)NN 73, No. 4	146	124 (84.9%)	121 (82.9%)	119 (81.5%)	110 (75.3%)	97 (66.4%)	78.2
	(N)NN 73, No. 3	[(N)NC 65♀, No. 4 × (N)NN 65♂, No. 4]							
		71(2n), No. 1	92	81 (88.0%)	59 (64.1%)	59 (64.1%)	58 (63.0%)	29 (31.5%)	35.8
		71(2n), No. 2	43	33 (76.7%)	25 (58.1%)	23 (53.5%)	23 (53.5%)	21 (48.8%)	63.6
		71(2n), No. 3	44	27 (61.4%)	18 (40.9%)	17 (38.6%)	16 (36.4%)	16 (36.4%)	59.3
	(N)NN 73, No. 3	[(N)NC 65♀, No. 5 × (N)NN 65♂, No. 4]							
		71(2n), No. 4	221	186 (84.2%)	185 (83.7%)	180 (81.4%)	176 (79.6%)	153 (69.2%)	82.3
		71(2n), No. 5	326	204 (62.6%)	201 (61.7%)	199 (61.0%)	152 (46.6%)	134 (41.1%)	65.7
		71(2n), No. 6	232	171 (73.7%)	163 (70.3%)	157 (67.7%)	140 (60.3%)	129 (55.6%)	75.4
Total	(N)NN (1)	71(2n), Nos. 1~6	958	702 (73.3%)	651 (68.0%)	635 (66.3%)	565 (59.0%)	482 (50.3%)	68.7

PMC, Percentage of metamorphosed frogs to normally cleaved eggs

2. Triploid backcrosses

a. Backcrosses from (C)CN♀ × (N)NN♂

Five 2-year-old triploid male backcrosses, Nos. 1~5, produced in 1975 from a

TABLE 17

Developmental capacity of the offspring of triploid males of backcrosses, (C)CN♀ × (N)NN♂, by mating with female *Rana nigromaculata* and the controls

Year	Parents		No. of eggs	No. of normal cleavages	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally feeding tadpoles
	Female	Male					
1977	(N)NN 75,	(N)NN 75, No. 1	142	125 (88.0%)	121 (85.2%)	119 (83.8%)	115 (81.0%)
	(N)NN 75, Nos. 1, 2	[(C)CN 73♀, No. 16 × (N)NN 73♂, No. 2]					
		75 (3n), No. 1	427	12 (2.8%)	3 (0.7%)	2 (0.5%)	1 (0.2%)
		75 (3n), No. 2	600	30 (5.0%)	7 (1.2%)	5 (0.8%)	1 (0.2%)
		75 (3n), No. 3	500	0	0	0	0
		75 (3n), No. 4	500	7 (1.4%)	5 (1.0%)	1 (0.2%)	0
		75 (3n), No. 5	500	4 (0.8%)	2 (0.4%)	2 (0.4%)	1 (0.2%)
Total	(N)NN (2)	75 (3n), Nos. 1~5	2527	53 (2.1%)	17 (0.7%)	10 (0.4%)	3 (0.12%)

female hybrid, (C)CN 73♀, No. 16, by mating with a male *Rana nigromaculata*, (N)NN 73♂, No. 2, were mated in 1977 with two female *Rana nigromaculata*, (N)NN 75♀, Nos. 1 and 2 (Table 17). It was found that only 53 (2.1%) of 2527 eggs in total cleaved normally. Of these normally cleaved eggs, four became abnormal at the blastula stage, 27 showed incomplete invagination at the gastrula stage and five became microcephalous and edematous at the early embryonic stage, while 17 (0.7%) were normal at the tail-bud stage. Thereafter, seven died of edema or underdevelopment, while 10 (0.4%) hatched normally. Of the latter, only three became normally feeding tadpoles, being 20~30 mm in total length, while the others died of edema or some other abnormalities without taking food. No tadpoles attained completion of metamorphosis. One of the three feeding tadpoles was a diploid and the other two were triploids.

In the control mating between the above female *Rana nigromaculata*, (N)NN 75♀, No. 1 and a male *Rana nigromaculata*, (N)NN 75♂, No. 1, 125 (88.0%) of 142 eggs cleaved normally, 119 (83.8%) hatched normally and 115 (81.0%) became normally feeding tadpoles.

b. Backcrosses from (N)NC♀ × (N)NN♂

Five 2-year-old triploid male backcrosses, Nos. 1~5, produced in 1975 from a female hybrid, (N)NC 73♀, No. 8, by mating with a male *Rana nigromaculata*, (N)NN 73♂, No. 2, were mated in 1977 with two female *Rana nigromaculata*, (N)NN 75♀, Nos. 3 and 4 (Table 18). The results indicated that 26 (1.1%) of 2447 eggs in total cleaved normally. Of these normally cleaved eggs, 12 showed incomplete invagination at the gastrula stage and became abnormal, while the other 14 (0.6%) became normal tail-bud embryos, of which eight (0.3%) hatched

TABLE 18

Developmental capacity of the offspring of triploid males of backcrosses, (N)NC♀ × (N)NN♂, by mating with female *Rana nigromaculata* and the controls

Year	Parents		No. of eggs	No. of normal cleavages	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally feeding tadpoles
	Female	Male					
1977	(N)NN 75, Nos. 3, 4	(N)NN 75, No. 2	233	217 (93.1%)	204 (87.6%)	199 (85.4%)	192 (82.4%)
	(N)NN 75, Nos. 3, 4	[(N)NC 73♀, No. 8 × (N)NN 73♂, No. 2] 75 (3n), No. 1	467	3 (0.6%)	1 (0.2%)	1 (0.2%)	0
		75 (3n), No. 2	480	11 (2.3%)	5 (1.0%)	3 (0.6%)	1 (0.2%)
		75 (3n), No. 3	500	5 (1.0%)	4 (0.8%)	2 (0.4%)	0
		75 (3n), No. 4	500	0	0	0	0
		75 (3n), No. 5	500	7 (1.4%)	4 (0.8%)	2 (0.4%)	1 (0.2%)
Total	(N)NN (2)	75 (3n), Nos. 1～5	2447	26 (1.1%)	14 (0.6%)	8 (0.3%)	2 (0.08%)

normally and the other six had ill-developed gills at the hatching stage and died of edema. Only two of the normally hatched embryos became normally feeding tadpoles, while the other six died of edema without taking food. No tadpoles attained completion of metamorphosis. Of the two feeding tadpoles, one was a diploid and the other was a triploid.

In the control matings between the above two female *Rana nigromaculata*, (N)NN 75♀, Nos. 3 and 4, and a male *Rana nigromaculata*, (N)NN 75♂, No. 2, 217 (93.1%) of 233 eggs cleaved normally, 199 (85.4%) hatched normally and 192 (82.4%) became normally feeding tadpoles.

V. Reproductive capacity of female backcrosses from female hybrids

1. Diploid backcrosses

a. Backcrosses from (C)CN♀ × (N)NN♂

Five 2-year-old diploid female backcrosses, Nos. 1～5, produced in 1975 from a female hybrid, (C)CN 73♀, No. 16, by mating with a male *Rana nigromaculata*, (N)NN 73♂, No. 2, were mated in 1977 with a male *Rana nigromaculata*, (N)NN 75♂, No. 1 (Table 19). It was found that 67.6～93.5% of the respective number of eggs, 2140 (73.8%) of 2899 eggs in total, cleaved normally, 49.0～74.9%, 1748 (60.3%) in total, hatched normally, 25.5～53.7%, 1112 (38.4%) in total, became normally feeding tadpoles and 23.9～49.1%, 973 (33.6%) in total, attained completion of metamorphosis. The metamorphosed frogs corresponded to 33.3～65.3%, 45.5% on the average, of the normally cleaved eggs. It was evident that the female backcrosses were considerably inferior in reproductive

TABLE 19

Developmental capacity of the offspring of diploid females of backcrosses, (C)CN♀ × (N)NN♂ or × (C)CC♂, by mating with male *Rana nigromaculata* or *Rana plancyi chosenica*

Year	Parents		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	PMC
	Female	Male							
1977	[(C)CN 73♀, No. 16 × (N)NN 73♂, No. 2]								
	75(2n), No. 1	(N)NN 75, No. 1	901	646 (71.7%)	619 (68.7%)	570 (63.3%)	230 (25.5%)	215 (23.9%)	33.3
	75(2n), No. 2	(N)NN 75, No. 1	615	416 (67.6%)	387 (62.9%)	347 (56.4%)	252 (41.0%)	230 (37.4%)	55.3
	75(2n), No. 3	(N)NN 75, No. 1	521	487 (93.5%)	447 (85.8%)	390 (74.9%)	280 (53.7%)	256 (49.1%)	52.6
	75(2n), No. 4	(N)NN 75, No. 1	217	150 (69.1%)	137 (63.1%)	125 (57.6%)	114 (52.5%)	98 (45.2%)	65.3
	75(2n), No. 5	(N)NN 75, No. 1	645	441 (68.4%)	390 (60.5%)	316 (49.0%)	236 (36.6%)	174 (27.0%)	39.5
Total			2899	2140 (73.8%)	1980 (68.3%)	1748 (60.3%)	1112 (38.4%)	973 (33.6%)	44.5
1977	[(C)CN 73♀, No. 16 × (C)CC 73♂, No. 1]								
	75(2n), No. 1	(C)CC 73, No. 3	756	634 (83.9%)	578 (76.5%)	554 (73.3%)	432 (57.1%)	396 (52.4%)	62.5
	75(2n), No. 2	(C)CC 73, No. 3	672	589 (87.6%)	504 (75.0%)	486 (72.3%)	433 (64.4%)	389 (57.9%)	66.0
	75(2n), No. 3	(C)CC 73, No. 3	647	452 (69.9%)	421 (65.1%)	402 (62.1%)	394 (60.9%)	360 (55.6%)	79.6
	75(2n), No. 4	(C)CC 73, No. 3	643	598 (93.0%)	404 (62.8%)	340 (52.9%)	225 (35.0%)	188 (29.2%)	31.4
	75(2n), No. 5	(C)CC 73, No. 3	341	303 (88.9%)	298 (87.4%)	259 (76.0%)	217 (63.6%)	195 (57.2%)	64.4
Total			3059	2576 (84.2%)	2205 (72.1%)	2041 (66.7%)	1701 (55.6%)	1528 (50.0%)	59.3

PMC, Percentage of metamorphosed frogs to normally cleaved eggs

capacity to the female *Rana nigromaculata*, (N)NN 75♀, Nos. 1 and 2, which were mated in 1977 with the above male *Rana nigromaculata*, (N)NN 75♂, No. 1, in the control series (Table 17).

b. Backcrosses from (C)CN♀ × (C)CC♂

Five 2-year-old diploid female backcrosses, Nos. 1~5, produced in 1975 from a female hybrids, (C)CN 73♀, No. 16, by mating with a male *Rana plancyi chosenica*, (C)CC 73♂, No. 1, were mated in 1977 with a male *Rana plancyi chosenica*, (C)CC 73♂, No. 3 (Table 19). It was found that 69.9~93.0% of the respective number of eggs, 2576 (84.2%) of 3059 in total, cleaved normally, 52.9~76.0%, 2041 (66.7%) in total, hatched normally, 35.0~64.4%, 1701 (55.6%) in total, became normally feeding tadpoles and 29.2~57.9%, 1528 (50.0%) in total, attained completion of metamorphosis. The metamorphosed frogs corresponded to 31.4~79.6%, 59.3% on the average, of the normally cleaved eggs.

c. Backcrosses from (N)NC♀ × (N)NN♂

Five 2-year-old diploid female backcrosses, Nos. 1~5, produced in 1975 from a female hybrid, (C)CN 73♀, No. 8 by mating with a male *Rana nigromaculata*, (N)NN 73♂, No. 2, were mated in 1977 with a male *Rana nigromaculata*, (N)NN

75♂, No. 2 (Table 20). It was found that 70.1~93.5% of the respective number of eggs, 1837 (82.3%) of 2231 eggs in total, cleaved normally, 19.7~85.4%, 1288 (57.7%) in total, hatched normally, 16.3~75.9%, 1158 (51.9%) in total, became normally feeding tadpoles and 12.7~71.1%, 1033 (46.3%) in total, attained completion of metamorphosis. The metamorphosed frogs corresponded to 18.1~77.7%, 56.2% on the average, of the normally cleaved eggs.

