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Production of Autotetraploids and Amphidiploids from Auto- and Allotriploids in *Rana nigromaculata* and *Rana brevipoda*

By

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(With 13 Text-figures)

CONTENTS

INTRODUCTION

Amphidiploid frogs were produced for the first time by KAWAMURA and NISHIOKA (1960). Four in all were obtained by three different methods; one by transplantation of a blastula nucleus of *Rana nigromaculata* into a fertilized egg of *Rana brevipoda*, one by transplantation of a blastula nucleus of an allotriploid

consisting of two *Rana brevipoda* genomes and one *Rana nigromaculata* genome into an unfertilized egg of *Rana nigromaculata* and two by heat-shock treatment of *Rana brevipoda* eggs inseminated with sperm of *Rana nigromaculata*. The amphidiploid obtained from a *Rana brevipoda* egg transplanted with a *Rana nigromaculata* nucleus was a fertile male and produced allotriploids by mating with diploid female *Rana nigromaculata* and *Rana brevipoda* (KAWAMURA and NISHIOKA, 1963a). KAWAMURA and NISHIOKA (1963b) produced thereafter six metamorphosed autotetraploid males from fertilized eggs by heat-shock treatment in *Rana nigromaculata*. Three of them attained sexual maturity and two produced autotetraploids and amphidiploids from matings with female *Rana nigromaculata* and *Rana brevipoda* by refrigeration of inseminated eggs. A number of amphidiploids were produced from matings of these autotetraploid male *Rana nigromaculata* with diploid female *Rana brevipoda* by cold treatment of inseminated eggs (KAWAMURA and NISHIOKA, 1983). Numerous amphidiploid offspring were also obtained from matings between amphidiploid males and females. They were almost completely fertile and produced amphidiploid male and female offspring approximately at a rate of 1 : 1.

Recently, the present authors found that auto- and allotriploid females in *Rana nigromaculata* and *Rana brevipoda* produced fairly many auto- and allotetraploids by mating with diploid males of these species. This finding is almost attributable to an increase in reproductive capacity of triploid females owing to a change of frog food from mosquitoes and domestic flies to crickets. In the present study, autotriploids of *Rana nigromaculata* and *Rana brevipoda* as well as two kinds of allotriploids between these two species were produced at the beginning. Then, autotriploid females were mated with diploid males of their own species in order to obtain autotetraploids on one side, and allotriploid females were mated with diploid males of the two species in order to obtain amphidiploids on the other. The developmental capacity, chromosome numbers and sex ratios of the offspring obtained from the above matings were observed. Lastly, the reproductive capacity of amphidiploid males and females was examined.

MATERIALS AND METHODS

In the breeding season of 1975, two mature males and two mature females of *Rana nigromaculata* HALLOWELL were collected from the suburbs of Hiroshima, and two mature males and two mature females of *Rana brevipoda* ITO were collected from Konko-cho near Okayama. Ovulation was accelerated by pituitary injection. Eggs were refrigerated after insemination in order to suppress extrusion of the second polar body. Autotriploids of *Rana nigromaculata* were produced by refrigerating eggs at 0.5～2°C for 2.5～3 hours, 20～25 minutes after insemination with sperm of the own species, while allotriploids from *Rana nigromaculata* eggs were produced by insemination with sperm of *Rana brevipoda* and refrigeration by the same way. Auto- and allotriploids were also produced from eggs of *Rana brevipoda* by refrigeration at 1～2°C for 2～2.5 hours, 20～25 minutes after in-

semination with sperm of the own or foreign species (NISHIOKA, 1971, 1972).

The chromosomes of tadpoles were examined in tail-tips by the squash method with water-pretreatment (MAKINO and NISHIMURA, 1952) modified by NISHIOKA (1972). In this case, the tadpoles had not been treated with colchicine, as it was necessary to rear them continuously. The tail-tip of each tadpole was clipped once, twice or sometimes thrice. The chromosomes of mature frogs were examined by the blood culture method or the bone marrow method (VOLPE and GEBHARDT, 1968; WU and YANG, 1980; OMURA, 1967). The meiotic chromosomes in the testes were prepared by the method of 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) and NISHIOKA, OHTANI and SUMIDA (1980).

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

In the present paper the following signs are used.

N.....A set of *Rana nigromaculata* chromosomes
B.....A set of *Rana brevipoda* chromosomes
(N) .....*Rana nigromaculata* cytoplasm
(B) .....*Rana brevipoda* cytoplasm
(N)NN.....Diploid *Rana nigromaculata*
(B)BB .....Diploid *Rana brevipoda*
(N)NNN ...Autotriploid *Rana nigromaculata*
(B)BBB .....Autotriploid *Rana brevipoda*
(N)NNB ...Allotriploid consisting of two *Rana nigromaculata* genomes and one *Rana brevipoda* genome
(B)BBN.....Allotriploid consisting of two *Rana brevipoda* genomes and one *Rana nigromaculata* genome
(B)BBNN...Amphidiploid between *Rana brevipoda* and *Rana nigromaculata*, having *Rana brevipoda* cytoplasm

OBSERVATION

I. Production of autotetraploids from autotriploids

1. Offspring of (N)NNN autotriploid females

a. Developmental capacity

i) Autotriploid females produced from (N)NN♀, No. 1 × (N)NN♂, No. 1

Many (N)NNN autotriploid females produced in 1975 by refrigerating eggs after insemination attained sexual maturity in the breeding season of 1977. Of these 2-year-old autotriploids, the largest 16 were injected with frog pituitaries.

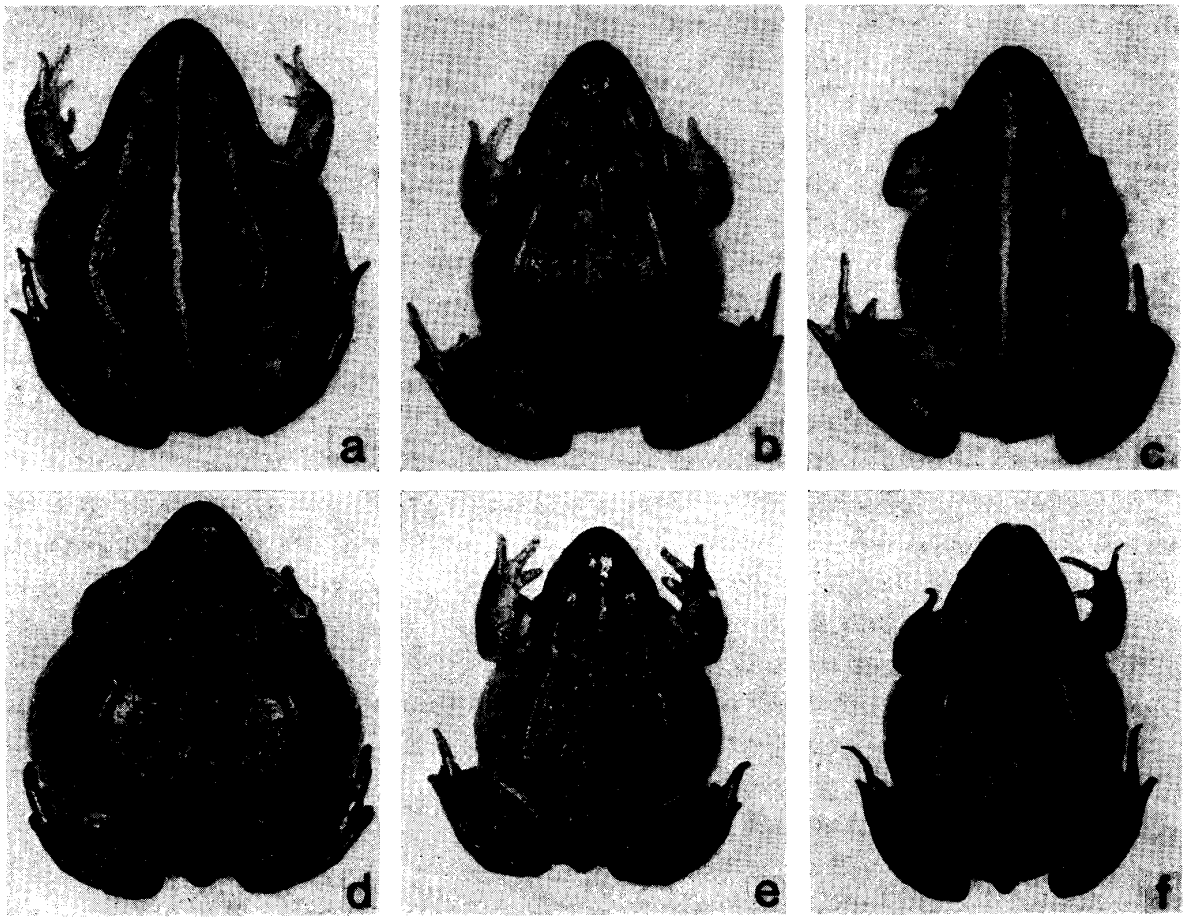


Fig. 1. Three-year-old diploid and triploid *Rana nigromaculata* and *Rana brevipoda*. ×0.6

a, b. Control diploid female and male *Rana nigromaculata* produced from (N)NN♀, No. 1 × (N)NN♂, No. 1

c. Triploid female *Rana nigromaculata* produced from (N)NN♀, No. 1 × (N)NN♂, No. 1 by refrigeration of the egg

d, e. Control diploid female and male *Rana brevipoda* produced from (B)BB♀, No. 1 × (B)BB♂, No. 1

f. Triploid female *Rana brevipoda* produced from (B)BB♀, No. 1 × (B)BB♂, No. 1 by refrigeration of the egg

It was found that normal ovulation occurred only in three, (N)NNN♀, Nos. 1～3. Females Nos. 1 and 2 laid 714 and 765 eggs, respectively. Of these eggs, 23 and 16 were large, being about 2.3 mm in diameter, while the others were normal-sized, being 1.7～2.0 mm. Female No. 3 laid 1026 eggs which were 1.6～2.2 mm in diameter and continuous from larger to smaller.