TABLE 20

Developmental capacity of the offspring of diploid females of backcrosses, (N)NC♀ × (N)NN♂ or × (C)CC♂, by mating with male *Rana nigromaculata* or *Rana plancyi chosenica*

Year	Parents		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	PMC
	Female	Male							
1977	[(N)NC 73♀, No. 8× (N)NN 73♂, No. 2]								
	75(2n), No. 1	(N)NN 75, No. 2	750	526 (70.1%)	361 (48.1%)	148 (19.7%)	122 (16.3%)	95 (12.7%)	18.1
	75(2n), No. 2	(N)NN 75, No. 2	534	495 (92.9%)	472 (88.4%)	456 (85.4%)	391 (73.2%)	338 (63.3%)	68.3
	75(2n), No. 3	(N)NN 75, No. 2	475	396 (83.4%)	381 (80.2%)	323 (68.0%)	305 (64.2%)	278 (58.5%)	70.2
	75(2n), No. 4	(N)NN 75, No. 4	232	217 (93.5%)	210 (90.5%)	183 (78.9%)	176 (75.9%)	165 (71.1%)	76.0
	75(2n), No. 5	(N)NN 75, No. 2	240	202 (84.2%)	194 (80.8%)	178 (74.2%)	160 (66.7%)	157 (65.4%)	77.7
Total			2231	1836 (82.3%)	1618 (72.5%)	1288 (57.7%)	1154 (51.7%)	1033 (46.3%)	56.3
1977	[(N)NC 73♀, No. 8× (C)CC 73♂, No. 2]								
	75(2n), No. 1	(C)CC 73, No. 4	499	421 (84.4%)	393 (78.8%)	276 (55.3%)	214 (42.9%)	113 (22.6%)	26.8
	75(2n), No. 2	(C)CC 73, No. 4	313	239 (76.4%)	212 (67.7%)	120 (38.3%)	104 (33.2%)	62 (19.8%)	25.9
	75(2n), No. 3	(C)CC 73, No. 4	367	318 (86.6%)	276 (75.2%)	224 (61.0%)	175 (47.7%)	101 (27.5%)	31.8
	75(2n), No. 4	(C)CC 73, No. 4	253	231 (91.3%)	196 (77.5%)	96 (37.9%)	92 (36.4%)	87 (34.4%)	37.7
	75(2n), No. 5	(C)CC 73, No. 4	241	195 (80.9%)	172 (71.4%)	153 (63.5%)	100 (41.5%)	76 (31.5%)	39.0
Total			1673	1404 (83.9%)	1249 (74.7%)	869 (51.9%)	685 (40.9%)	439 (26.2%)	31.3

PMC, Percentage of metamorphosed frogs to normally cleaved eggs

d. Backcrosses from (N)NC♀ × (C)CC♂

Five 2-year-old diploid female backcrosses, Nos. 1~5, produced in 1975 from a female hybrid, (N)NC 73♀, No. 8, by mating with a male *Rana plancyi chosenica*, (C)CC 73♂, No. 2, were mated in 1977 with a male *Rana plancyi chosenica*, (C)CC 73♂, No. 4 (Table 20). The results indicated that 76.4~91.3% of the respective number of eggs, 1404 (83.9%) of 1673 eggs in total, cleaved normally, 37.9~63.5%, 869 (51.9%) in total, hatched normally, 33.2~47.7%, 685 (40.9%) in total, became normally feeding tadpoles and 19.8~34.4%, 439 (26.2%) in total, attained completion of metamorphosis. The metamorphosed frogs corresponded to 25.9~39.0%, 31.3% on the average, of the normally cleaved eggs.

2. Triploid backcrosses

a. Backcrosses from (N)NC♀ × (N)NN♂ in 1978

Fourteen 3-year-old triploid female (N)NNC backcrosses produced in 1975 from large eggs of a female hybrid, (N)NC 73♀, No. 8, by mating with a male *Rana nigromaculata*, (N)NN 73♂, No. 2, were injected with frog pituitaries in the breeding season of 1978. The results showed that ovulation occurred in six of the triploid females, (N)NNC 75♀, Nos. 1～6.

Each of the triploid females laid 41～523 eggs, 287.7 eggs on the average. The eggs were 1.3～3.3 mm in diameter and generally divided into three groups of normal-sized, large and huge eggs. Of 1726 eggs laid by the six females, 81 were normal-sized, 1462 were large and 183 were huge eggs (Table 21). Of these eggs, 922 large and 146 huge ones, 1068 in total, were inseminated with sperm of a male *Rana plancyi chosenica*, (C)CC 76♂, No. 1. The results indicated that 533 of the

TABLE 21

Number of the eggs laid by (N)NNC allotriploid females obtained by backcrossing a female hybrid with a male *Rana nigromaculata*

Year	Kinds	Individual no.	Age (years)	Body length (mm)	No. of eggs			
					Total	normal (mm)	large (mm)	huge (mm)
1978	[(N)NC 73♀, No. 8× (N)NN 73♂, No. 2] 75, (N)NNC	1	3	57.5	267	7 (1.3～1.6)	257 (2.1～2.5)	3 (2.7～3.0)
		2	3	57.0	41	3 (1.5～1.7)	18 (2.0～2.3)	20 (2.7～3.2)
		3	3	58.5	442	25 (1.4～1.6)	412 (1.9～2.7)	5 (3.0～3.2)
		4	3	59.0	174	3 (1.3)	156 (2.2～2.5)	15 (2.8～3.3)
		5	3	60.0	523	11 (1.2～1.5)	375 (1.8～2.2)	137 (2.5～2.7)
		6	3	59.5	279	32 (1.3～2.0)	244 (2.2～2.5)	3 (3.0～3.2)
1980	[(N)NC 73♀, No. 8× (N)NN 73♂, No. 2] 75, (N)NNC	7	5	60.5	494	10 (1.5～1.7)	466 (2.0～2.7)	18 (3.0～3.3)
		8	5	68.0	1215	0	1203 (2.0～2.5)	12 (3.0～3.2)
		9	5	68.5	536	23 (1.3～1.6)	494 (2.0～2.7)	19 (3.0～3.5)
		10	5	62.5	948	7 (1.4～1.6)	921 (2.0～2.7)	20 (3.0～3.5)
		11	5	64.0	825	3 (1.4～1.5)	810 (2.0～2.5)	12 (2.7～3.0)
1982	[(N)NC 76♀, No. 14× (N)NN76♂, No. 3] 80, (N)NNC	12	2	76.0	1214	19 (1.3～1.5)	1186 (2.0～2.5)	9 (3.0～3.3)
		13	2	73.0	1082	13 (1.6～1.7)	923 (2.0～2.6)	146 (3.0～3.2)
		14	2	72.0	984	16 (1.4～1.6)	909 (2.1～2.7)	59 (3.0～3.5)
		15	2	75.5	1160	17 (1.3～1.5)	975 (1.9～2.8)	168 (3.0～3.3)

large eggs and 31 of the huge eggs, 564 (52.8%) in total, cleaved normally. However, most of the normally cleaved eggs died of incomplete invagination, edema, microcephaly or various other abnormalities during the embryonic stage, while 83 from the large eggs and 9 from the huge eggs, 92 (8.6%) in total, hatched normally. After more than half of the normally hatched tadpoles died of edema or various abnormalities without taking food, 33 from the large and five from the huge eggs, 38 (3.6%) in total, became normally feeding tadpoles, being more than 30 mm in total length. Eventually, 16 from the large and four from the huge eggs, 20 (1.9%) in total, attained completion of metamorphosis (Table 22).

TABLE 22

Developmental capacity of the offspring of triploid females of backcrosses, (N)NC♀ × (N)NN♂, by mating with male *Rana plancyi chosenica*

Year	Parents		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						
1978	(N)NNC 75, No. 1	(C)CC 76, No. 1	238	67 (28.2%)	25 (10.5%)	13 (5.5%)	3 (1.3%)	0
	(N)NNC 75, No. 2	(C)CC 76, No. 1	10	2 (20.0%)	2 (20.0%)	2 (20.0%)	2 (20.0%)	0
	(N)NNC 75, No. 3	(C)CC 76, No. 1	164	74 (45.1%)	25 (15.2%)	12 (7.3%)	7 (4.3%)	4 (2.4%)
	(N)NNC 75, No. 4	(C)CC 76, No. 1	128	109 (85.2%)	29 (22.7%)	14 (10.9%)	9 (7.0%)	5 (3.9%)
	(N)NNC 75, No. 5	(C)CC 76, No. 1	355	268 (75.5%)	106 (29.9%)	38 (10.7%)	15 (4.2%)	11 (3.1%)
	(N)NNC 75, No. 6	(C)CC 76, No. 1	173	44 (25.4%)	17 (9.8%)	13 (7.5%)	2 (1.2%)	0
1980	(N)NNC 75, No. 7	(C)CC 76, No. 2	484	93 (19.2%)	75 (15.5%)	72 (14.9%)	8 (1.7%)	0
	(N)NNC 75, No. 8	(C)CC 76, No. 2	1215	302 (24.9%)	283 (23.3%)	277 (22.8%)	23 (1.9%)	13 (1.1%)
	(N)NNC 75, No. 9	(C)CC 76, No. 2	336	80 (23.8%)	65 (19.3%)	56 (16.7%)	53 (15.8%)	32 (9.5%)
	(N)NNC 75, No. 10	(C)CC 76, No. 2	546	508 (93.0%)	362 (66.3%)	344 (63.0%)	38 (7.0%)	27 (4.9%)
	(N)NNC 75, No. 11	(C)CC 76, No. 2	822	357 (43.4%)	337 (41.0%)	274 (33.3%)	25 (3.0%)	9 (1.1%)
1982	(N)NNC 80, No. 12	(C)CC 76, No. 3	584	312 (53.4%)	234 (40.1%)	110 (18.8%)	54 (9.2%)	42 (7.2%)
	(N)NNC 80, No. 13	(C)CC 76, No. 3	1012	993 (98.1%)	619 (61.2%)	245 (24.2%)	131 (12.9%)	76 (7.5%)
	(N)NNC 80, No. 14	(C)CC 76, No. 3	984	703 (71.4%)	510 (51.8%)	152 (15.4%)	86 (8.7%)	48 (4.9%)
	(N)NNC 80, No. 15	(C)CC 76, No. 3	740	321 (43.4%)	231 (31.2%)	79 (10.7%)	49 (6.6%)	18 (2.4%)
Total	(N)NNC(15)	(C)CC (3)	7791	4233 (54.3%)	2920 (37.5%)	1701 (21.8%)	505 (6.5%)	285 (3.7%)

b. Backcrosses from (N)NC♀ × (N)NN♂ in 1980

Six 5-year-old triploid female (N)NNC backcrosses produced in 1975 from the

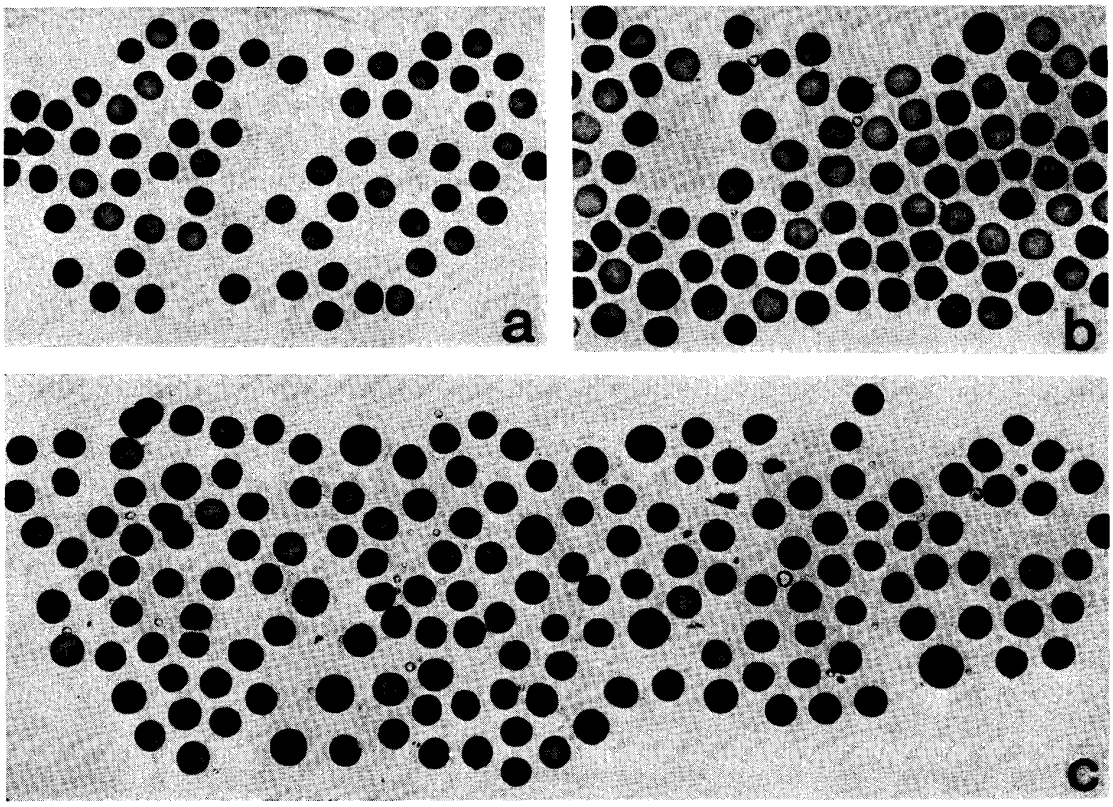


Fig. 4. Eggs of triploid female backcrosses obtained from a female (N)NC hybrid by mating with a male *Rana nigromaculata*, and the control diploid female *Rana nigromaculata*. ×1.3

a. Control diploid *Rana nigromaculata*, (N)NN 75♀, No. 5
b. Triploid backcross, (N)NNC 75♀, No. 8
c. Triploid backcross, (N)NNC 75♀, No. 9

same mating as the above were injected with frog pituitaries in the breeding season of 1980. Ovulation occurred in five of the triploid females, (N)NNC 75♀, Nos. 7～11, each of which laid 494～1215 eggs, 803.6 eggs on the average. These eggs were 1.3～3.5 mm in diameter and generally divided into three groups of normal-sized, large and huge eggs (Fig. 4). Of 4018 eggs obtained from the five triploid females, 43 were normal-sized, 3894 were large and 81 were huge eggs (Table 21).

A total of 3403 eggs including 3324 large and 79 huge eggs, obtained from the five triploid female backcrosses, Nos. 7～11, were inseminated with sperm of a male *Rana plancyi chosenica*, (C)CC 76♂, No. 2. It was found that 1328 large and 12 huge eggs, 1340 (39.4%) in total, cleaved normally. After many of the normally cleaved eggs died of incomplete invagination, edema or various other abnormalities during the embryonic stage, 1017 from the large and six from the huge eggs, 1023 (30.1%) in total, hatched normally. Most of the normally hatched tadpoles died of edema or underdevelopment without taking food, while 145 from the large and two from the huge eggs, 147 (4.3%) in total, became normally feeding tadpoles, being more than 30 mm in total length. Eventually, 81 (2.4%) tadpoles including 79 from the large and two from the huge eggs attained the completion of metamorphosis (Table 22).

c. Backcrosses from (N)NC♀ × (N)NN♂ in 1982

Five of 28 two-year-old triploid female (N)NNC backcrosses produced in 1980 from large eggs of a female hybrid, (N)NC 76♀, No. 14, by mating with a male *Rana nigromaculata*, (N)NN 76♂, No. 3, were injected with frog pituitaries in the breeding season of 1982. The results showed that ovulation occurred in four of the triploid females, (N)NNC 80♀, Nos. 12~15.

Each of the triploid females laid 984~1214 eggs, 1110 eggs on the average. These eggs were 1.3~3.5 mm in diameter and generally divided into three groups of normal-sized, large and huge eggs. Of 4440 eggs obtained from the four triploid females, 65 were normal-sized, 3993 were large and 382 were huge eggs (Table 21).

A total of 3320 eggs, including 3126 large and 194 huge eggs, obtained from the four triploid female backcrosses, Nos. 12~15, were inseminated with sperm of a male *Rana plancyi chosenica*, (C)CC 76♂, No. 3. It was found that 2303 large and 26 huge eggs, 2329 (70.2%) in total, cleaved normally. After 1636 of the normally cleaved eggs died of incomplete invagination, edema or various other abnormalities during the embryonic stage, 680 from the large and 13 from the huge eggs, 693 (20.9%) in total, hatched normally. Most of the normally hatched tadpoles died of edema or underdevelopment without taking food, while 309 from the large and 11 from the huge eggs, 320 (9.6%) in total, became normally feeding tadpoles, being more than 30 mm in total length. Eventually, 184 (5.5%) tadpoles, including 173 from the large and 11 from the huge eggs, attained the completion of metamorphosis (Table 22).

3. Chromosomes

a. Offspring of triploid female backcrosses in 1978

The chromosomes of 38 feeding tadpoles, being more than 30 mm in total

TABLE 23

Chromosome number of the offspring of triploid females of backcrosses, (N)NC♀ × (N)NN♂, by mating with male *Rana plancyi chosenica*

Year	Parents		Number of tadpoles																
			Ana-lyzed	Number of chromosomes															
	Female	Male		26 (2n)	27	28	29	32	33	34	35	36	37	38	39 (3n)	40	42	43	52 (4n)
1978	(N)NNC 75, No. 1	(C)CC 76, No. 1	1												1				
	(N)NNC 75, No. 2	(C)CC 76, No. 1	2												1				1
	(N)NNC 75, No. 3	(C)CC 76, No. 1	7	1		1	1								1				3
	(N)NNC 75, No. 4	(C)CC 76, No. 1	9							1			1	1	1				5
	(N)NNC 75, No. 5	(C)CC 76, No. 1	15		1	1	1			2				1	1	1			7
	(N)NNC 75, No. 6	(C)CC 76, No. 1	2						1						1				
1980	(N)NNC 75, No. 7	(C)CC 76, No. 2	8	1		1								1	2				3
	(N)NNC 75, No. 8	(C)CC 76, No. 2	22	2		1	1	1		1		1	1	1	4	1	1		7
	(N)NNC 75, No. 9	(C)CC 76, No. 2	48	2	2	1	1		1	1				2	5	1		1	31
	(N)NNC 75, No. 10	(C)CC 76, No. 2	38	3											11				24
	(N)NNC 75, No. 11	(C)CC 76, No. 2	25	2	2	2	1	1	1	4	2		2	2	3				3
1982	(N)NNC 80, No. 12	(C)CC 76, No. 3	43	1			1			1					7	1	1		31
	(N)NNC 80, No. 13	(C)CC 76, No. 3	119	14	1	1	2	1	1			2	1		19	2	2	1	72
	(N)NNC 80, No. 14	(C)CC 76, No. 3	80	2	1	2	1			1	1			2	5		1		64
	(N)NNC 80, No. 15	(C)CC 76, No. 3	47	2		1						1			2			1	40
Total	(N)NNC (15)	(C)CC (3)	466	30	7	11	9	3	4	11	3	4	5	10	64	6	5	3	291
				30						67					64		14		291

length, produced in 1978 from six triploid female backcrosses, (N)NNC 75♀, Nos. 1~6, by mating with a male *Rana plancyi chosenica*, (C)CC 76♂, No. 1, were examined in the tail-tips by the squash method with water-pretreatment (Table 23). It was found that one was diploid, six were triploid, 16 were tetraploid, 12 were aneuploid between 2n and 3n, one was 40 (3n+1) in chromosome number and the remaining two were not analyzable. Five of the 16 tetraploids were raised from huge eggs, while the other 11 were from large eggs. Of 20 normally metamorphosed frogs, three were triploids, 14 were tetraploids and three were aneuploids. One of the latter was 27 (2n+1), another was 28 (2n+2) and the remainder was 40 (3n+1) in chromosome number. More specifically, the three triploids were produced from (N)NNC 75♀, Nos. 3, 4 and 5. Of the 14 tetraploids, three, four and seven were produced from (N)NNC 75♀, Nos. 3, 4 and 5, respectively. The three aneuploids were all derived from (N)NNC 75♀, No. 5.

b. Offspring of triploid female backcrosses in 1980

The chromosomes of 147 feeding tadpoles, being more than 30 mm in total length, produced in 1980 from five triploid female backcrosses, (N)NNC 75♀, Nos. 7~11, by mating with a male *Rana plancyi chosenica*, (C)CC 76♂, No. 2, were examined in the tail-tips (Table 23). Of 141 feeding tadpoles whose chromosomes were definitely analyzed, 10 were diploids, 25 were triploids, 68 were tetraploids, 34 were aneuploids between 2n and 3n and the remaining four were aneuploids between 3n and 4n. Two of the 68 tetraploids were raised from huge eggs, while the other 66 were from large eggs.

Of 81 metamorphosed frogs, two were diploids, 15 were triploids, 61 were tetraploids and three were aneuploids. More specifically, the two diploids came from (N)NNC♀, No. 11. Of the 15 triploids, four, three, five and three were produced from (N)NNC♀, Nos. 8, 9, 10 and 11, respectively. Of the 61 tetraploids, 7, 29, 22 and 3 were produced from (N)NNC♀, Nos. 8, 9, 10 and 11, respectively. Of the three aneuploids, one hyperdiploid (2n+2) and one hypertriploid (3n+1) came from (N)NNC 75♀, No. 8, and one hyperdiploid (2n+1) came from (N)NNC 75♀, No. 11.

c. Offspring of triploid female backcrosses in 1982

The chromosomes of 320 feeding tadpoles, being more than 30 mm in total length, produced in 1982 from four triploid female backcrosses, (N)NNC 80♀, Nos. 12~15, by mating with a male *Rana plancyi chosenica*, (C)CC 76♂, No. 3, were examined in the tail-tips (Table 23). Of 289 feeding tadpoles whose chromosomes were definitely analyzed, 19 were diploids, 33 were triploids, 207 were tetraploids, 21 were aneuploids between 2n and 3n and the remaining nine were aneuploids between 3n and 4n. Eleven of the 207 tetraploids were raised from huge eggs, while the other 196 were large eggs.

Of the 184 metamorphosed frogs, six were diploids, 20 were triploids, 151 were tetraploids and seven were aneuploids. More specifically, one diploid came

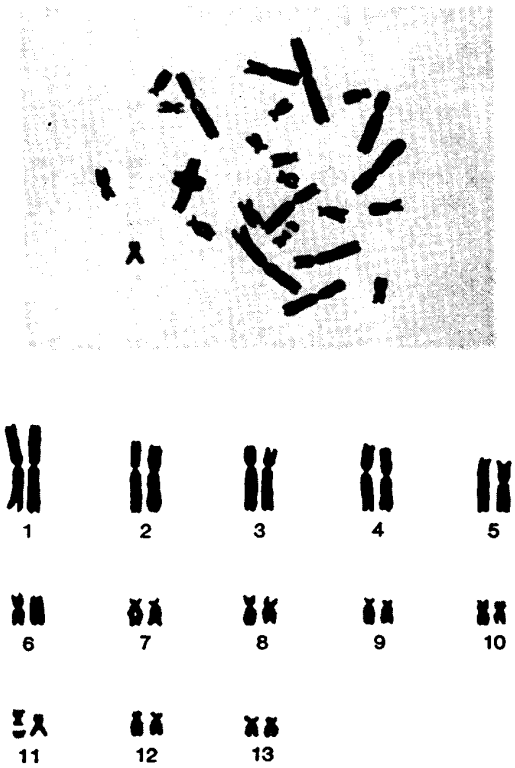


Fig. 5. Metaphase plate and the karyotype of a control diploid female *Rana nigromaculata.* ×900

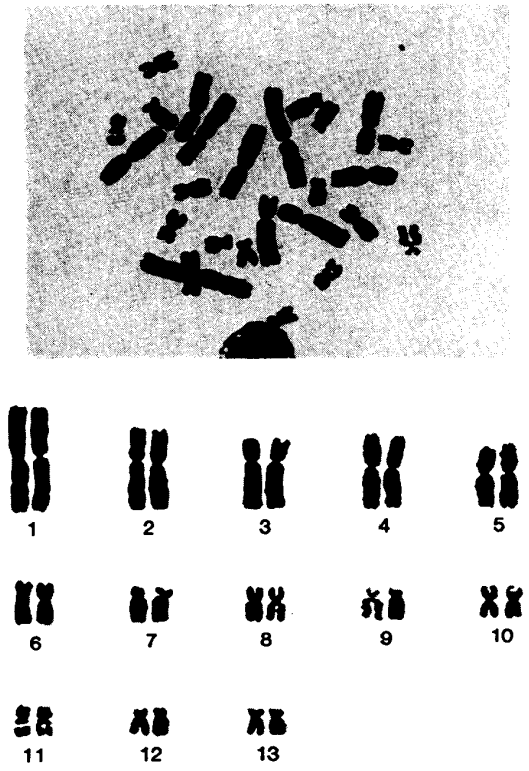


Fig. 6. Metaphase plate and the karyotype of a control diploid female *Rana plancyi chosenica.* ×900

Fig. 7. Metaphase plate and the karyotype of a triploid male backcross, (N)NNC 80♂, No. 6, obtained from a female (N)NC hybrid by mating with a male *Rana nigromaculata.* ×900

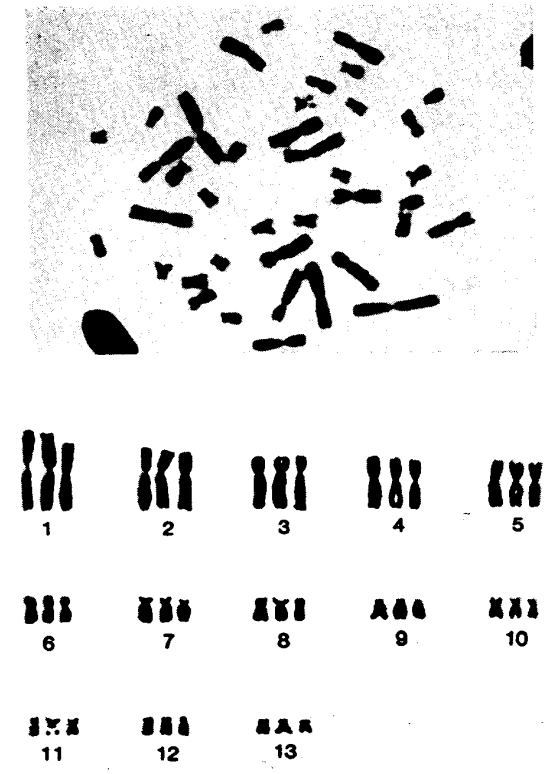


Fig. 8. Metaphase plate and the karyotype of a triploid male backcross, (C)CCN 80♂, No. 6, obtained from a female (C)CN hybrid by mating with a male *Rana plancyi chosenica.* ×900

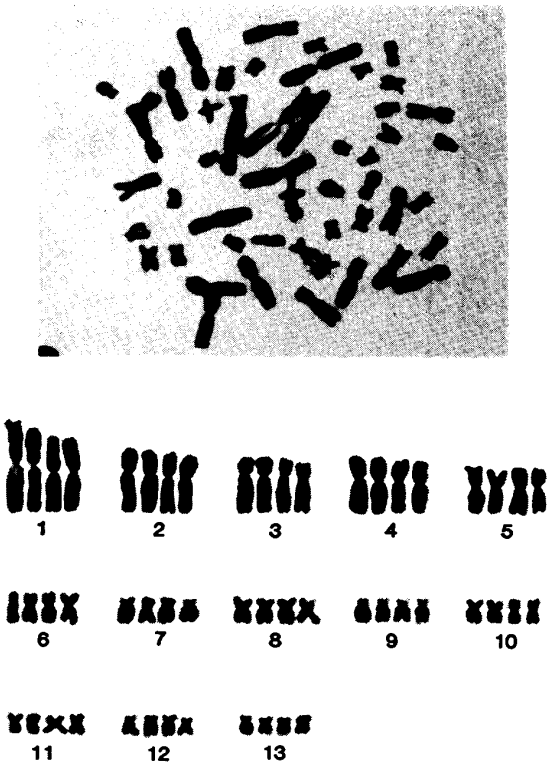


Fig. 9. Metaphase plate and the karyotype of a (N)NNCC amphidiploid male produced from a (N)NNC triploid female backcross by mating with a male *Rana plancyi chosenica*. ×900

from (N)NNC♀, No. 12 and five diploids came from (N)NNC♀, No. 13. Of the 20 triploids, 7, 10 and 3 were produced from (N)NNC♀, Nos. 12, 13 and 14, respectively. Of the 151 tetraploids, 31, 57, 45 and 18 were produced from (N)NNC♀, Nos. 12, 13, 14 and 15, respectively. Eleven of these tetraploids were raised from huge eggs, while the other 140 were from large eggs. Of the seven aneuploids, one hyperdiploid (2n+3), one hypotriploid (3n−5) and one hypertriploid (3n+1) came from (N)NNC♀, No. 12 and two hyperdiploids (2n+3) and two hypertriploids (3n+1) came from (N)NNC♀, No. 13.