In the breeding season of 1978, nine 3-year-old female autotriploids were injected with frog pituitaries (Fig. 1). It was found that only two of them, (N)NNN♀, Nos. 4 and 5, laid eggs. They laid 511 and 320 eggs, of which 21 and 14 were large, being about 2.3～2.5 mm in diameter and the others were normal-sized, being 1.7～2.0 mm.

Most of the eggs laid by the five autotriploid females, (N)NNN♀, Nos. 1～5, were inseminated with sperm of two diploid males, (N)NN♂, Nos. 1 and 2

TABLE 1

Developmental capacity of the eggs of female autotriploids inseminated with sperm of male diploids in *Rana nigromaculata* or *Rana brevipoda*

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)NNN, No. 1	(N)NN, No. 1	634	522(82.3%)	234(36.9%)	59(9.3%)	14(2.2%)
	(N)NNN, No. 2	(N)NN, No. 1	715	679(95.0%)	307(42.9%)	93(13.0%)	20(2.8%)
	(N)NNN, No. 3	(N)NN, No. 1	864	675(78.1%)	452(52.3%)	190(22.0%)	45(5.2%)
1978	(N)NNN, No. 4	(N)NN, No. 2	511	426(83.4%)	265(51.9%)	99(19.4%)	22(4.3%)
	(N)NNN, No. 5	(N)NN, No. 2	320	219(68.4%)	172(53.8%)	57(17.8%)	3(0.9%)
1977	(B)BBB, No. 1	(B)BB, No. 1	482	380(78.8%)	238(49.4%)	109(22.6%)	15(3.1%)
	(B)BBB, No. 2	(B)BB, No. 1	616	572(92.9%)	294(47.7%)	136(22.1%)	43(7.0%)
	(B)BBB, No. 3	(B)BB, No. 2	512	457(89.3%)	269(52.5%)	150(29.3%)	30(5.9%)
	(B)BBB, No. 4	(B)BB, No. 2	1230	1215(98.8%)	124(10.1%)	89(7.2%)	38(3.1%)
1978	(B)BBB, No. 5	(B)BB, No. 3	455	405(89.0%)	210(46.2%)	97(21.3%)	26(5.7%)
	(B)BBB, No. 6	(B)BB, No. 3	913	816(89.4%)	233(25.5%)	115(12.6%)	47(5.1%)
1980	(B)BBB, No. 1	(B)BB, No. 4	533	175(32.8%)	46(8.6%)	7(1.3%)	0
	(B)BBB, No. 2	(B)BB, No. 4	974	220(22.6%)	76(7.8%)	13(1.3%)	3(0.3%)
	(B)BBB, No. 3	(B)BB, No. 4	2274	358(15.7%)	135(5.9%)	26(1.1%)	3(0.1%)
	(B)BBB, No. 4	(B)BB, No. 4	1241	492(39.6%)	115(9.3%)	16(1.3%)	6(0.5%)
	(B)BBB, No. 5	(B)BB, No. 5	2444	537(22.0%)	491(20.1%)	116(4.7%)	24(1.0%)
	(B)BBB, No. 6	(B)BB, No. 5	404	243(60.1%)	102(25.2%)	16(4.0%)	7(1.7%)
	(B)BBB, No. 7	(B)BB, No. 5	118	74(62.7%)	18(15.3%)	14(11.9%)	3(2.5%)
	(B)BBB, No. 8	(B)BB, No. 6	1284	145(11.3%)	114(8.9%)	26(2.0%)	2(0.2%)
	(B)BBB, No. 9	(B)BB, No. 6	1940	253(13.0%)	98(5.1%)	18(0.9%)	5(0.3%)
	(B)BBB, No. 10	(B)BB, No. 6	1927	184(9.5%)	83(4.3%)	30(1.6%)	5(0.3%)
	(B)BBB, No. 11	(B)BB, No. 7	1860	1073(57.7%)	430(23.1%)	96(5.2%)	6(0.3%)
	(B)BBB, No. 12	(B)BB, No. 7	600	267(44.5%)	79(13.2%)	14(2.3%)	0
	(B)BBB, No. 13	(B)BB, No. 7	967	460(47.6%)	39(4.0%)	27(2.8%)	3(0.3%)
	(B)BBB, No. 14	(B)BB, No. 7	1533	562(36.7%)	110(7.2%)	65(4.2%)	3(0.2%)
Total (1977, 1978)	(N)NNN, Nos. 1~5	(N)NN, Nos. 1, 2	3044	2521(82.8%)	1430(47.0%)	498(16.4%)	104(3.4%)
	(B)BBB, Nos. 1~6	(B)BB, Nos. 1~3	4208	3845(91.4%)	1368(32.5%)	696(16.5%)	199(4.7%)
(1980)	(B)BBB, Nos. 1~14	(B)BB, Nos. 4~7	18099	5043(27.9%)	1936(10.7%)	484(2.7%)	70(0.4%)

(Table 1). The results showed that 68.4~95.0% of the respective number of eggs, 2521 (82.8%) of 3044 eggs in total, cleaved normally. After 1091 of the normally cleaved eggs died of incomplete invagination at the gastrula stage, edema or microcephaly before the tail-bud stage, 36.9~53.8%, 1430 (47.0%) in total, became normal tail-bud embryos. Thereafter, 932 embryos died of microcephaly, blisters, curvature of the body or underdevelopment, while 9.3~22.0%, 498 (16.4%) in total, hatched normally. The hatched tadpoles mostly became edematous or emaciated and died without taking food, and eventually 0.9~5.2%, 104 (3.4%) in total, became normally feeding tadpoles.

ii) Autotriploid females produced from (N)NN♀, No. 2 × (N)NN♂, No. 2

Eleven mature autotriploid females which were produced in 1975 by refrigerating eggs after insemination were injected with frog pituitaries in the breeding season of 1980. However, no ovulation occurred in all of them. When the abdominal walls of three of these autotriploid females were cut open, it was found that full-grown ova were scarce in their ovaries. Although the remaining 8 females were repeatedly injected with frog pituitaries in 1981 and 1982, none of

them laid eggs.

b. Chromosome number

The chromosomes of mature autotriploids and diploids of *Rana nigromaculata* were examined by the blood culture method. As shown in Figs. 2 and 3, the three chromosomes of each of the 13 triplets in the autotriploids as well as the two chromosomes of each of the 13 pairs in the diploids were nearly equal to each other in size and shape.

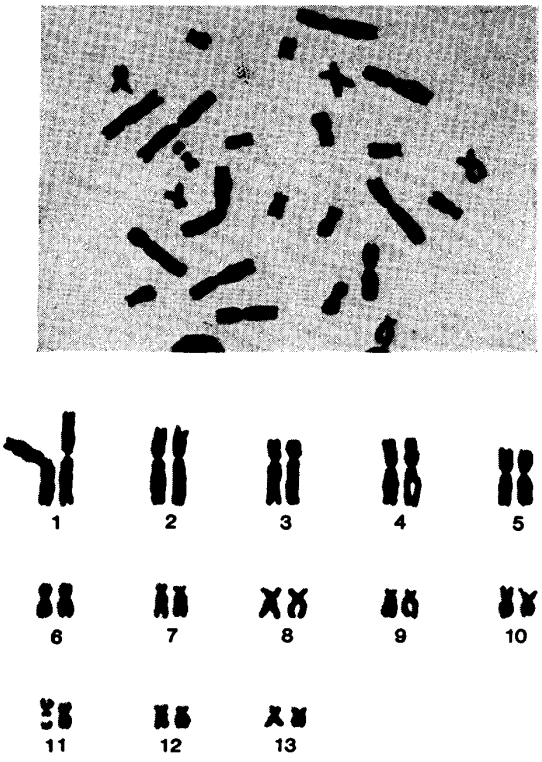


Fig. 2. Metaphase plate and the karyotype of a control diploid female *Rana nigromaculata*. ×900

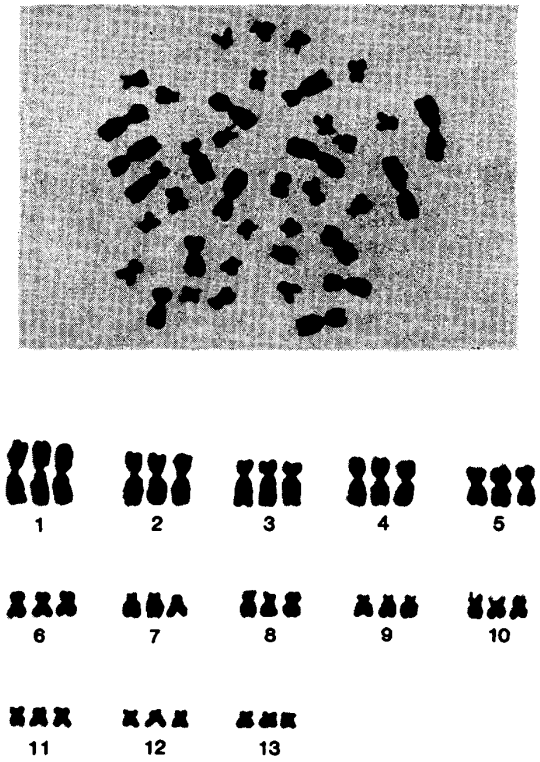


Fig. 3. Metaphase plate and the karyotype of a triploid female *Rana nigromaculata*. ×900

The chromosomes of the offspring of autotriploid females mated with diploid males were examined in the tail-tips of normally feeding tadpoles which were more than 20 mm in total length by the squash method with water pretreatment. The autotriploid females were those that had been produced from a mating, (N)NN♀, No. 1×(N)NN♂, No. 1. The results are presented in Table 2.