The chromosomes of mature female allotriploids, (N)NNC, (N)NCC, (C)CCN and (C)CNN, and mature female amphidiploids, (N)NNCC, were examined by the blood culture method. The chromosomes of mature diploid male *Rana nigromaculata* and *Rana plancyi chosenica* were also examined by the same method (Figs. 5~9).

4. Sex

a. Offspring of triploid female backcrosses in 1978

The sex of the metamorphosed frogs produced in 1978 from six triploid female backcrosses, (N)NNC 75♀, Nos. 1~6, by mating with a male *Rana plancyi chosenica*, (C)CC 76♂, No. 1, was examined. One of three mature triploids was a female and the other two were males. Three of 14 tetraploids died within one week after completion of metamorphosis. The sex of these three was undetermined owing to postmortem changes. The other 11 tetraploids were matured.

TABLE 24

Sex of the offspring of female triploid backcrosses mated with male *Rana plancyi chosenica*

Year	Parents		Ploidy	No. of metamorphosed frogs	Sex of immature frogs			Sex of mature frogs			Sex of all frogs examined		
	Female	Male			No. of frogs	Fem.	Male	No. of frogs	Fem.	Male	No. of frogs	Fem.	Male
1978	(N)NNC 75, No. 3	(C)CC 76, No. 1	3n	1	0	0	0	1	1	0	1	1	0
			4n	3	0	0	0	1	0	1	1	0	1
	(N)NNC 75, No. 4	(C)CC 76, No. 1	3n	1	0	0	0	1	0	1	1	0	1
			4n	4	0	0	0	3	0	3	3	0	3
	(N)NNC 75, No. 5	(C)CC 76, No. 1	3n	1	0	0	0	1	0	1	1	0	1
			4n	7	0	0	0	7	1	6	7	1	6
			aneu.	3	2	0	2	0	0	0	2	0	2
1980	(N)NNC 75, No. 8	(C)CC 76, No. 2	3n	4	4	2	2	0	0	0	4	2	2
			4n	7	2	0	2	5	1	4	7	1	6
			aneu.	2	0	0	0	0	0	0	0	0	0
	(N)NNC 75, No. 9	(C)CC 76, No. 2	3n	3	3	1	2	0	0	0	3	1	2
			4n	29	8	1	7	21	3	18	29	4	25
	(N)NNC 75, No. 10	(C)CC 76, No. 2	3n	5	5	2	3	0	0	0	5	2	3
			4n	22	5	2	3	17	3	14	22	5	17
	(N)NNC 75, No. 11	(C)CC 76, No. 2	2n	2	0	0	0	0	0	0	0	0	0
			3n	3	3	1	2	0	0	0	3	1	2
			4n	3	0	0	0	3	0	3	3	0	3
			aneu.	1	0	0	0	0	0	0	0	0	0
1982	(N)NNC 80, No. 12	(C)CC 76, No. 3	2n	1	1	1	0	0	0	0	1	1	0
			3n	7	5	2	3	0	0	0	5	2	3
			4n	31	4	1	3	27	7	20	31	8	23
			aneu.	3	2	0	2	0	0	0	2	0	2
	(N)NNC 80, No. 13	(C)CC 76, No. 3	2n	5	5	2	3	0	0	0	5	2	3
			3n	10	7	3	4	0	0	0	7	3	4
			4n	57	1	1	0	56(7)	15(4)	41(3)	57	16	41
			aneu.	4	4	0	4	0	0	0	4	0	4
	(N)NNC 80, No. 14	(C)CC 76, No. 3	3n	3	3	2	1	0	0	0	3	2	1
			4n	45	15	4	11	30	9	21	45	13	32
	(N)NNC 80, No. 15	(C)CC 76, No. 3	4n	18	5	1	4	13(4)	3(2)	10(2)	18	4	14
Total	(N)NNC (15)	(C)CC (3)	2n	8	6	3	3	0	0	0	6	3	3
			3n	38	30	13	17	3	1	2	33	14	19
			4n	226	40	10	30	183(11)	42(6)	141(5)	223	52	171
			aneu.	13	8	0	8	0	0	0	8	0	8
		Sum total		285	84	26	58	186	43	143	270	69	201

Parentheses indicate the number of tetraploids which were raised from large eggs and became giant frogs.

One of them was a female and the other 10 were males. Of three aneuploids, one having 28 chromosomes and another having 40 chromosomes, died about 2 weeks and 3 weeks after completion of metamorphosis, respectively. These two were males. The sex of the other aneuploid having 27 chromosomes was undetermined owing to postmortem changes (Table 24).

b. Offspring of triploid female backcrosses in 1980

Of the metamorphosed frogs produced in 1980 from five triploid female backcrosses, (N)NNC 75♀, Nos. 7~11, by mating with a male *Rana plancyi chosenica*, (C)CC 76♂, No. 2, 15 triploid frogs died or were killed within 3 weeks after

metamorphosis. Six of them were females and the other nine were males. Of 61 metamorphosed tetraploids, 15 died within 5 weeks after metamorphosis. Of these tetraploids, three were females and 12 were males. Of 46 tetraploids which were matured, seven were females and 39 were males. More specifically, one female and four males were from (N)NNC♀, No. 8, three females and 18 males were from (N)NNC♀, No. 9, three females and 14 males were from (N)NNC♀, No. 10 and three males were from (N)NNC♀, No. 11. Three aneuploids and two diploids died about 2 weeks after completion of metamorphosis. The sex of these frogs was not determined owing to postmortem changes.

c. Offspring of triploid female backcrosses in 1982

Of the metamorphosed frogs produced in 1982 from four triploid female backcrosses, (N)NNC 80♀, Nos. 12~15, by mating with a male *Rana plancyi chosenica*, (C)CC 76♂, No. 3, six diploid frogs died or were killed within one month after metamorphosis. Three of them were females and the other three were males. Five of 20 metamorphosed triploids died within two weeks after completion of metamorphosis. The sex of these five was undetermined owing to postmortem changes. The other 15 triploids were killed within one month after metamorphosis. Seven of them were females and eight were males. Of 151 metamorphosed triploids, 25 died within two months after metamorphosis. Of these triploids, seven were females and 18 were males. Of 126 tetraploids which were sexually matured in appearance, 34 were females and 92 were males. Of seven aneuploids, three with 29 chromosomes, another with 34 chromosomes and two others with 40 chromosomes died within three weeks after metamorphosis. These six were males. The sex of the remaining aneuploid with 40 chromosomes was undetermined owing to postmortem changes.

d. Summary of sex

The sex of the metamorphosed offspring obtained in 1978, 1980 and 1982 from the 15 triploid female backcrosses by mating with three male *Rana plancyi chosenica* is summarized as follows. Of 223 of the 226 metamorphosed tetraploids, 52 were females and 171 (76.7%) were males. Of 33 of the 38 metamorphosed triploids, 14 were females and 19 (57.6%) were males. Of six of the eight metamorphosed diploids, three were females and three were males. Eight of the 13 metamorphosed aneuploids were all males.

VI. Characteristics of allotriploids and amphidiploids

Reciprocal hybrids, (C)CN and (N)NC, between *Rana plancyi chosenica* and *Rana nigromaculata* were intermediate between the two species in external character. The allotriploids produced from females of these hybrids by inseminating with sperm of male *Rana nigromaculata* or *Rana plancyi chosenica* were regarded as (C)CNN, (C)CCN, (N)NNC and (N)NCC in genome constitution principally on the basis of their origin.

Detailed observations in external character, inner structure of testes, spermatogenesis and electrophoretic patterns of various kinds of proteins were made in the above four kinds of allotriploids as well as tetraploids produced from matings between female (N)NNC allotriploids and male *Rana plancyi chosenica* to confirm more accurately their genome constitutions.

1. External characters

a. *Rana nigromaculata* and *Rana plancyi chosenica*

As already described briefly, *Rana nigromaculata* are larger and more slender than *Rana plancyi chosenica.* The dermal tubercles on the back are elongated rods in shape and numerous in number. The dorsal ground color is brown, grayish brown or yellowish brown. There is always a pale dorsomedian stripe running from the snout to the anus. As the dorsolateral folds are usually of a pale color similar to dorsomedian stripe, the frogs have three pale stripes on the back. The dorsolateral folds are narrow in width and comparatively steep in elevation. While the three stripes of males are usually yellowish brown or pale brown and somewhat similar to the dorsal ground color, those of females are whitish and stand out from the ground color. They are often of silver or golden color. The dorsal black spots of males are rather vague, while those of females are distinct, large and numerous. They are usually connected with each other and form wide black areas. Such a sexual difference in coloration is most distinct in the breeding season. The dorsal surfaces of males become uniformly yellowish ocher, while those of females become uniformly blackish except the three pale stripes. The ventral surfaces are white. The hind limbs are longer than those of *Rana plancyi chosenica.* The inner metatarsal tubercles are small, although they are distinct.

Rana plancyi chosenica are smaller than *Rana nigromaculata* and somewhat dumpy in body shape. Males of this species are especially small. The dermal tubercles on the back are small dots and few in number. The dorsal ground color is green, yellowish green or dark green. There is no dorsomedian stripe. The dorsolateral folds are wide and gentle in elevation. They are dark and coppery in color. Dorsal black spots are small circles and few, although they are obscure and often indistinguishable. There is no sexual difference in coloration. The ventral surfaces are yellow, white or dusky with gray. The hind limbs are shorter than those of *Rana nigromaculata.* The inner metatarsal tubercles are large.

b. Reciprocal hybrids

Reciprocal hybrids, (C)CN and (N)NC, were intermidiate between the two species in almost all the external characters. However, the dorsal ground color was yellowish green or pale brown. All the hybrids had a dorsomedian stripe. The ventral surfaces were white. The dorsal black spots were clearcut in outline, as found in *Rana nigromaculata.*

c. Allotriploids

Two kinds of allotriploids, (C)CNN and (N)NNC, were similar to the diploid

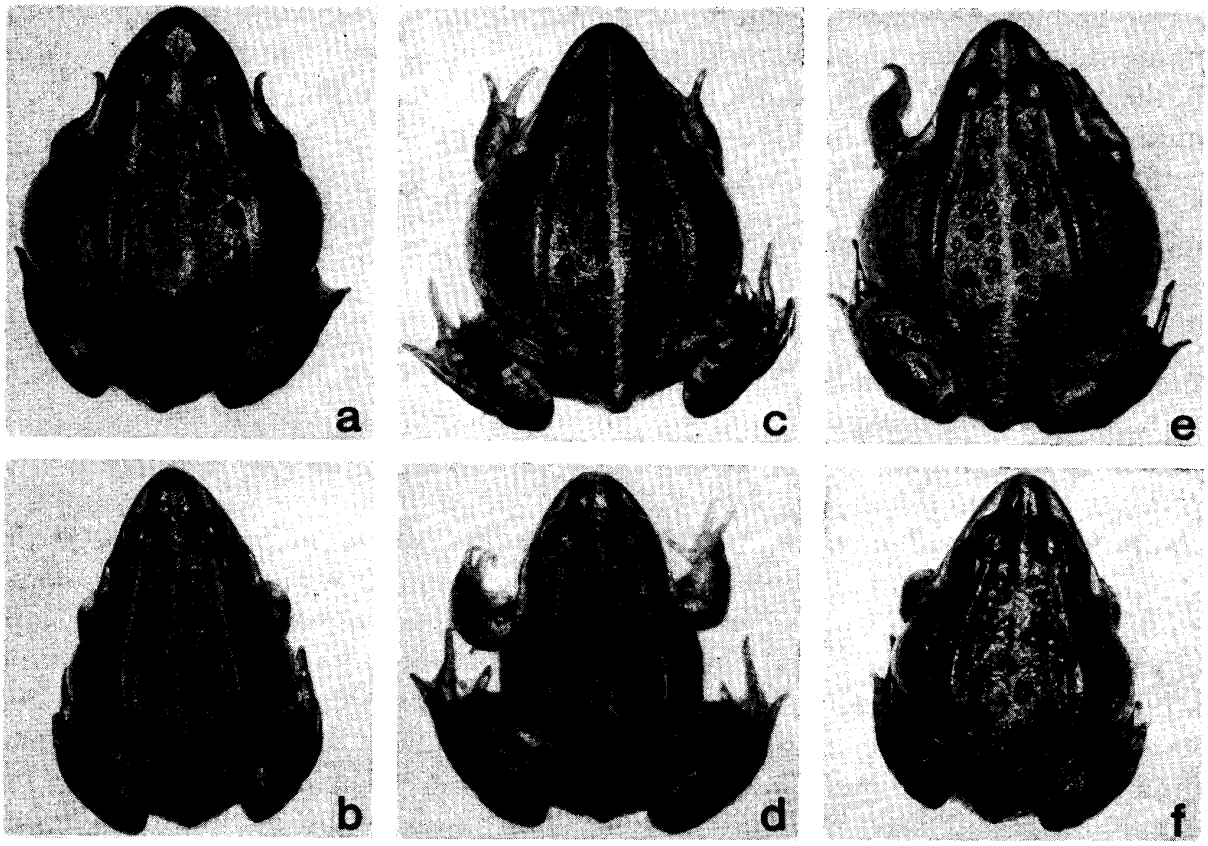


Fig. 10. Two-year-old allotriploids and amphidiploids between *Rana nigromaculata* and *Rana plancyi chosenica*. ×0.6

a, b. Female and male (N)NCC triploid backcrosses produced from (N)NC 73♀, No. 8× (C)CC 73♂, No. 2

c, d. Female and male (N)NNC triploid backcrosses produced from (N)NC 73♀, No. 8× (N)NN 73♂, No. 2

e, f. Female and male (N)NNCC amphidiploids produced from (N)NNC 75♀, No. 9× (C)CC 75♂, No. 2

hybrids in dorsal ground color, dorsal black spots, dorsomedian stripe, ventral color as well as in width, elevation and color of the dorsolateral folds. However, these allotriploids were intermediate between *Rana nigromaculata* and diploid (N)NC hybrids in the other external characters. There was a distinct sexual difference in coloration (Fig. 10c, d).