Of 97 feeding tadpoles produced from five autotriploid females, (N)NNN♀, Nos. 1~5, mated with two diploid males, (N)NN♂, Nos. 1 and 2, 11 were diploids, 17 were triploids and 12 were tetraploids. The other 57 consisted of 23 hyperdiploids, 19 hypotriploids, three hypertriploids, three hypotetraploids, five hypertetraploids and four mosaics. More specifically, of the 23 hyperdiploids, five were 27 (2n+1), eight were 28 (2n+2), one was 29 (2n+3), two were 30 (2n+4), three were 31 (2n+5) and the remaining four were 32 (2n+6) in chromosome number. Of the 19 hypotriploids, four were 38 (3n−1), three were 37

TABLE 2

Chromosome number of the offspring of female autotriploids mated with male diploids in *Rana nigromaculata* or *Rana brevipoda*

Year	Parents		Number of tadpoles									
				Number of chromosomes								
	Female	Male	Analyzed	26 (2n)	27~32 (2n+)	33~38 (3n−)	39 (3n)	40~45 (3n+)	46~51 (4n−)	52 (4n)	53~58 (4n+)	Mosaics
1977	(N)NNN, No. 1	(N)NN, No. 1	12	2	3	1	5	1				
	(N)NNN, No. 2	(N)NN, No. 1	16	1	2	2	3	1	1	4	2	
	(N)NNN, No. 3	(N)NN, No. 1	44	5	12	11	3		2	6	2	3
1978	(N)NNN, No. 4	(N)NN, No. 2	22	2	6	5	4	1		2	1	1
	(N)NNN, No. 5	(N)NN, No. 2	3	1			2					
1977	(B)BBB, No. 1	(B)BB, No. 1	12	1	3	2	2	1	1	2		
	(B)BBB, No. 2	(B)BB, No. 1	43	4	13	7	6	2	6	3	2	
	(B)BBB, No. 3	(B)BB, No. 2	28	5	6	4	4	1		7	1	
	(B)BBB, No. 4	(B)BB, No. 2	37	3	4	5	2	3	2	12	3	3
1978	(B)BBB, No. 5	(B)BB, No. 3	25	3	7	5	5	2				3
	(B)BBB, No. 6	(B)BB, No. 3	47	9	6	7	10	4	3	4	2	2
1980	(B)BBB, No. 2	(B)BB, No. 4	3		1	1	1					
	(B)BBB, No. 3	(B)BB, No. 4	3	1		1	1					
	(B)BBB, No. 4	(B)BB, No. 4	6	1	2	1	2					
	(B)BBB, No. 5	(B)BB, No. 5	21	5	8	2	6					
	(B)BBB, No. 6	(B)BB, No. 5	6	2	1	1	2					
	(B)BBB, No. 7	(B)BB, No. 5	3			1	2					
	(B)BBB, No. 8	(B)BB, No. 6	2	1			1					
	(B)BBB, No. 9	(B)BB, No. 6	3		1	1	1					
	(B)BBB, No. 10	(B)BB, No. 6	4	2	1		1					
	(B)BBB, No. 11	(B)BB, No. 7	6	2	0	2	2					
	(B)BBB, No. 13	(B)BB, No. 7	3				3					
	(B)BBB, No. 14	(B)BB, No. 7	3			1	2					
Total (1977, 1978)	(N)NNN, Nos. 1~5	(N)NN, Nos. 1, 2	97	11	23	19	17	3	3	12	5	4
	(B)BBB, Nos. 1~6	(B)BB, Nos. 1~3	192	25	39	30	29	13	12	28	8	8
(1980)	(B)BBB, Nos. 2~11, 13, 14	(B)BB, Nos. 4~7	63	14	14	11	24					

(3n−2), five were 36 (3n−3), three were 35 (3n−4), three were 34 (3n−5) and the remainder was 33 (3n−6). Of the three hypertriploids, one was 40 (3n+1) and the others were 42 (3n+3). Of the three hypotetraploids, one was 51 (4n−1), another was 48 (4n−4) and the remainder was 47 (4n−5). Of the five hypertetraploids, two were 53 (4n+1), one was 55 (4n+3), one was 56 (4n+4) and the remainder was 57 (4n+5). Of the four mosaics, three were n-3n and the remainder was 2n-3n in chromosome combination.

Of the above tadpoles, 12 triploids, five tetraploids, 11 hyperdiploids including four (2n+1), five (2n+2) and two (2n+6), eight hypotriploids including three (3n−1), one (3n−3), three (3n−2) and one (3n−6), and two mosaics with n-3n, completed metamorphosis, while diploids were all preserved shortly after chromosome analysis. However, all these metamorphosed frogs died of underdevelopment, edema or infectious diseases without attaining sexual maturity.

The sex was examined in nine triploids, three tetraploids, and four hyperdiploids. Of the nine triploids, six were males and three were females. The three tetraploids and one (2n+1) of the hyperdiploids were males, while the remaining three hyperdiploids (2n+2) consisted of two males and one female. The single hyperdiploid female was underdeveloped and had no growing auxocyte in the ovaries.

2. Offspring of (B)BBB autotriploid females

a. Developmental capacity

i) Autotriploid females produced from (B)BB♀, No. 1 × (B)BB♂, No. 1

Autotriploid females which were produced in 1975 by refrigerating eggs after insemination attained sexual maturity in the breeding season of 1977 (Fig. 1). After five of these 2-year-old females were injected with frog pituitaries, normal ovulation occurred in four, (B)BBB♀, Nos. 1～4, of them. The eggs of them were not uniform in size. These four females laid 482, 616, 512 and 1230 eggs, respectively. The eggs were usually 1.2～2.0 mm in diameter and continuous in size from larger to smaller.

In the breeding season of 1978, four 3-year-old female autotriploids were injected with frog pituitaries. Normal ovulation occurred in two, (B)BBB♀, Nos. 5 and 6, of them. These females laid 455 and 913 eggs which were usually 1.3～2.2 mm and 1.2～2.0 mm in diameter, respectively. The eggs were continuous in size from larger to smaller.

The eggs of the above six females, (B)BBB♀, Nos. 1～6, were inseminated with sperm of three males, (B)BB♂, Nos. 1～3, which were produced in 1975 from a mating, (B)BB♀, No. 1 × (B)BB♂, No. 1. The results are presented in Table 1. Of the respective number of eggs, 78.8～98.8%, 3845 (91.4%) of 4208 eggs in total, cleaved normally. After 2477 eggs died of incomplete invagination at the gastrula stage, edema, microcephaly or some other abnormalities, 10.1～52.5%, 1368 (32.5%) in total, became normal tail-bud embryos. Thereafter, 672 embryos became edematous, microcephalous or emaciated and died, while 7.2～29.3%, 696 (16.5%) in total, hatched normally. After the hatching stage, 497 died of edema, curvature of the body or ill-development of gills and various organs without taking food. Eventually, 3.1～7.0%, 199 (4.7%) in total, became normally feeding tadpoles which were more than 20 mm in total length.

ii) Autotriploid females produced from (B)BB♀, No. 2 × (B)BB♂, No. 2

Many autotriploid females produced in 1975 by refrigerating eggs after insemination attained sexual maturity. When 18 of them were injected with frog pituitaries in the breeding season of 1980, normal ovulation occurred in 14 females, (B)BBB♀, Nos. 1～14, which were five years old. These females usually laid eggs which were not uniform in size.

Female No. 1 laid especially abundant eggs, being about 5700 in number and 1.2～2.0 mm in diameter. Of the other 13 females, Nos. 7 and 13 laid 118 and 967 eggs, respectively. These eggs were about 1.8 mm in diameter and almost uniform in size. Females Nos. 2, 4, 5, 6, 9 and 11 laid 1164, 1242, 2444, 404, 1940 and 1860 eggs, respectively. These eggs were usually 1.2～2.2 mm and continuous in size from larger to smaller. Females Nos. 3, 8, 10, 12 and 14 laid 2274, 1284, 1927, 600 and 1560 eggs, respectively. In contrast to the eggs of the above females, those laid by each of these females were divided into two groups.

One group included large eggs, being usually 1.8～2.1 mm in diameter, while the other included normal-sized eggs, being 1.3～1.5 mm. The proportion of large eggs to normal-sized ones was various with female. The large eggs of females Nos. 3, 8, 10, 12 and 14 were 1021, 96, 900, 48 and 264 in number, respectively.

Most of the eggs of the above 14 autotriploid females, (B)BBB♀, Nos. 1～14, were inseminated with sperm of four diploid males, (B)BB♂, Nos. 4～7. As presented in Table 1, 9.5～62.7% of the respective number of eggs, 5043 (27.9%) of 18099 eggs in total, cleaved normally. Most of the normally cleaved eggs died of incomplete invagination at the gastrula stage, edema, microcephaly or some other abnormalities, and 4.0～25.2%, 1936 (10.7%) in total, became normal tail-bud embryos. However, 1452 of these embryos died of edema, microcephaly or ill-development of gills and various organs, while 0.9～11.9%, 484 (2.7%) in total, hatched normally. Thereafter, many tadpoles died of edema or under-development without taking food. Eventually, 0～2.5%, 70 (0.4%) in total, became normally feeding tadpoles which were more than 20 mm in total length. It was found that only four feeding tadpoles were raised from the large eggs of three autotriploid females, (B)BBB♀, Nos. 3, 10 and 14, while no feeding tadpoles were obtained from the large eggs of the other two females, (B)BBB♀, Nos. 8 and 12.

b. Chromosome number

The chromosomes of mature (B)BBB autotriploid females and (B)BB diploid males of *Rana brevipoda* were examined by the blood culture method (Figs. 4 and 5). Those of the offspring of autotriploid females were examined by the squash method with water-pretreatment in the tail-tips of feeding tadpoles which were more than 20 mm in total length. The results are presented in Table 2.

i) Autotriploid females produced from (B)BB♀, No. 1 × (B)BB♂, No. 1

A total of 199 feeding tadpoles were produced from six autotriploid females, (B)BBB♀, Nos. 1～6, by mating with three diploid males, (B)BB♂, Nos. 1～3. Of these feeding tadpoles the chromosomes of 192 were analyzed, while those of the other seven were not analyzed owing to scarcity of good mitotic figures. It was found that 25 were diploids, 29 were triploids, 28 were tetraploids, 39 were hyperdiploids, 30 were hypotriploids, 13 were hypertriploids, 12 were hypotetraploids, eight were hypertetraploids and the remaining eight were mosaics (Table 2). More specifically, of the 39 hyperdiploids, 21 were 27 (2n+1), 9 were 28 (2n+2), two were 29 (2n+3), three were 30 (2n+4), three were 31 (2n+5) and the remainder was 32 (2n+6) in chromosome number. Of the 30 hypotriploids, 19 were 38 (3n−1), six were 37 (3n−2), two were 36 (3n−3), one was 35 (3n−4), one was 34 (3n−5) and the remainder was 33 (3n−6). Of the 13 hypertriploids, three were 40 (3n+1), four were 41 (3n+2), three were 43 (3n+4), two were 44 (3n+5) and the remainder was 45 (3n+6). Of the 12 hypotetraploids, four were 51 (4n−1), two were 50 (4n−2), one was 49 (4n−3), two were 48 (4n−4), two were 47 (4n−5) and the remainder was 46 (4n−6). Of the eight

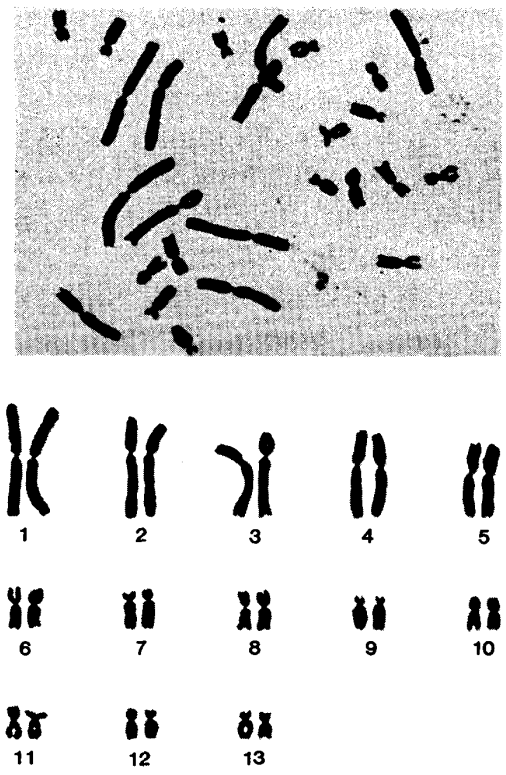


Fig. 4. Metaphase plate and the karyotype of a control diploid female *Rana brevipoda*. ×900

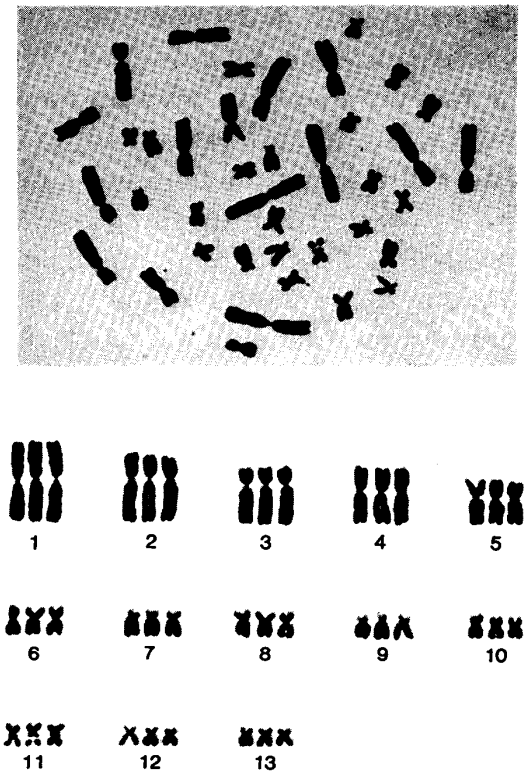


Fig. 5. Metaphase plate and the karyotype of a triploid female *Rana brevipoda*. ×900

hypertetraploids, three were 53 (4n+1), one was 54 (4n+2), two were 55 (4n+3), one was 57 (4n+5) and the remainder was 58 (4n+6). Of the eight mosaics, two were n-3n, one was 2n-3n, one was a mixture of 3n+1 and 3n−1, one was 3n-4n, one was 2n-4n, one was a mixture of 2n, 2n+1 and 2n−1, and the remainder was n-3n-4n (Table 2).

The 25 diploid tadpoles were all preserved shortly after chromosome analysis, while the others were continuously reared. Of the latter, 21 triploids, 13 tetraploids, 10 hyperdiploids including six (2n+1) and four (2n+2), 10 hypotriploids including eight (3n−1) and two (3n−2), three hypertriploids including two (3n+2) and one (3n+4), and one hypotetraploid (4n−1), completed metamorphosis. The remaining tadpoles died of underdevelopment or edema during the tadpole or metamorphosing stage.

Of the metamorphosed frogs, 19 triploids took food and grew further. However, they were preserved within one month after metamorphosis in order to examine their sex. It was found that eight were females and 11 were males. The other metamorphosed frogs were all underdeveloped and died without taking food.

ii) Autotriploid females produced from (B)BB♀, No. 2×(B)BB♂, No. 2

Of 70 feeding tadpoles produced from 12 autotriploid females, (B)BBB♀, Nos. 2~11, 13 and 14, by mating with four diploid males, (B)BB♂, Nos. 4~7, 63 were analyzed in terms of chromosomes, while the remaining seven were not analyzed owing to scarcity of good mitotic figures. As presented in Table 2, 14 were

diploids, 24 were triploids, 14 were hyperdiploids and the remaining 11 were hypotriploids. More specifically, of the 14 hyperdiploids, seven were 27 (2n+1), five were 28 (2n+2), and the remaining two were 32 (2n+6) in chromosome number. Of the 11 hypotriploids, two were 38 (3n−1), three were 37 (3n−2), one was 36 (3n−3), two were 35 (3n−4), two were 34 (3n−5) and the remaining one was 33 (3n−6).

While four diploids and 12 triploids completed metamorphosis, all the others died before or during metamorphosis. All the metamorphosed frogs were preserved.

II. Production of allotetraploids from allotriploids

1. Offspring of (N)NNB allotriploid females

a. Developmental capacity

i) Allotriploid females produced from (N)NN♀, No. 1 × (B)BB♂, No. 1

As (N)NNB allotriploid females produced in 1975 by refrigerating eggs after insemination attained sexual maturity in 1977, five of them were injected with frog pituitaries. Normal ovulation occurred in two, (N)NNB♀, Nos. 1 and 2. These two females laid 3213 and 2064 eggs which were 1.6～2.3 mm in diameter and continuous in size from larger to smaller.

In the breeding season of 1978, seven 3-year-old females were injected with frog pituitaries, and normal ovulation occurred in three, (N)NNB♀, Nos. 3～5. Females Nos. 3, 4 and 5 laid 2005, 1690 and 236 eggs, respectively. These eggs were usually 1.7～2.4 mm in diameter and continuous in size from larger to smaller.

Most of the eggs of four allotriploid females, (N)NNB♀, Nos. 1～4, were inseminated with sperm of two diploid male *Rana nigromaculata*, (N)NN♂, Nos. 1 and 2, or three diploid male *Rana brevipoda*, (B)BB♂, Nos. 1～3. The results are presented in Table 3.

In four matings between the four allotriploid females and two male *Rana nigromaculata*, 30.1～88.2% of the respective number of eggs, 2010 (51.9%) of 3873 eggs in total, cleaved normally. After 937 eggs died of incomplete invagination at the gastrula stage, edema or some other abnormalities, 23.2～41.3%, 1073 (27.7%) in total, became normal tail-bud embryos. Many of these embryos died of edema, blisters, microcephaly or underdevelopment before the hatching stage, while 7.7～23.4%, 546 (14.1%) in total, hatched normally. However, most of the tadpoles died of ill-development of gills and other organs, edema or some other abnormalities without taking food. Eventually, only 1.8～3.0%, 82 (2.1%) in total, became normally feeding tadpoles which were more than 20 mm in length.