The other two kinds of allotriploids, (C)CCN and (N)NCC, were green or yellowish green and similar to *Rana plancyi chosenica* in dorsal ground color. They were also similar to the latter species in circular shape of the dorsal black spots, width and color of the dorsolateral folds and body shape. However, they were similar to *Rana nigromaculata* in that the outlines of the dorsal black spots were distinct. On the other hand, they were intermediate between *Rana plancyi chosenica* and diploid (C)CN hybrids in length of the hind limbs and size of the inner metatarsal tubercles. There was no sexual difference in coloration (Fig. 10a, b).

d. Amphidiploids

Amphidiploids, (N)NNCC, did not differ from reciprocal diploid hybrids in

external characters, except that the outlines of the dorsal black spots were very distinct and the black color was remarkably deep (Fig. 10e, f).

2. Inner structure of testes

a. Allotriploids

The inner structure of testes was observed in five 2-year-old mature male allotriploids, (C)CNN 75♂, Nos. 1～5, obtained in 1975 from a female hybrid, (C)CN 73♀, No. 16, by inseminating with sperm of a male *Rana nigromaculata*, (N)NN 73♂, No. 2. One of the testes of each male was fixed and sectioned to observe the inner structure, while the other was used in mating experiments performed in 1977 (cf. Table 17). The testes of five 2-year-old male allotriploids, (C)CNN 80♂, Nos. 1～5, raised in 1980 from a female hybrid, (C)CN 76♀, No. 18, by inseminating with sperm of a male *Rana nigromaculata*, (N)NN 76♂, No. 1, were also fixed and sectioned to observe the inner structure (cf. Table 13). It was found that the testes of these ten male (C)CNN allotriploids were all abnormal in inner structure. No normal spermatozoa were observed in the testes. The seminiferous tubules contained abnormally shaped spermatozoa and pycnotic nuclei. In the peripheral parts, there were many first spermatocytes and spermatogonia.

The testes of five 2-year-old mature male allotriploids, (C)CCN 75♂, Nos. 1～5, raised in 1975 from a female hybrid, (C)CN 73♀, No. 16, by inseminating with sperm of a male *Rana plancyi chosenica*, (C)CC 73♂, No. 1, were very similar to those of the above male (C)CNN allotriploids in inner structure. These testes were very abnormal and contained no normal spermatozoa. There were some abnormally shaped spermatozoa and pycnotic nuclei in the seminiferous tubules.

The inner structure of testes was observed in five 2-year-old mature male allotriploids, (N)NNC 75♂, Nos. 1～5, obtained in 1975 from a female hybrid, (N)NC 73♀, No. 8, by inseminating with sperm of a male *Rana nigromaculata*, (N)NN 73♂, No. 2. One of the testes of each male was fixed and sectioned in order to examine the inner structure, while the other was used in mating experiments of 1977 (cf. Table 18). The testes of five 2-year-old mature male allotriploids, (N)NNC 80♂, Nos. 1～5, obtained in 1980 from a female hybrid, (N)NC 76♀, No. 14, by inseminating with sperm of a male *Rana nigromaculata*, (N)NN 76♂, No. 3, were examined (cf. Table 14). The testes of these 10 male (N)NNC allotriploids were very abnormal in inner structure and had no normal spermatozoa. The seminiferous tubules contained abnormally shaped spermatozoa and pycnotic nuclei. In the peripheral parts, there were many first spermatocytes and spermatogonia (Fig. 11).

The testes of three 2-year-old mature male allotriploids, (N)NCC 75♂, Nos. 1～3, obtained in 1975 from a female hybrid, (N)NC 73♀, No. 8, by inseminating with sperm of a male *Rana plancyi chosenica*, (C)CC 73♂, No. 2, were also very abnormal in inner structure (cf. Table 14). The seminiferous tubules had no normal spermatozoa, while they contained some abnormally shaped spermatozoa and pycnotic nuclei (Fig. 11).

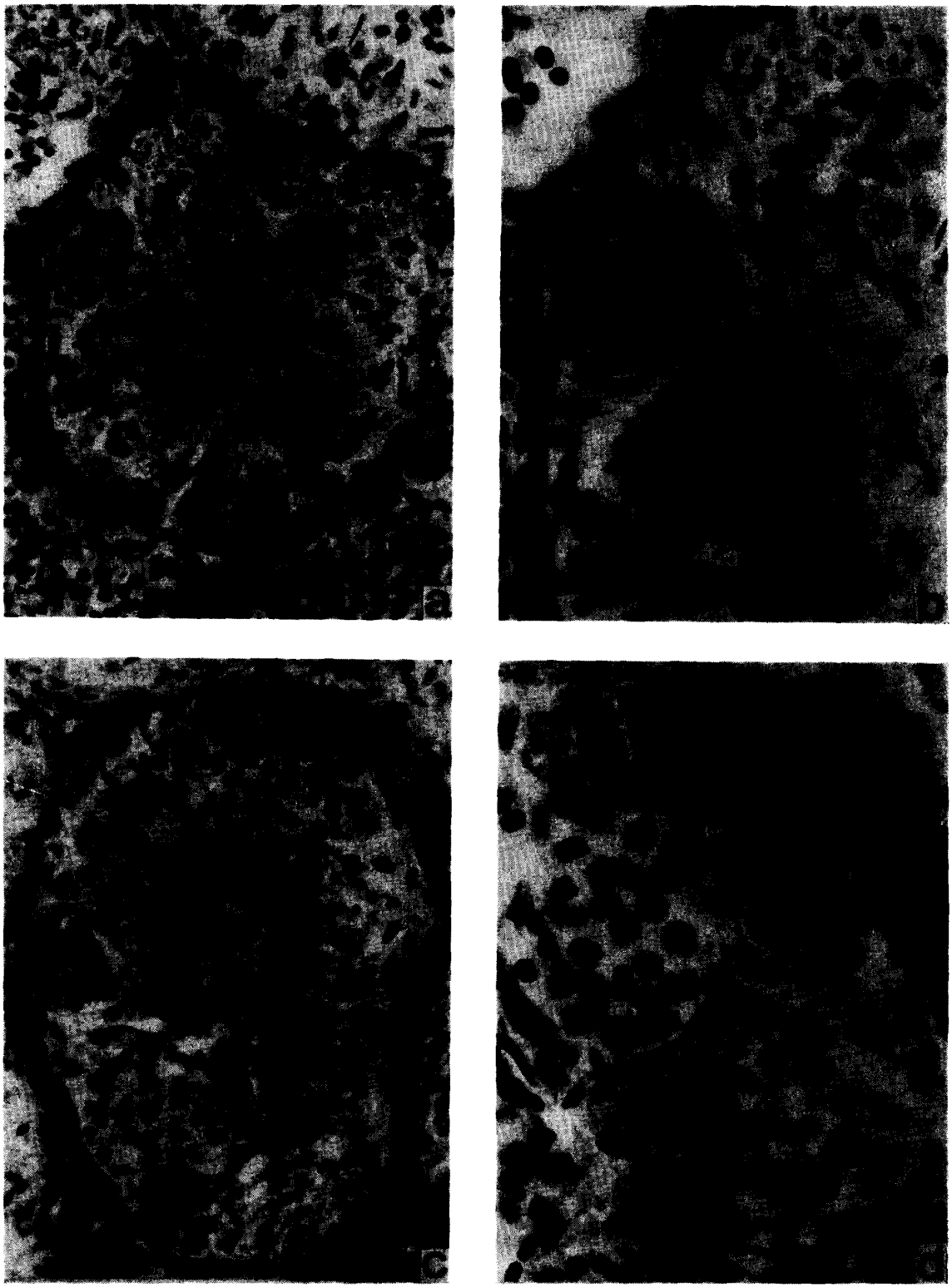


Fig. 11. Cross-sections of the testes of triploid male backcrosses between *Rana nigromaculata* and *Rana plancyi chosenica*.

a. (N)NCC triploid backcross produced from (N)NC 73♀, No. 8×(C)CC 73♂, No. 2 ×260

b. Ditto ×520

c. (N)NNC triploid backcross produced from (N)NC 73♀, No. 8×(N)NN 73♂, No. 2 ×260

d. Ditto ×520

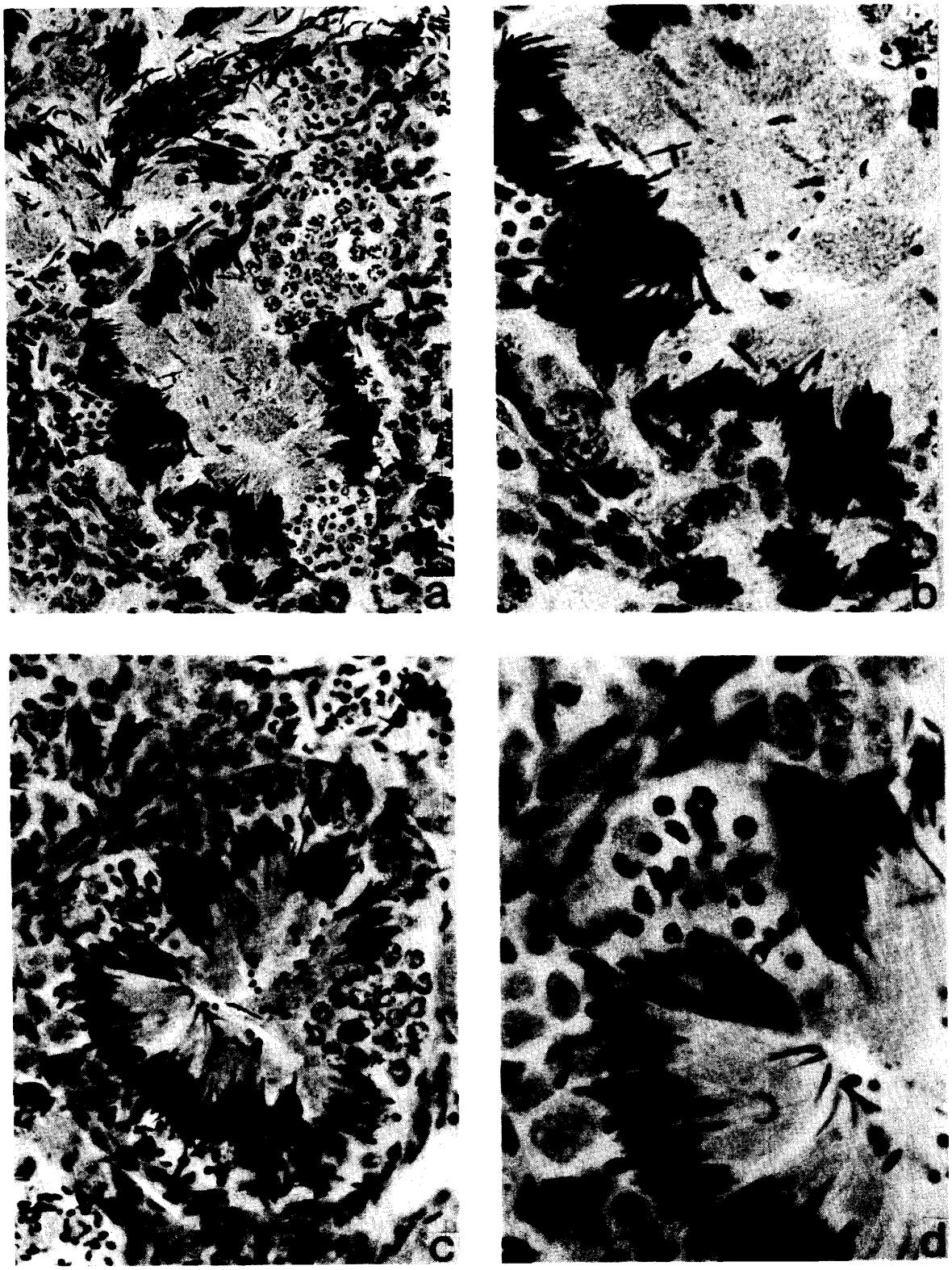


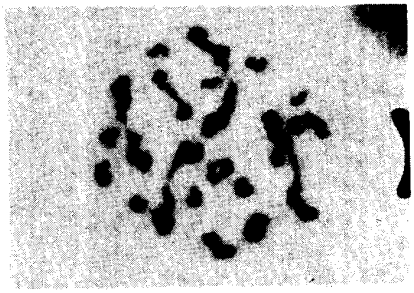
Fig. 12. Cross-sections of the testes of a male amphidiploid and the control diploid.

a. Control diploid *Rana nigromaculata* produced from (N)NN 75♀, No. 7×(N)NN 75♂, No. 7 ×260
b. Ditto ×520
c. (N)NNCC amphidiploid produced from (N)NNC 75♀, No. 9×(C)CC 75♂, No. 2 ×260
d. Ditto ×520

b. Amphidiploids

The inner structure of testes was observed in three 2-year-old mature male amphidiploids, (N)NNCC 78♂, Nos. 1~3, produced in 1978 from a mating between an allotriploid female backcross (N)NNC 75♀, No. 5, and a male *Rana plancyi chosenica*, (C)CC 76♂, No. 1, and in five 2-year-old mature male amphidiploids, (N)NNCC 80♂, Nos. 1~5, produced in 1980 from a mating between an allotriploid female backcross, (N)NNC 75♀, No. 9 and a male *Rana plancyi chosenica*, (C)CC 76♂, No. 2. Of these eight male amphidiploids, only two, (N)NNCC 78♂, Nos. 1 and 2, were used in mating experiments besides microscopical observations (NISHIOKA and OKUMOTO, 1983; Table 1). In each of them, one of the testes was fixed and sectioned to examine the inner structure.