In four matings between four allotriploid females and three male *Rana brevipoda*, 44.7～81.5% of the respective number of eggs, 2986 (61.0%) of 4895

TABLE 3

Developmental capacity of the eggs of female allotriploids inseminated with sperm of male diploid *Rana nigromaculata* or *Rana brevipoda*

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)NNB, No. 1	(N)NN, No. 1	1368	691(50.5%)	358(26.2%)	183(13.4%)	24(1.8%)
		(B)BB, No. 1	1812	1477(81.5%)	473(26.1%)	265(14.6%)	30(1.7%)
	(N)NNB, No. 2	(N)NN, No. 1	935	281(30.1%)	224(24.0%)	219(23.4%)	19(2.0%)
		(B)BB, No. 2	1072	479(44.7%)	93(8.7%)	91(8.5%)	7(0.7%)
1978	(N)NNB, No. 3	(N)NN, No. 2	867	418(48.2%)	201(23.2%)	90(10.4%)	26(3.0%)
		(B)BB, No. 3	1042	499(47.9%)	136(13.1%)	46(4.4%)	20(1.9%)
	(N)NNB, No. 4	(N)NN, No. 2	703	620(88.2%)	290(41.3%)	54(7.7%)	13(1.8%)
		(B)BB, No. 3	969	531(54.8%)	114(11.8%)	35(3.6%)	7(0.7%)
Total	(N)NNB, Nos. 1~4	(N)NN, Nos. 1, 2	3873	2010(51.9%)	1073(27.7%)	546(14.1%)	82(2.1%)
		(B)BB, Nos. 1~3	4895	2986(61.0%)	816(16.7%)	437(8.9%)	64(1.3%)

eggs in total, cleaved normally. However, most of the normally cleaved eggs died of incomplete invagination at the gastrula stage, edema or some other abnormalities, while 8.7~26.1%, 816 (16.7%) in total, and 3.6~14.6%, 437 (8.9%) in total, became normal tail-bud embryos and normally hatched tadpoles, respectively. Shortly after hatching, most of the tadpoles died of ill-development of gills or some other organs, edema and various abnormalities without taking food. Eventually, 0.7~1.9%, 64 (1.3%) in total, became normally feeding tadpoles which were more than 20 mm in total length.

ii) Allotriploid females produced from (N)NN♀, No. 2×(B)BB♂, No. 2

As (N)NNB allotriploid females produced in 1975 by refrigerating eggs after insemination attained sexual maturity, seven of them were injected with frog pituitaries in the breeding season of 1980. However, no ovulation occurred in all of them. Although 9 and 13 allotriploid females were also injected with frog pituitaries in 1981 and 1982, respectively, no ovulation occurred even in one of them.

b. Chromosome number

The chromosomes of mature (N)NNB allotriploid females were examined by the blood culture method (Fig. 6). Those of the offspring of allotriploid females were examined in the tail-tips of tadpoles by the squash method with water-pretreatment. The results are presented in Table 4.

i) Offspring of (N)NNB♀, Nos. 1~4×(N)NN♂, Nos. 1 and 2

Of 82 feeding tadpoles whose chromosomes were analyzed, two were diploids, 16 were triploids, 32 were tetraploids, three were hyperdiploids, four were hypotriploids, eight were hypertriploids, 12 were hypotetraploids, one was hypertetraploid and the remaining four were mosaics. More specifically, one of the three hyperdiploids was 27 (2n+1) and the other two were 28 (2n+2) in chromosome number. Of the four hypotriploids, one was 38 (3n−1), another

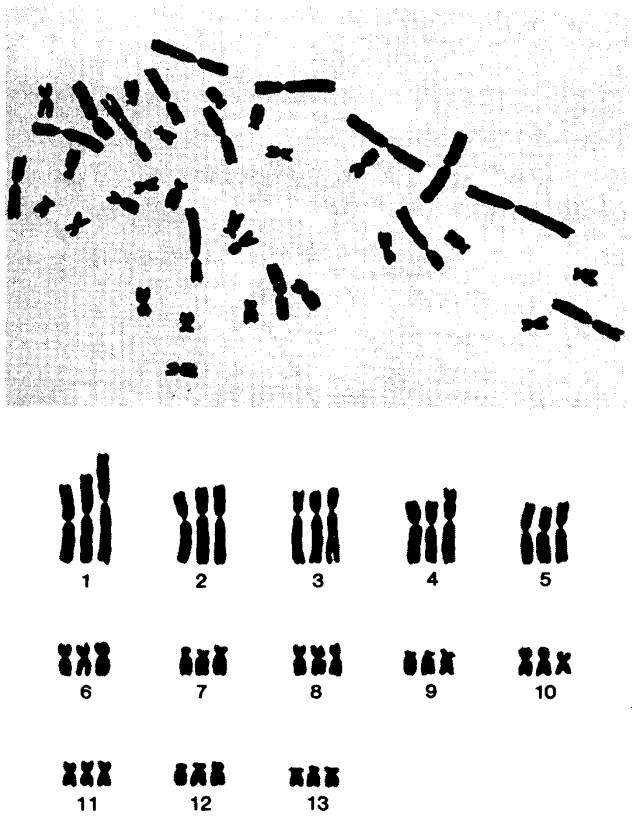


Fig. 6. Metaphase plate and the karyotype of (N)NNB allotriploid female between *Rana nigromaculata* and *Rana brevipoda*. ×900

TABLE 4

Chromosome number of the offspring of female allotriploids mated with male diploid *Rana nigromaculata* or *Rana brevipoda*

Year	Parents		Number of tadpoles								
				Number of chromosomes							
	Female	Male	Analyzed	26 (2n)	27~32 (2n+)	33~38 (3n−)	39 (3n)	40~45 (3n+)	46~51 (4n−)	52 (4n)	53~58 (4n+)	Mosaics
1977	(N)NNB, No. 1	(N)NN, No. 1	24		1	1	5	1	3	12	1	
		(B)BB, No. 1	30	2		1	3	4	3	9	1	7
	(N)NNB, No. 2	(N)NN, No. 1	19	1			5	3	2	7		1
		(B)BB, No. 2	7			1	1	1	1	2		1
1978	(N)NNB, No. 3	(N)NN, No. 2	26	1		3	4	3	5	9		1
		(B)BB, No. 3	20			3	3	2	2	8		2
	(N)NNB, No. 4	(N)NN, No. 2	13		2		2	1	2	4		2
		(B)BB, No. 3	7				1	1	1	2	1	1
Total	(N)NNB, Nos. 1~4	(N)NN, Nos. 1, 2	82	2	3	4	16	8	12	32	1	4
		(B)BB, Nos. 1~3	64	2	0	5	8	8	7	21	2	11

was 37 (3n−2) and the remaining two were 35 (3n−4). Of the eight hypertriploids, three were 40 (3n+1), four were 41 (3n+2) and the remainder was 45 (3n+6). Of the 12 hypotetraploids, two were 51 (4n−1), one was 50 (4n−2), three were 49 (4n−3), one was 48 (4n−4), four were 47 (4n−5) and the remainder was 46 (4n−6). The single hypertetraploid was 54 (4n+2). Of the four mosaics,

one was 2n-4n, two were n-3n and the remainder was 2n-3n in chromosome number.

Of these tadpoles, one hypotriploid (3n−1), five triploids, two hypertriploid including one (3n+1) and one (3n+2), seven tetraploids and two mosaics including one (2n-3n) and one (2n-4n), completed metamorphosis. The other tadpoles died of edema or underdevelopment before or during metamorphosis. Of the metamorphosed frogs, four triploids, five tetraploids and two mosaics died during or immediately after hibernation.

The sex of 13 metamorphosed frogs in total was observed. Of four triploids, two were males and two were females. Six tetraploids included five males and one female. Two of these five male tetraploids attained sexual maturity. The single hypotriploid and the two mosaics were males.

ii) Offspring of (N)NNB♀, Nos. 1～4×(B)BB♂, Nos. 1～3

Of 64 feeding tadpoles whose chromosomes were observed, two were diploids, eight were triploids, 21 were tetraploids, five were hypotriploids, eight were hypertriploids, seven were hypotetraploids, two were hypertetraploids, three were hypohexaploids and the remaining eight were mosaics. More specifically, of the five hypotriploids, two were 38 (3n−1), one was 37 (3n−2), one was 36 (3n−3) and the remainder was 33 (3n−6) in chromosome number. Of the eight hypertriploids, three were 40 (3n+1), one was 41 (3n+2), two were 42 (3n+3) and the remaining two were 43 (3n+4). Of the seven hypotetraploids, one was 51 (4n−1), one was 50 (4n−2), two were 49 (4n−3), one was 48 (4n−4) and the remaining two were 46 (4n−6). Of the two hypertetraploids, one was 53 (4n+1) and the other was 55 (4n+3). Of the three hypohexaploids, one was 77 (6n−1), one was 76 (6n−2) and the remainder was 74 (6n−4). Of the eight mosaics, four were 2n-3n, one was 2n-4n, one was n-2n-4n, one was n-4n and the remainder was 3n-4n (Table 4).

Of the above tadpoles, 15 tetraploids, five triploids, one hypotriploid (3n−1), six hypertriploids including two (3n+1), one (3n+2), two (3n+3) and one (3n+4), two hypotetraploids including one (4n−1) and one (4n−3), and two mosaics including one (2n-3n) and one (2n-4n), completed metamorphosis. The other tadpoles died of edema or underdevelopment before or duirng metamorphosis. All the metamorphosed aneuploids and mosaics also died of underdevelopment without taking food. Although 13 tetraploids and three triploids grew further and six of the tetraploids and all the three triploids lived for one year, all of them were remarkably slow in growth and died without attaining sexual maturity.

When the sex of metamorphosed polyploids was examined, it was found that five triploids consisted of three males and two females, while 11 tetraploids consisted of 9 males and two females.

2. Offspring of (B) BBN allotriploid females

a. Developmental capacity

i) Allotriploid females produced from (B)BB♀, No. 1 × (N)NN♂, No. 1

As (B)BBN allotriploid females produced in 1975 by refrigerating eggs after insemination attained sexual maturity in 1977, three of them were injected with frog pituitaries; ovulation occurred in two females, (B)BBN♀, Nos. 1 and 2. Female No. 1 laid 666 eggs which divided into three groups of six huge, 435 large and 225 normal-sized eggs. Female No. 2 laid 1342 eggs which were also divided into three groups of 19 huge, 1210 large and 113 normal-sized eggs. The huge, large and normal-sized eggs were usually 2.5～2.8 mm, 2.0～2.3 mm and 1.6～1.8 mm in diameter, respectively.

In 1978, five 3-year-old female allotriploids were injected with frog pituitaries. The results showed that normal ovulation occurred in two females, (B)BBN♀, Nos. 3 and 4. Female No. 3 laid 1507 eggs, which were divided into three groups of 73 huge, being 2.4～2.6 mm, 1122 large, being 1.9～2.2 mm, and 312 normal-sized eggs, being 1.6～1.7 mm in diameter. Female No. 4 laid 1860 eggs, which were divided into three groups of 26 huge, being 2.5～2.8 mm, 1324 large, being 2.0～2.3 mm, and 510 normal-sized eggs, being 1.6～1.8 mm in diameter.

About half of the eggs of the above four allotriploid females, (B)BBN♀, Nos. 1～4, was used in insemination with sperm of two male *Rana nigromaculata*, (N)NN♂ Nos. 1～2. As presented in Table 5, 48.6～89.2% of the respective number of eggs, 2152 (75.9%) of 2837 eggs in total, cleaved normally. After 1020 eggs died of incomplete invagination at the gastrula stage, edema, microcephaly or under-development, 28.9～46.3%, 1132 (39.9%) in total, became normal tail-bud embryos. Thereafter, 262 embryos died of edema, blisters, microcephaly or various other abnormalities, while 22.5～34.6%, 870 (30.7%) in total, hatched normally. Most of the hatched tadpoles died of ill-development of gills and some other organs, edema or various other abnormalities without taking food. Eventually, 2.2～12.1%, 176 (6.2%) in total, became normal feeding tadpoles which were more than 20 mm in total length.

The other eggs of the four allotriploid females, (B)BBN♀, Nos. 1～4, were inseminated with sperm of three male *Rana brevipoda*, (B)BB♂, Nos. 1～3. As presented in Table 5, 30.9～58.2% of the respective number of eggs, 1236 (48.7%) of 2538 eggs in total, cleaved normally. After 656 and 203 eggs died of incomplete invagination of gastrulae, microcephaly, edema, curvature of the body or various other abnormalities before the tail-bud and the hatching stage, respectively, 2.8～40.0%, 580 (22.9%) in total, and 1.0～23.5%, 377 (14.9%) in total, became normal tail-bud embryos and normally hatched tadpoles, respectively. Most of the hatched tadpoles died of ill-development of gills and some other organs or edema without taking food. Eventually, 0.4～7.0%, 86 (3.4%) in total, became feeding tadpoles which were more than 20 mm in total length.

TABLE 5

Developmental capacity of the eggs of female allotriploids inseminated with sperm of male diploid *Rana nigromaculata* or *Rana brevipoda*

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	(B)BBN, No. 1	(N)NN, No. 1	346	168(48.6%)	123(35.5%)	97(28.0%)	24(6.9%)
		(B)BB, No. 1	320	99(30.9%)	61(19.1%)	50(15.6%)	11(3.4%)
	(B)BBN, No. 2	(N)NN, No. 1	602	537(89.2%)	279(46.3%)	201(33.4%)	73(12.1%)
		(B)BB, No. 2	740	431(58.2%)	296(40.0%)	174(23.5%)	52(7.0%)
1978	(B)BBN, No. 3	(N)NN, No. 2	679	445(65.5%)	196(28.9%)	153(22.5%)	15(2.2%)
		(B)BB, No. 3	828	377(45.5%)	23(2.8%)	8(1.0%)	3(0.4%)
	(B)BBN, No. 4	(N)NN, No. 2	1210	1002(82.8%)	534(44.1%)	419(34.6%)	64(5.3%)
		(B)BB, No. 3	650	329(50.6%)	200(30.8%)	145(22.3%)	20(3.1%)
1980	(B)BBN, No. 1	(N)NN, No. 3	891	669(75.1%)	477(53.5%)	446(50.1%)	108(12.1%)
	(B)BBN, No. 2	(N)NN, No. 3	372	253(68.0%)	134(36.0%)	61(16.4%)	7(1.9%)
	(B)BBN, No. 3	(N)NN, No. 3	614	473(77.0%)	356(58.0%)	243(39.6%)	11(1.8%)
	(B)BBN, No. 4	(N)NN, No. 4	1367	1133(82.9%)	494(36.1%)	293(21.4%)	18(1.3%)
	(B)BBN, No. 5	(N)NN, No. 4	1547	1206(78.0%)	890(57.5%)	825(53.3%)	72(4.7%)
	(B)BBN, No. 6	(N)NN, No. 5	550	346(62.9%)	155(28.2%)	46(8.4%)	3(0.5%)
	(B)BBN, No. 7	(N)NN, No. 5	1132	934(82.5%)	271(23.9%)	190(16.8%)	11(1.0%)
Total (1977, 1978)	(B)BBN, Nos. 1~4	(N)NN, Nos. 1, 2	2837	2152(75.9%)	1132(39.9%)	870(30.7%)	176(6.2%)
		(B)BB, Nos. 1~3	2538	1236(48.7%)	580(22.9%)	377(14.9%)	86(3.4%)
(1980)	(B)BBN, Nos. 1~7	(N)NN, Nos. 3~5	6473	5014(77.5%)	2777(42.9%)	2104(32.5%)	230(3.6%)

ii) Allotriploid females produced from (B)BB♀, No. 2×(N)NN♂, No. 2

When 9 (B)BBN allotriploid females produced in 1975 by refrigerating eggs after insemination were injected with frog pituitaries in the breeding season of 1980, normal ovulation occurred in seven females, (B)BBN♀, Nos. 1~7. Females Nos. 1, 6 and 7 laid 930, 550 and 1132 eggs which were 1.6~2.0 mm, 1.9~2.3 mm, 2.0~2.3 mm in diameter, respectively. These eggs were continuous in size from larger to smaller. The other four females laid 577, 746, 1503 and 1637 eggs. The eggs laid by each of these females were divided into three groups of huge, large and normal-sized ones. The eggs of females Nos. 2 and 3 included 19 and 7 huge, being about 2.8 mm, 356 and 226 large, being 2.0~2.5 mm and 202 and 513 normal-sized eggs, being 1.5~1.8 mm in diameter, respectively. The eggs of females Nos. 4 and 5 included three and 90 huge, being 2.4~2.5 mm, 1364 and 1377 large, being 1.8~2.2 mm, and 136 and 170 normal-sized eggs, being 1.5~1.6 mm in diameter, respectively.

Most of the eggs of the above seven allotriploid females were inseminated with sperm of three male *Rana nigromaculata*, (N)NN♂, Nos. 3~5, reared in the control series of 1975. As presented in Table 5, 62.9~82.9% of the respective number of eggs, 5014 (77.5%) of 6473 eggs in total, cleaved normally. Of the normally cleaved eggs, 2237 and 673 died of incomplete invagination of gastrulae, edema, microcephaly, curvature of the body or various other abnormalities before the tail-bud and the hatching stage, respectively, while 23.9~58.0%, 2777 (42.9%) in total, and 8.4~53.3% 2104 (32.5%) in total, became tail-bud embryos and hatched tadpoles, respectively. Thereafter, most of the tadpoles died of edema or various other abnormalities without taking food, and eventually, 0.5~12.1%,

230 (3.6%) in total, became normal feeding tadpoles which were more than 20 mm in total length. It was noted that the embryos raised from large eggs usually became abnormal at the early embryonic stage. None of them became a feeding tadpole.

b. Chromosome number

The chromsomes of mature (B)BBN allotriploid females were examined by the blood culture method. Those of the offspring of allotriploid females were examined in the tail-tips of feeding tadpoles which were more than 20 mm in total length by the squash method with water-pretreatment. The results are presented in Table 6.