The testes of the eight male amphidiploids were all completely normal in inner structure and did not differ from those of male diploid *Rana nigromaculata* and *Rana plancyi chosenica*, except that the spermatozoa were larger than those of the diploid males. The seminiferous tubules were filled with compact bundles of large spermatozoa. In the peripheral parts, there were many first and second spermatocytes at various stages of meiosis. Along the inner walls of seminiferous tubules, there were many primary and secondary spermatogonia. All these germ cells were evidently larger than those of the control diploid males. It was also remarkable that spermatocytes at a definite meiotic stage were uniform



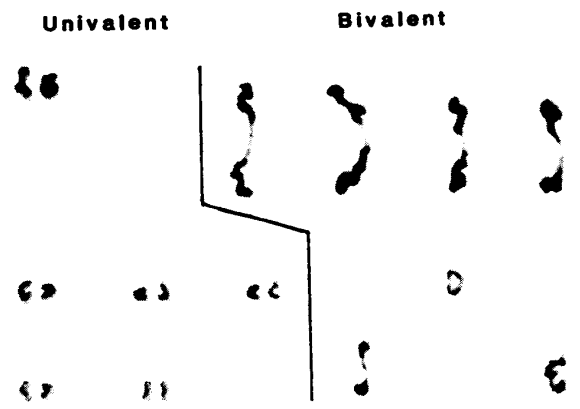
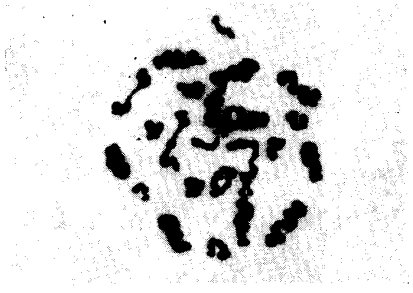


Fig. 13. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 4 large and 3 small bivalents and 12 univalents in a male (N)NC hybrid produced from (N)NN 75♀, No. 10× (C)CC 76♂, No. 10. ×670



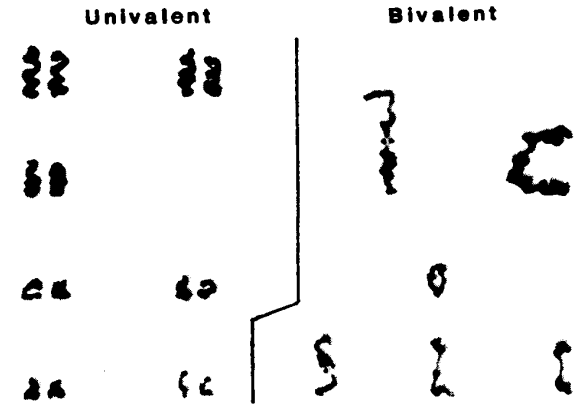


Fig. 14. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 2 large and 4 small bivalents and 14 univalents in a male (C)CN hybrid produced from (C)CC 76♀, No. 8× (N)NN 76♂, No. 8. ×670

in size in contrast to those of the male allotriploids (Fig. 12c, d).

First meiotic divisions were observed by the method of SCHMID et al. (1979) in two male amphidiploids. It was found that the first metaphases had usually 26 bivalent chromosomes or one or two tetravalent chromosomes besides bivalents (Figs. 17, 18). On the other hand, first meiotic divisions were observed in both three male (N)NNC allotriploids and three male (C)CCN allotriploids. The results showed that most of the first metaphases had a mixture of bivalent and univalent chromosomes together with one or two trivalents (Figs. 15, 16). The first meiotic divisions in the testes of three male (N)NC and three male (C)CN diploid hybrids almost conclusively contained a mixture of univalents and bivalents (Figs. 13, 14).

Further details about the meiotic divisions in male allotriploids and amphidiploids will be reported in a separate paper.

3. Electrophoretic patterns

The four kinds of allotriploids and one kind of allotetraploid produced from reciprocal hybrids between *Rana nigromaculata* and *Rana plancyi chosenica* by backcrossing were regarded as (C)CNN, (C)CCN, (N)NNC, (N)NCC and (N)NNCC in genome constitution principally on the basis of their origin and external

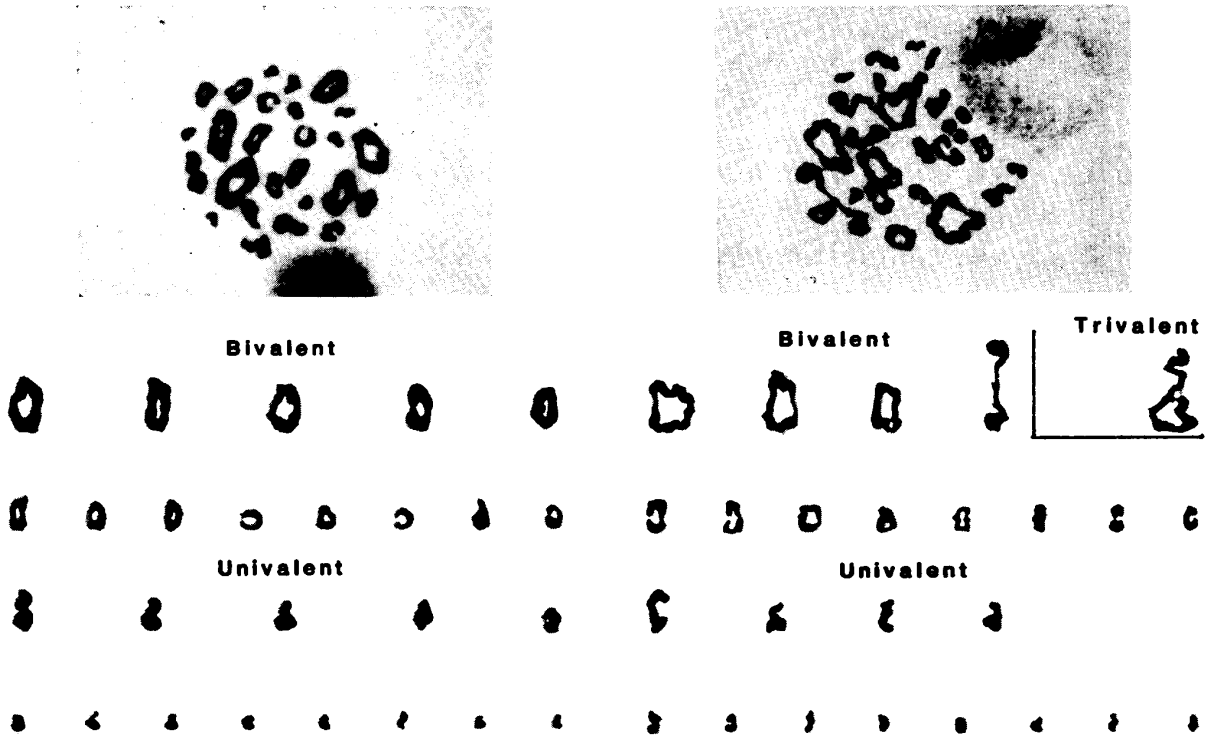
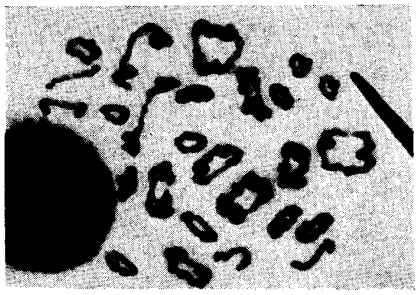


Fig. 15. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 5 large and 8 small bivalents and 13 univalents in a (N)NNC triploid male backcross produced from (N)NC 73♀, No. 8×(N)NN 73♂, No. 2. ×670

Fig. 16. 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 (C)CCN triploid male backcross produced from (C)CN 73♀, No. 16×(C)CC 73♂, No. 1. ×670



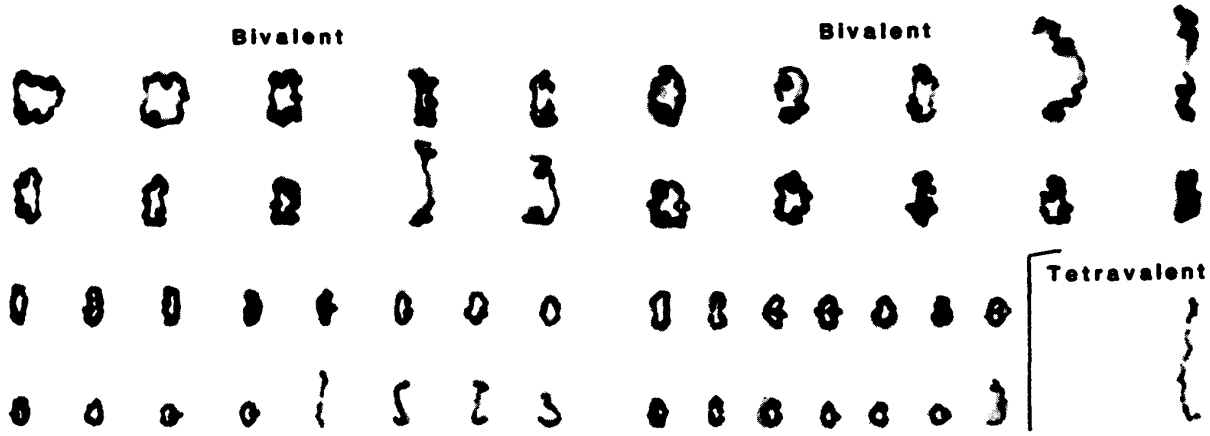


Fig. 17. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 10 large and 16 small bivalents in a male amphidiploid produced from (N)NNC 75♀, No. 9× (C)CC 76♂, No. 2. ×670

Fig. 18. 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)NNC 75♀, No. 9× (C)CC 76♂, No. 2. ×670

characters. These genome constitutions were confirmed by observing inner structure of their testes and spermatogenesis. In order to confirm more definitely the assumed genome constitutions of allotriploids or allotetraploids, the following 12 kinds of proteins and enzymes extracted from them were analyzed by starch-gel electrophoresis, together with those from *Rana nigromaculata, Rana plancyi chosenica* and reciprocal hybrids between them; protein C and albumin (Ab) from blood serum, hemoglobin (Hb) from erythrocytes and nine kinds of enzymes extracted from skeletal muscles, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), α-glycerophosphate dehydrogenase (α-GDH), isocitrate dehydrogenase (IDH), superoxide dismutase (SOD), aspartate aminotransferase (AAT), creatine kinase (CK), phosphoglucomutase (PGM) and glucosephosphate isomerase (GPI).

The results indicated that nine components of the 12 kinds of proteins and enzymes, that is, protein C, Ab, Hb, LDH-B, MDH-A, MDH-B, α-GDH, IDH-B and CK of *Rana nigromaculata* differed from those of *Rana plancyi chosenica* in electrophoretic patterns. The genes controlling these nine protein components of *Rana nigromaculata* and *Rana plancyi chosenica* were named as follows.

Components	*Rana nigromaculata*	*Rana plancyi chosenica*
Protein C	C^nC^n	C^cC^c
Ab	A^nA^n	A^cA^c

Hb	H^nH^n	H^cH^c
LDH-B	L^nL^n	L^cL^c
MDH-A	$M\text{-}A^n$ $M\text{-}A^n$	$M\text{-}A^c$ $M\text{-}A^c$
MDH-B	$M\text{-}B^n$ $M\text{-}B^n$	$M\text{-}B^c$ $M\text{-}B^c$
α-GDH	G^nG^n	G^cG^c
IDH-B	I^nI^n	I^cI^c
CK	K^nK^n	K^cK^c

The electrophoretic patterns of the nine protein components extracted from reciprocal hybrids, (C)CN and (N)NC, showed that the genes for these components were all heterozygous, being C^nC^c, A^nA^c, H^nH^c, L^nL^c, $M\text{-}A^n$ $M\text{-}A^c$, $M\text{-}B^n$ $M\text{-}B^c$, G^nG^c, I^nI^c and K^nK^c.

In two kinds of (C)CNN and (N)NNC allotriploids raised from female (C)CN and (N)NC hybrids, respectively, by inseminating with sperm of male *Rana nigromaculata*, the electrophoretic patterns of the nine protein components showed that the band derived from two *Rana nigromaculata* genomes was distinctly darker than that derived from one *Rana plancyi chosenica* genome, in other words, that the genes for the nine components of these allotriploids were $C^nC^nC^c$, $A^nA^nA^c$, $H^nH^nH^c$, $L^nL^nL^c$, $M\text{-}A^n$ $M\text{-}A^n$ $M\text{-}A^c$, $M\text{-}B^n$ $M\text{-}B^n$ $M\text{-}B^c$, $G^nG^nG^c$, $I^nI^nI^c$ and

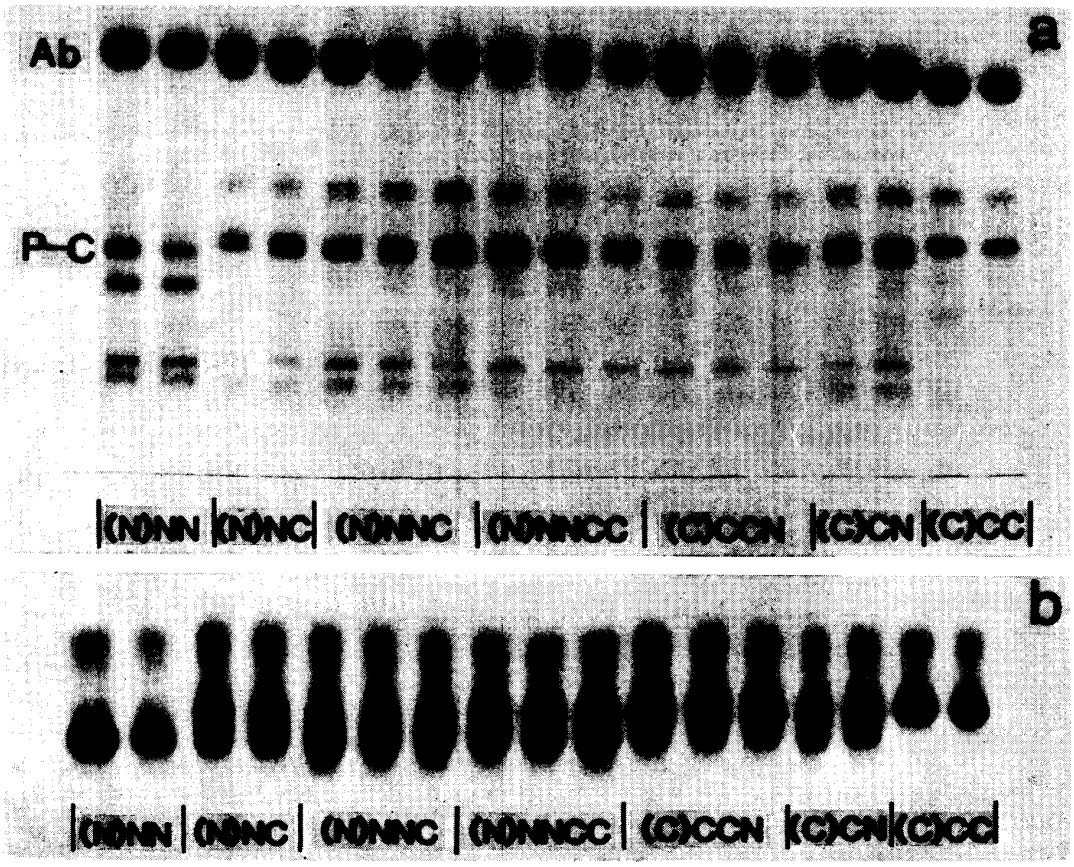


Fig. 19. Electrophoretic patterns of three blood proteins from reciprocal diploid hybrids, reciprocal triploid backcrosses and amphidiploids between *Rana nigromaculata* and *Rana plancyi chosenica*, and the control diploids.

a. Serum albumin (Ab) and protein C (P-C) b. Hemoglobin (Hb)

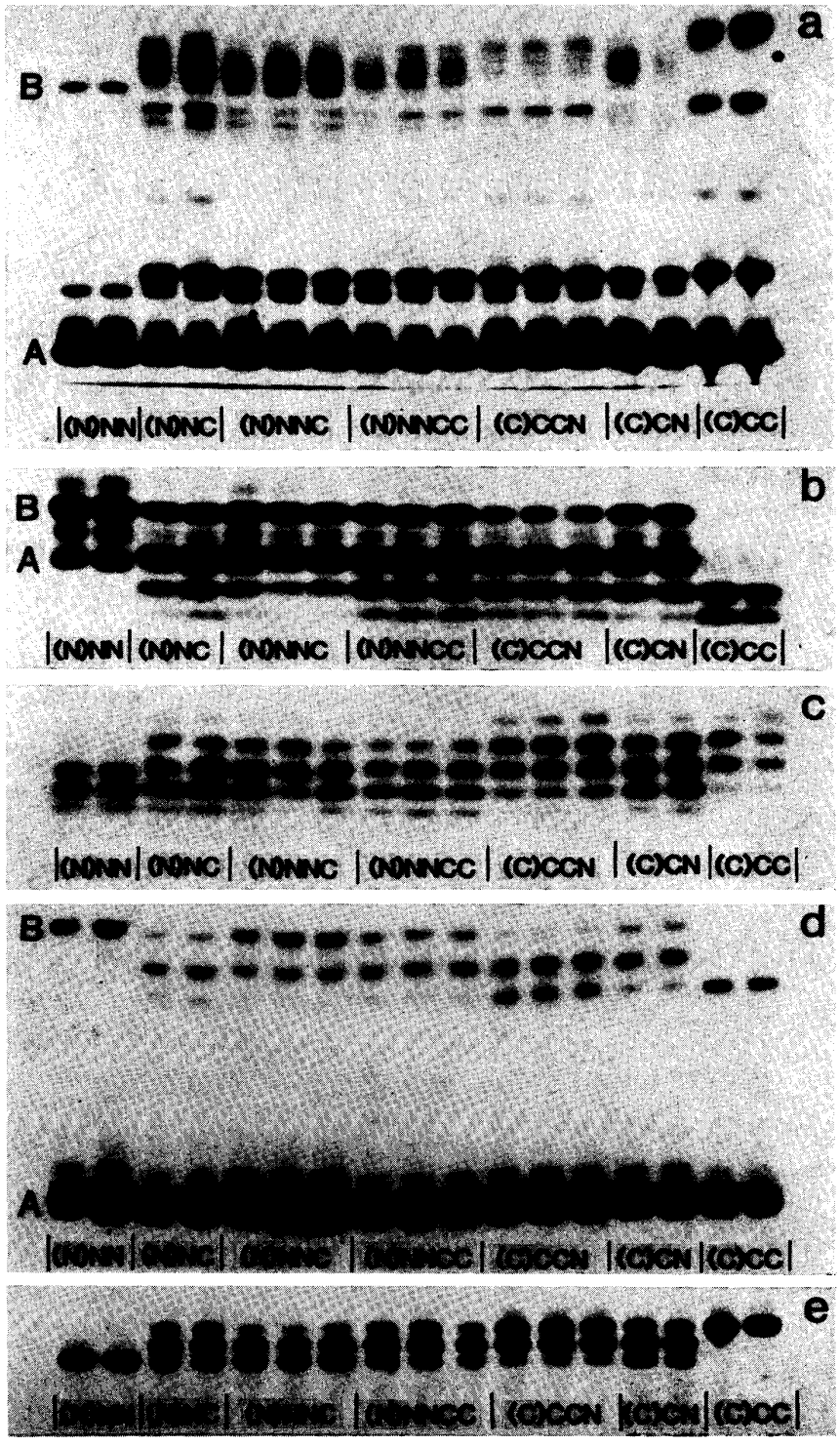


Fig. 20. Electrophoretic patterns of five enzymes from reciprocal diploid hybrids, reciprocal triploid backcrosses and amphidiploids between *Rana nigromaculata* and *Rana plancyi chosenica*, and the control diploids.

a. Lactate dehydrogenase (LDH) b. Malate dehydrogenase (MDH)
c. α-Glycerophosphate dehydrogenase (α-GDH)
d. Isocitrate dehydrogenase (IDH) e. Creatine kinase (CK)

$K^nK^nK^c$. It was remarkable that the results of electrophoretic analyses of the nine protein components completely agreed with the assumed genome constitutions.

In the other two kinds of (C)CCN and (N)NCC allotriploids, raised from female (C)CN and (N)NC hybrids, respectively, by inseminating with sperm of male *Rana plancyi chosenica*, the electrophoretic patterns of the nine protein components also showed that the band derived from two *Rana plancyi chosenica* genomes was remarkably darker than that derived from one *Rana nigromaculata* genome. From these electrophoretic patterns, it was evident that the genes for the nine protein components of the two kinds of (C)CCN and (N)NCC allotriploids were $C^nC^cC^c$, $A^nA^cA^c$, $H^nH^cH^c$, $L^nL^cL^c$, $M\text{-}A^n\ M\text{-}A^c\ M\text{-}A^c$, $M\text{-}B^n\ M\text{-}B^c\ M\text{-}B^c$, $G^nG^cG^c$, $I^nI^cI^c$ and $K^nK^cK^c$. These gene constitutions completely agreed with the assumed genome constitutions. It was also confirmed that the electrophoretic patterns of (N)NNC and (C)CCN allotriploids raised from diploid female hybrids were very similar to those of the same kinds of allotriploids raised from eggs of one species by refrigeration after inseminating with sperm of the other species.

The amphidiploids, (N)NNCC, produced from matings between female (N)NNC allotriploids and male *Rana plancyi chosenica* were completely the same as the diploid (N)NC hybrids in electrophoretic patterns of the nine protein components. Thus, the genes for these components were considered to be $C^nC^nC^cC^c$, $A^nA^nA^cA^c$, $H^nH^nH^cH^c$, $L^nL^nL^cL^c$, $M\text{-}A^n\ M\text{-}A^n\ M\text{-}A^c\ M\text{-}A^c$, $M\text{-}B^n\ M\text{-}B^n\ M\text{-}B^c\ M\text{-}B^c$, $G^nG^nG^cG^c$, $I^nI^nI^cI^c$ and $K^nK^nK^cK^c$, and it was evident that the gene constitutions completely agreed with the assumed genome constitution, (N)NNCC (Figs. 19, 20).

DISCUSSION

Reciprocal hybrids between *Rana nigromaculata* and *Rana plancyi chosenica* are very similar to those between *Rana nigromaculata* and *Rana brevipoda*. When female *Rana plancyi chosenica* were mated in 1962, 1965, 1973 and 1976 with male *Rana nigromaculata*, 91.1% of 1096 eggs obtained from eight females cleaved normally and 64.3% became metamorphosed frogs, while 79.3% of 823 eggs cleaved normally and 51.9% became metamorphosed frogs in the control *Rana plancyi chosenica*. When female *Rana nigromaculata* were mated in 1965, 1973, 1975 and 1976 with male *Rana plancyi chosenica*, 53.0% of 2402 eggs obtained from 10 females cleaved normally and 31.0% became metamorphosed frogs, while 93.8% of 2336 eggs cleaved normally and 77.9% became metamorphosed frogs in the control *Rana nigromacucta*. Thus, it is evident that there is neither gametic isolation nor hybrid inviability between female *Rana plancyi chosenica* and male *Rana nigromaculata*, whereas these two kinds of isolating mechanisms seem to exist to a slight degree in the reciprocal combination. The fact that there was nearly an equal number of males and females in each of reciprocal hybrids as found in the controls seems to show that the two species are the same in sex determining mechanism.

Males of reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda* were almost completely sterile and no viable embryos were produced (KAWAMURA

and NISHIOKA, 1978; NISHIOKA, 1971). In contrast with these, most males of reciprocal hybrids between *Rana plancyi chosenica* and *Rana nigromaculata* were slightly fertile. When 12 male hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata* were backcrossed with female *Rana nigromaculata*, 18.9% of 2069 cleaved normally, and 12.9% and 11.0% became feeding tadpoles and metamorphosed frogs, respectively. On the other hand, when five males of the reciprocal hybrids were backcrossed with female *Rana nigromaculata*, 10.5% of 2138 eggs cleaved normally, and 8.7% and 7.8% became feeding tadpoles and metamorphosed frogs, respectively.

Like females of reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda*, those between *Rana plancyi chosenica* and *Rana nigromaculata* were fertile to a large extent and usually laid a number of normal-sized eggs together with some large ones. It was evident that the percentage of large eggs was greatly affected by food. In 1965 and 1971 when the frogs were fed on domestic flies and bagworms, only 0.2~1.6% of eggs laid by eight of 10 female hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata* were large, while the other two females laid no large eggs. In 1971, only 0.3~0.7% of eggs laid by five of six females of the reciprocal hybrids were large, while the other female laid no large eggs. In contrast to these female hybrids, those raised in 1980 when the frogs were fed on crickets laid far more numerous large eggs. Of the eggs of four female hybrids between female *Rana plancyi chosenica* and male *Rana nigromaculata*, 7.2~39.7% were large, and 3.9~19.8% of four females of the reciprocal hybrids were also large. Large eggs laid by females of reciprocal hybrids were nearly equal to normal-sized eggs laid by the same females in rate of normal cleavages as well as in developmental capacity.

Triploid frogs have been reported in European *Rana esculenta* by HERTWIG and HERTWIG (1920), WICKBOM (1945) and GÜNTHER (1970, 1975a, b). According to GÜNTHER (1975b), 39.6% of the frogs collected from 19 districts in the northern and eastern parts of East Germany were triploids. Especially in two of these districts, more than 80% of the frogs were triploids. Since BERGER (1966) insisted that *Rana esculenta* are hybrids between *Rana lessonae* and *Rana ridibunda*, the hybridism of *Rana esculenta* has been widely believed. UZZELL, BERGER and GÜNTHER (1975), BERGER and UZZELL (1977), BERGER, ROGUSKI and UZZELL (1978), and BERGER and ROGUSKI (1978) confirmed that triploids were produced from large eggs of diploid female *Rana esculenta* by fertilization with sperm of male *Rana lessonae* or *Rana ridibunda*.