TABLE 6

Chromosome number of the offspring of female allotriploids mated with male diploid *Rana nigromaculata* or *Rana brevipoda*

Year	Parents		Number of tadpoles									
				Number of chromosomes								
	Female	Male	Ana-lyzed	26 (2n)	27~32 (2n+)	33~38 (3n−)	39 (3n)	40~45 (3n+)	46~51 (4n−)	52 (4n)	53~58 (4n+)	Mosaics
1977	(B)BBN, No. 1	(N)NN, No. 1	24	1		1	5	4	4	6	1	2
		(B)BB, No. 1	11			1	1	1	3	5		
	(B)BBN, No. 2	(N)NN, No. 1	73	2	4	1	6	8	8	36	4	4
		(B)BB, No. 2	52		1	4	7	10	7	14	2	5
1978	(B)BBN, No. 3	(N)NN, No. 2	15	2	3	2	1	2	2	3		
		(B)BB, No. 3	3					1	1	1		
	(B)BBN, No. 4	(N)NN, No. 2	64	1	3	4	3	11	7	25	5	5
		(B)BB, No. 3	20			2	3	3	1	7	2	2
1980	(B)BBN, No. 1	(N)NN, No. 3	78	46	6	4	17					5
	(B)BBN, No. 2	(N)NN, No. 3	5	1	1	2	1					
	(B)BBN, No. 3	(N)NN, No. 3	10		5	2		2		1		
	(B)BBN, No. 4	(N)NN, No. 4	18		4	3				11		
	(B)BBN, No. 5	(N)NN, No. 4	63	11	6	17	13	11	3	2		
	(B)BBN, No. 6	(N)NN, No. 5	3					1		2		
	(B)BBN, No. 7	(N)NN, No. 5	11	3	1	2		1	1	3		
Total (1977, 1978)	(B)BBN, Nos. 1~4	(N)NN, Nos. 1, 2	176	6	10	8	15	25	21	70	10	11
		(B)BB, Nos. 1~3	86	2	1	7	11	15	12	27	4	7
(1980)	(B)BBN, Nos. 1~7	(N)NN, Nos. 3~5	188	61	23	30	31	15	4	19	0	5

i) Allotriploid females produced from (B)BB♀, No. 1 × (N)NN♂, No. 1

Of 176 feeding tadpoles produced from four allotriploid females, (B)BBN♀, Nos. 1~4, by mating with two male *Rana nigromaculata*, (N)NN♂, Nos. 1 and 2, six were diploids, 15 were triploids, 70 were tetraploids, 10 were hyperdiploids, eight were hypotriploids, 25 were hypertriploids, 21 were hypotetraploids, 10 were hypertetraploids and the remaining 11 were mosaics. More specifically, of the 10 hyperdiploids, three were 27 (2n+1), four were 28 (2n+2), one was 30 (2n+4), one was 31 (2n+5) and the remainder was 32 (2n+6) in chromosome number. Of the eight hypotriploids, two were 38 (3n−1), one was 37 (3n−2), three were 36 (3n−3), one was 35 (3n−4) and the remainder was 33 (3n−6). Of the 25 hypertriploids, six were 40 (3n+1), five were 41 (3n+2), two were 42

(3n+3), five were 43 (3n+4), five were 44 (3n+5) and the remaining two were 45 (3n+6). Of the 21 hypotetraploids, seven were 51 (4n−1), three were 50 (4n−2), four were 49 (4n−3), two were 48 (4n−4), two were 47 (4n−5) and the remaining three were 46 (4n−6). Of the 10 hypertetraploids, three were 53 (4n+1), one was 54 (4n+2), two were 55 (4n+3), two were 56 (4n+4), one was 57 (4n+5) and the remainder was 58 (4n+6). Of the 11 mosaics, four were n-3n, one was 2n-3n-4n, two were 2n-3n, two were n-4n, one was 3n-4n and the remainder was n-2n-3n in chromosome number.

Of the above tadpoles whose chromosome numbers were elucidated, only 47 completed metamorphosis. Of these metamorphosed frogs, two were diploids, six were triploids, 26 were tetraploids, two were hyperdiploids including one (2n+1) and one (2n+2), one was a hypotriploid (3n−1), five were hypertriploids including two (3n+1) and three (3n+2), three were hypotetraploids including two (4n−1) and one (4n−4), and the remaining two were hypertetraploids (4n+1) in chromosome number. The other tadpoles all died of underdevelopment or edema before or during metamorphosis.

While most of the metamorphosed frogs died of underdevelopment, edema or various other abnormalities without taking food, one diploid, two triploids and 14 tetraploids took food and grew further. However, most of these metamorphosed frogs died within one month after metamorphosis. Eventually, seven tetraploids hibernated.

The sex of two triploids and 13 tetraploids was examined. It was found that one of the triploids was a male and the other was a female. One of the tetraploids was a female, while the other 12 were males.

Of 86 feeding tadpoles produced from four allotriploid females, (B)BBN♀, Nos. 1∼4, mated with three male *Rana brevipoda*, (B)BB♂, Nos. 1∼3, two were diploids, 11 were triploids, 27 were tetraploids, one was a hyperdiploid (2n+2), seven were hypotriploids, 15 were hypertriploids, 12 were hypotetraploids, four were hypertetraploids and the remaining seven were mosaics. More specifically, of the seven hypotriploids, three were 38 (3n−1), one was 37 (3n−2), two were 35 (3n−4) and the remainder was 34 (3n−5) in chromosome number. Of the 15 hypertriploids, six were 40 (3n+1), four were 41 (3n+2), one was 42 (3n+3), two were 43 (3n+4), one was 44 (3n+5) and the remainder was 45 (3n+6). Of the 12 hypotetraploids, two were 51 (4n−1), four were 50 (4n−2), one was 48 (4n−4), three were 47 (4n−5) and the remaining two were 46 (4n−6). Of the four hypertetraploids, two were 53 (4n+1), another was 54 (4n+2) and the remainder was 56 (4n+4). Of the seven mosaics, four were n-3n, one was n-2n-3n, one was n-4n and the remainder was 2n-3n in chromosome number.

Only 20 of the above tadpoles whose chromosome numbers were elucidated completed metamorphosis. The metamorphosed frogs included five triploids, 11 tetraploids and four aneuploids, of which one was 38 (3n−1), two were 40 (3n+1), and one was 50 (4n−2) in chromosome number. The other tadpoles all died of edema or various other abnormalities before or during metamorphosis. Most of the metamorphosed frogs also died of underdevelopment or edema without

taking food shortly after metamorphosis. Eventually, three triploids and six tetraploids lived for more than one month, although all of them died before hibernation.

The sex of the triploids and tetraploids was examined. It was found that four triploids consisted of three males and one female, while seven tetraploids consisted of six males and one female. The sex of the other metamorphosed frogs were not elucidated.

ii) Allotriploid females produced from (B)BB♀, No. 2×(N)NN♂, No. 2

The chromosomes of 230 feeding tadpoles produced from seven allotriploid females (B)BBN♀, Nos. 1～7, by mating with three male *Rana nigromaculata*, (N)NN♂, Nos. 3～5, were examined. The chromosome number was elucidated in 188 of them, while it was obscure in the other 42, owing to absence of analyzable metaphase spreads. The results are presented in Table 6.

Of these tadpoles, 61 were diploids, 31 were triploids, 19 were tetraploids, 23 were hyperdiploids, 30 were hypotriploids, 15 were hypertriploids, four were hypotetraploids and the remaining five were mosaics. More specifically, 11 of the 23 hyperdiploids were 27 (2n+1), five were 28 (2n+2), three were 29 (2n+3), two were 30 (2n+4) and the remaining two were 32 (2n+6) in chromosome number. Of the 30 hypotriploids, three were 38 (3n−1), one was 37 (3n−2), 15 were 36 (3n−3), six were 35 (3n−4), four were 34 (3n−5) and the remainder was 33 (3n−6). Of the 15 hypertriploids, two were 40 (3n+1), two were 41 (3n+2), five were 42 (3n+3), two were 43 (3n+4), three were 44 (3n+5) and the remainder was 45 (3n+6). Of the four hypotetraploids, two were 51 (4n−1), another was 50 (4n−2) and the remainder was 46 (4n−6). Of the five mosaics, four were n-3n and the other was 2n-3n in chromosome number.

Ninety-five of the 188 tadpoles whose chromosome numbers were elucidated completed metamorphosis, while the others died of edema, underdevelopment or various other abnormalities before or during metamorphosis. The metamorphosed frogs consisted of 45 diploids, 19 triploids, 14 tetraploids, 9 hyperdiploids including six (2n+1), two (2n+2) and one (2n+6), one hypotriploid (3n−1), four hypertriploid including one (3n+1) and three (3n+3), and three mosaics (n-3n).

The metamorphosed diploids and triploids were all preserved shortly after metamorphosis. Although the other frogs were continuously reared, those other than tetraploids were dwarfs and gradually died of underdevelopment or edema without taking food. Only 11 of the tetraploids attained sexual maturity. Of these mature tetraploids, nine were males and two were females. When the tetraploids produced from the above four (B)BBN allotriploid females derived from (B)BB♀, No. 1×(N)NN♂, No. 1, were added to these tetraploids, there were 24 tetraploids in total which were of a similar origin. Of these tetraploids, three were females and 21 were males. In 1982, two of the tetraploid females derived from (B)BB♀, No. 2×(N)NN♂, No. 2 were mated with the two tetraploid males to produce their offspring. It was evident that these tetraploids were amphidiploids, (B)BBNN, on the basis of their origin and morphology.

III. Offspring of (B)BBNN amphidiploids

1. Developmental capacity

Two mature amphidiploid females, (B)BBNN♀, Nos. 1 and 2, produced in 1980 from a mating, (B)BBN♀, No. 4×(N)NN♂, No. 4, were injected with frog pituitaries in the breeding season of 1982. Normal ovulation occurred in both females. Female No. 1 laid 581 eggs which were divided into two groups of 432 large eggs, being 2.0~2.1 mm and 149 normal-sized eggs, being 1.5~1.6 mm in diameter. In contrast, female No. 2 laid 475 eggs which were large, being about 2.2 mm in diameter and almost uniform in size.