In the present study, 14 of 20 diploid female (C)CN hybrids almost laid normal-sized eggs alone, while the other six laid a mixture of large and normal-sized eggs (Table 7). Of a total of 319 normal-sized eggs laid by four of the former 14 females, 289, 25 and 4 became diploid, triploid and aneuploid tadpoles, respectively, by mating with diploid male *Rana nigromaculata* or *Rana plancyi chosenica*. A total of 276 large eggs laid by five of the latter six females all grew into triploid tadpoles, except that two became hypotriploids, by mating with diploid male *Rana nigromaculata* or *Rana plancyi chosenica*. Of a total of 422 normal-sized eggs

laid by the same females, 385, 23 and 15 became diploid, triploid and aneuploid tadpoles, respectively, by mating with diploid males (Table 11).

On the other hand, one of 12 diploid female (N)NC hybrids laid normal-sized eggs alone, while the other 15 laid a mixture of large and normal-sized eggs (Table 9). Of 391 normal-sized eggs laid by three of these 15 females, 379, 5 and 7 became diploid, triploid and aneuploid tadpoles, respectively, by mating with diploid male *Rana nigromaculata* or *Rana plancyi chosenica*. In contrast, a total of 227 large eggs laid by nine of the remaining 12 females became triploid tadpoles without exception by mating with diploid male *Rana nigromaculata* or *Rana plancyi chosenica*, while 634, 39 and 21 of 694 normal-sized eggs became diploid, triploid and aneuploid tadpoles, respectively (Table 12).

It seems evident that diploid backcrosses are raised from haploid eggs fertilized with haploid spermatozoa. These haploid eggs seem to have been produced from diploid oocytes of female hybrids by normal meiosis, as the diploid backcrosses between female hybrids and male *Rana nigromaculata* were various from the *Rana nigromaculata* type to the hybrid type in appearance, and those between female hybrids and male *Rana plancyi chosenica* were various from the *Rana plancyi chosenica* type to the hybrid type. These phenomena seem to show that the two species are very closely related and have genomes being very similar to each other.

In contrast to diploid backcrosses, triploid backcrosses were uniform in appearance. Those produced from backcrossings of diploid female hybrids with diploid male *Rana nigromaculata* were intermediate between the hybrid type and the *Rana nigromaculata* type on the whole, while those produced from backcrossings with *Rana plancyi chosenica* were intermediate between the hybrid type and the *Rana plancyi chosenica* type on the whole. This seems to indicate that the large eggs of diploid female hybrids were diploid and contained the genomes of the two species. Such a large egg seems to have derived from a tetraploid oocyte whose choromosomes were doubled at the oogonium stage. It is very probable that this tetraploid oocyte becomes diploid ovum by normal meiosis and then becomes triploid by fertilization with a haploid spermatozoon. It is natural that there was nearly an equal number of males and females in triploid backcrosses, as the sex is determined by spermatozoa as far as the eggs develop without any physiological disturbance.

Diploid male backcrosses obtained from (C)CN♀ × (N)NN♂ were fertile to a large extent when they were mated with female *Rana nigromaculata*, although they were somewhat inferior to the control male *Rana nigromaculata* in reproductive capacity. Of 1482 eggs of two female *Rana nigromaculata*, 61.7% cleaved normally, and 55.1% and 43.2% became feeding tadpoles and metamorphosed frogs, respectively, by insemination with sperm of six diploid male backcrosses. While 89.4% of normally cleaved eggs became metamorphosed frogs in the control matings of *Rana nigromaculata*, 70.0% did so in the matings of the six diploid male backcrosses with diploid female *Rana nigromaculata*. Diploid male backcrosses obtained from (N)NC♀ × (N)NN♂ were very similar to the above diploid male backcrosses in reproductive capacity. Of 958 eggs of one female *Rana nigro-*

maculata, 73.3% cleaved normally, and 59.0% and 50.3% became feeding tadpoles and metamorphosed frogs, respectively, by insemination with sperm of six diploid male backcrosses. While 78.2% of normally cleaved eggs became metamorphosed frogs in the control matings of *Rana nigromaculata*, 68.7% did so in the matings of the six diploid male backcrosses with diplid female *Rana nigromaculata*.

In contrast to diploid male backcrosses, triploid male backcrosses were almost sterile. When five triploid male backcrosses obtained from (C)CN♀ × (N)NN♂ were mated with diploid female *Rana nigromaculata*, only 2.1% of 2527 eggs cleaved normally and 0.12% became feeding tadpoles. Triploid male backcrosses obtained from (N)NC♀ × (N)NN♂ were almost sterile, too. When five triploid male backcrosses were mated with diploid female *Rana nigromaculata*, 1.1% of 2447 eggs cleaved normally and 0.08% became feeding tadpoles.

Diploid female backcrosses, (C)CN♀ × (N)NN♂, (N)NC♀ × (N)NN♂, (C)CN ♀ × (C)CC♂ and (N)NC♀ × (C)CC♂, were fertile to a large extent when they were mated with diploid male *Rana nigromaculata* or *Rana plancyi chosenica*, although they were somewhat inferior to the control diploid *Rana nigromaculata* in reproductive capacity. In the former two kinds of crossings, 73.8% of 2899 eggs and 82.3% of 2231 eggs cleaved normally, 38.4% and 51.7% became feeding tadpoles and 33.6% and 46.3% completed metamorphosis, while in the latter two kinds of crossings, 84.2% of 3059 eggs and 83.9% of 1673 eggs cleaved normally, 55.6% and 40.9% became feeding tadpoles and 50.0% and 26.2% attained completion of metamorphosis. The diploid female backcrosses obtained from (C)CN♀ × (N)NN♂ and (N)NC♀ × (N)NN♂ seemed to be somewhat inferior to the diploid male backcrosses obtained from the same kinds of matings in percentage of matamorphosed frogs to normally cleaved eggs, although they seemed to be slightly superior in rate of normally cleaved eggs.

In contrast to triploid male backcrosses, triploid female backcrosses were somewhat fertile. Of 7791 eggs of 15 (N)NNC triploid female backcrosses mated with diploid male *Rana plancyi chosenica*, 54.3% cleaved normally, and 6.5% and 3.7% became feeding tadpoles and metamorphosed frogs, respectively. When chromosomes were examined in 466 feeding tadpoles raised from the eggs of the above 15 (N)NNC triploid female backcrosses, 30 (6.4%) were diploids, 64 (13.7%) were triploids, 291 (62.4%) were tetraploids, 67 were aneuploids between $2n+1 \sim 3n-1$ and 14 were hypertriploids. A total of 10184 eggs of these 15 triploid female backcrosses were divided into three groups of 189 (1.9%) normal-sized, 9349 (91.8%) large and 646 (6.3%) huge eggs. It was found that 18 of the 291 tetraploid tadpoles were raised from huge eggs, while the other 273 were from large ones. It seems evident that the tetraploids were derived from triploid eggs of triploid female backcrosses by fertilization with haploid spermatozoa of diploid male *Rana plancyi chosenica*. It is very probable that the triploid eggs were produced by normal meiosis from hexaploid oocytes whose chromosomes had been doubled at the oogonium stage.

Concerning the sex of the tetraploid and triploid offspring produced from the 15 triploid female backcrosses by mating with diploid male *Rana plancyi*

chosenica, it was found that 52 and 171 (76.7%) of 223 metamorphosed tetraploids were females and males, respectively, while 14 and 19 (57.8%) of 33 metamorphosed triploids were females and males, respectively. As the sex of the tetraploids should be determined by spermatozoa of the diploid male *Rana plancyi chosenica*, the preponderance of males in number may be attributable to sex-reversal of some genetic females.

The assumption of the above tetraploids and triploids as amphidiploids and allotriploids on the basis of their origin was confirmed by observing their external characters, inner structure of testes and electrophoretic patterns of three proteinic components extracted from blood as well as six enzymes from skeletal muscles.

SUMMARY

1. Reciprocal hybrids between *Rana plancyi chosenica* and *Rana nigromaculata* and their backcrosses were produced for the purpose of elucidating their sex, ploidy and reproductive capacity.

2. Of 1096 eggs of eight female *Rana plancyi chosenica* mated with eight male *Rana nigromaculata*, 998 (91.1%) cleaved normally, 705 (64.3%) attained completion of metamorphosis and 383 (34.9%) became sexually mature frogs. Of the latter, 197 were females and 186 (48.6%) were males. In the reciprocal hybridization, 1272 (53.0%) of 2402 eggs of 10 female *Rana nigromaculata* cleaved normally, 744 (31.0%) attained completion of metamorphosis and 318 (13.2%) became sexually mature frogs. Of the latter, 158 were females and 160 (50.3%) were males.

3. Males and females of reciprocal hybrids were backcrossed with *Rana nigromaculata* and *Rana plancyi chosenica*. Of 2069 eggs of four female *Rana nigromaculata* backcrossed with 12 male (C)CN hybrids, 392 (18.9%) cleaved normally, 267 (12.9%) became feeding tadpoles and 227 (11.0%) attained completion of metamorphosis. Of 2138 eggs of two female *Rana nigromaculata* backcrossed with five male (N)NC hybrids, 224 (10.5%) cleaved normally, 187 (8.7%) became feeding tadpoles and 167 (7.8%) attained completion of metamorphosis.

Of the 267 feeding tadpoles produced from two female *Rana nigromaculata* by backcrossing with the male (C)CN hybrids, 176 (65.9%) were triploids, 83 (31.1%) were diploids and the other eight were aneuploids. Of the 187 feeding tadpoles produced from the same females by backcrossing with the male (N)NC hybrids, 118 (63.1%) were triploids, 63 (33.7%) were diploids and the other six were aneuploids.

4. Females of reciprocal hybrids usually laid a few large eggs together with normal-sized ones. Of the eggs laid by 17 of 20 female (C)CN hybrids, 0.2~39.7% were large eggs, while 0.1~20.4% of the eggs laid by 15 of 16 female (N)NC hybrids were also large ones. The large eggs did not remarkably differ from the normal-sized eggs laid by the same females in developmental capacity when inseminated with sperm of *Rana nigromaculata* or *Rana plancyi chosenica*. Of the respective total number of large and normal-sized eggs of the female hybrids, 61.1~88.4% cleaved normally, 37.9~60.4% became feeding tadpoles and 34.7~

55.6% attained completion of metamorphosis.

5. All 503 feeding tadpoles produced from large eggs of female hybrids were triploids except two which were hypotriploids. Of 1826 feeding tadpoles produced from normal-sized eggs of these female hybrids, 1687 were diploids, 92 were triploids and the remaining 47 were aneuploids.

In mature diploid and triploid frogs produced from females of reciprocal hybrids by backcrossing with male *Rana nigromaculata* or *Rana plancyi chosenica*, there was nearly an equal number of males and females.

6. When six diploid male backcrosses produced from females of reciprocal hybrids by mating with male *Rana nigromaculata* were mated with three female *Rana nigromaculata*, 44.3~83.4% of the respective number of eggs cleaved normally, 55.0~93.1% became feeding tadpoles and 31.4~56.7% attained completion of metamorphosis. These percentages of metamorphosed frogs corresponded to 35.8~82.3% of normally cleaved eggs.

When 10 triploid male backcrosses produced from females of reciprocal hybrids by mating with male *Rana nigromaculata* were mated with four female *Rana nigromaculata*, two of them were completely sterile and did not give rise to cleavage. By the other eight males, only 0.6~5.0% of the respective number of eggs cleaved normally, 0.2~0.8% became normally hatched tadpoles and 0~0.2%, five in total, became feeding tadpoles.

7. When 20 diploid female backcrosses produced from females of reciprocal hybrids by mating with male *Rana nigromaculata* or *Rana plancyi chosenica* were mated again with a male *Rana nigromaculata* or *Rana plancyi chosenica*, 67.6~93.5% of the respective number of eggs cleaved normally, 16.3~75.9% became feeding tadpoles and 12.7~71.1% attained completion of metamorphosis. These percentages of metamorphosed frogs corresponded to 18.1~79.6% of normally cleaved eggs.

8. Fifteen triploid female (N)NNC backcrosses produced from diploid female (N)NC hybrids by mating with male *Rana nigromaculata* laid 41~1215 eggs which were not uniform in size and usually divided into normal-sized, large and huge eggs. The overwhelming majority of the eggs laid by each triploid female backcrosses was large ones.

9. Of 7791 eggs laid by the 15 triploid female backcrosses mated with three male *Rana plancyi chosenica*, 4233 (54.3%) cleaved normally, 1701 (21.8%) hatched, 505 (6.5%) became feeding tadpoles and 285 (3.7%) attained completion of metamorphosis. Of 466 of the feeding tadpoles, 291 (62.4%) were tetraploids, 64 (13.7%) were triploids, 30 (6.4%) were diploids and the remaining 81 were aneuploids.

10. The sex of 270 of the above 285 metamorphosed offspring obtained from the 15 triploid females was examined. Of 223 of 226 metamorphosed tetraploids, 52 were females and 171 (76.7%) were males. Of 33 of 38 metamorphosed triploids, 14 were females and 19 (57.6%) were males. Of six of eight metamorphosed diploids, three were females and three were males. Eight of 13 metamorphosed aneuploids were all males.

11. The genome constitutions of triploid backcrosses, (N)NNC, and tetraploid

offspring, (N)NNCC, of triploid female backcrosses were confirmed by observing external characters, inner structure of testes and electrophoretic patterns of nine protein components, although they were assumed from their origin and chromosome number.

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