All the eggs of the two amphidiploid females were inseminated with sperm of two mature amphidiploid males, (B)BBNN♂, Nos. 1 and 2, and a male *Rana nigromaculata*, (N)NN♂, No. 6. The results are presented in Table 7. When inseminated with sperm of the two amphidiploid males, 59.1~77.6% of the respective number of eggs, 576 (67.2%) of 857 eggs in total, cleaved normally. After many of the normally cleaved eggs died of incomplete invagination at the gastrula stage, edema, blisters or various other abnormalities, 45.6~58.9%, 431 (50.3%) in total, and 36.2~46.3%, 355 (41.4%) in total, became normal tail-bud embryos and normally hatched tadpoles, respectively. Of the normally hatched tadpoles, 61 became edematous and died without taking food. Eventually, 33.3~35.5%, 294 (34.3%) in total, and 29.3~32.3%, 267 (31.2%) became feeding tadpoles and metamorphosed frogs, respectively, while 27 tadpoles died of edema or various other abnormalities before or during metamorphosis (Table 7).

Of 199 eggs of the two amphidiploid females, (B)BBNN♀, Nos. 1 and 2,

TABLE 7

Developmental capacity of the eggs of female amphidiploids mated with male amphidiploids or diploid *Rana nigromaculata*

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						
(B)BBNN 80,No. 1	(B)BBNN 80, No. 1	214	162 (75.7%)	126 (58.9%)	99 (46.3%)	76 (35.5%)	67 (31.3%)
	(B)BBNN 80, No. 2	261	161 (61.7%)	125 (47.9%)	108 (41.4%)	90 (34.5%)	81 (31.0%)
	(N)NN, No. 6	106	58 (54.7%)	50 (47.2%)	48 (45.3%)	33 (31.1%)	30 (28.3%)
(B)BBNN 80, No. 2	(B)BBNN 80, No. 1	147	114 (77.6%)	67 (45.6%)	63 (42.9%)	49 (33.3%)	43 (29.3%)
	(B)BBNN 80, No. 2	235	139 (59.1%)	113 (48.1%)	85 (36.2%)	79 (33.6%)	76 (32.3%)
	(N)NN, No. 6	93	77 (82.8%)	68 (73.1%)	50 (53.8%)	44 (47.3%)	41 (44.1%)
(B)BBNN, Nos. 1, 2	(B)BBNN, Nos. 1, 2	857	576 (67.2%)	431 (50.3%)	355 (41.4%)	294 (34.3%)	267 (31.2%)
	(N)NN, No. 6	199	135 (67.8%)	118 (59.3%)	98 (49.2%)	77 (38.7%)	71 (35.7%)

inseminated with sperm of a diploid male *Rana nigromaculata*, (N)NN♂, No. 6, 135 (67.8%) cleaved normally. After 17 and 20 embryos died of incomplete invagination, edema or various other abnormalities before the tail-bud and the hatching stage, respectively, 118 (59.3%) and 98 (49.2%) became normal tail-bud embryos and normally hatched tadpoles, respectively. While 21 of the latter became abnormal soon afterwards, 77 (38.7%) became normally feeding tadpoles and eventually 71 (35.7%) completed metamorphosis (Table 7).

2. Chromosome number

The chromosomes of mature (B)BBNN amphidiploid males and females were examined by the blood culture method (Fig. 7). Those of the offspring of amphidiploid males and females were examined in the tail-tips of feeding tadpoles which were more than 30 mm in total length by the squash method with water-pretreatment.

Fourteen of 76 normally feeding tadpoles produced from an amphidiploid female, (B)BBNN♀, No. 1, by mating with an amphidiploid male, (B)BBNN♂, No. 1, were raised from normal-sized eggs, while the others were from large eggs. Fourteen of 90 normally feeding tadpoles produced from the same female by mating with the other amphidiploid male, (B)BBNN♂, No. 2, were also raised from normal-sized eggs, while the others were from large eggs. All the feeding tadpoles raised from normal-sized eggs as well as a small number of the feeding tadpoles raised from large eggs were examined in terms of chromosome number.

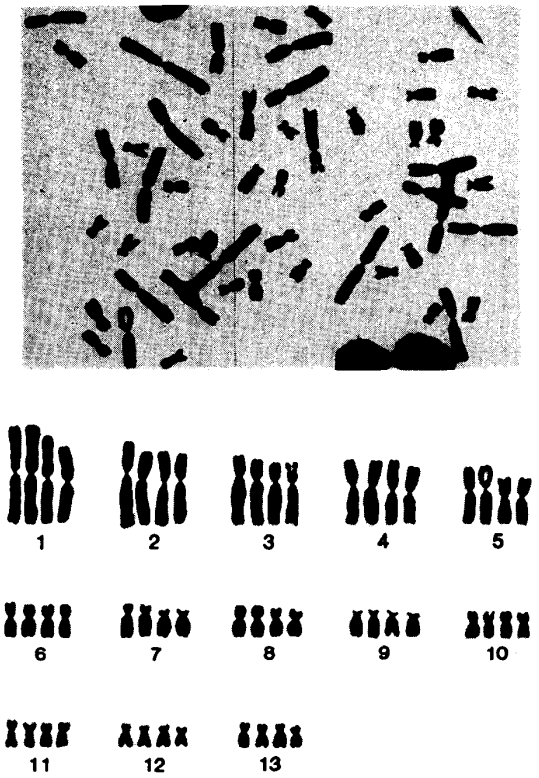


Fig. 7. Metaphase plate and the karyotype of a (B)BBNN amphidiploid female between *Rana brevipoda* and *Rana nigromaculata*. ×900

It was found that 12 of the 14 tadpoles produced from a mating, (B)BBNN♀, No. 1 × (B)BBNN♂, No. 1, and all the 14 tadpoles produced from a mating, (B)BBNN♀, No. 1 × (B)BBNN♂, No. 2, were tetraploids, while the remaining two were mosaics, 2n-3n-5n and 2n-4n-6n, in chromosome number. The feeding tadpoles raised from large eggs were all tetraploids as far as the chromosomes of their metaphase spreads were definitely analyzed, although only 12 tadpoles were examined.

Six of 33 feeding tadpoles produced from an amphidiploid female, (B)BBNN♀, No. 1, by mating with a diploid male *Rana nigromaculata*, (N)NN♂, No. 6, were raised from normal-sized eggs, while the others were from large eggs. The chromosomes of all the six tadpoles raised from normal-sized eggs as well as five tadpoles raised from large eggs were examined. The results indicated that the tadpoles raised from normal-sized eggs were all triploids and those raised from large eggs were also triploids, although two of them were slightly obscure in determining their chromosome number.

More detailed chromosome analyses of the offspring of amphidiploid males and females will be reported in a separate paper.

3. External characters

External characters were observed in mature amphidiploid male and female

TABLE 8

External characters of five kinds of mature frogs in *Rana nigromaculata* and *Rana brevipoda*

	(N)NN	(B)BB	(B)BNN	(B)BBN	(B)BBNN
Body shape	Slender	Dumpy	Intermediate near (N)NN	Intermediate near (B)BB	Intermediate between (N)NN & (B)BB
Dorsal tubercles: Shape	Elongated rod	Dot or irregular short rod	Intermediate near (N)NN	Intermediate near (B)BB	Intermediate between (N)NN & (B)BB
Number	Many	Few	Intermediate near (N)NN	Intermediate near (B)BB	Intermediate between (N)NN & (B)BB
Color of dorsolateral folds	Pale	Dark, coppery	Intermediate near (N)NN	Intermediate near (B)BB	Intermediate between (N)NN & (B)BB
Dorsal ground color in female	Pale brown	Brownish gray	Intermediate near (N)NN	Intermediate near (B)BB	Intermediate between (N)NN & (B)BB
Median stripe on the back	Present	Absent	Present	Present	Present
Dorsal black spots: Shape	Rod, connected in female	Round	Intermediate near (N)NN	Intermediate near (B)BB	Intermediate between (N)NN & (B)BB Clear-cut, deep black
Size	Small	Large	Intermediate near (N)NN	Intermediate near (B)BB	Intermediate between (N)NB & (B)BB
Number	Many	Few	Intermediate near (N)NN	Intermediate near (B)BB	Intermediate between (N)NN & (B)BB
Color of undersurfaces	White	Dusky with gray	White	White	White
Hind limbs	Long	Short	Intermediate near (N)NN	Intermediate near (B)BB	Intermediate between (N)NN & (B)BB
Nuptial color in male	Distinctly present	Absent	Present near (N)NN	Absent	Faintly present

offspring, (B)BBNN 82♂ and ♀, obtained from matings between two 2-year-old amphidiploid females, (B)BBNN 80♀, Nos. 1 and 2, and two 2-year-old amphidiploid males, (B)BBNN 80♂, Nos. 1 and 2, and in (B)BNN allotriploids obtained from matings between the above two parental amphidiploid females and a diploid male *Rana nigromaculata*, (N)NN♂, No. 6. The external characters of these amphidiploids and allotriploids were compared with those of allotriploids (B)BBN obtained in 1975 from a diploid female *Rana brevipoda* (B)BB♀, No. 1, whose eggs were refrigerated after insemination with sperm of a diploid male *Rana nigromaculata*, (N)NN♂, No. 1, those of (N)NNB allotriploids obtained in 1975 from a diploid female *Rana nigromaculata*, (N)NN♀, No. 1, whose eggs were refrigerated after insemination with sperm of a male *Rana brevipoda*, (B)BB♂,

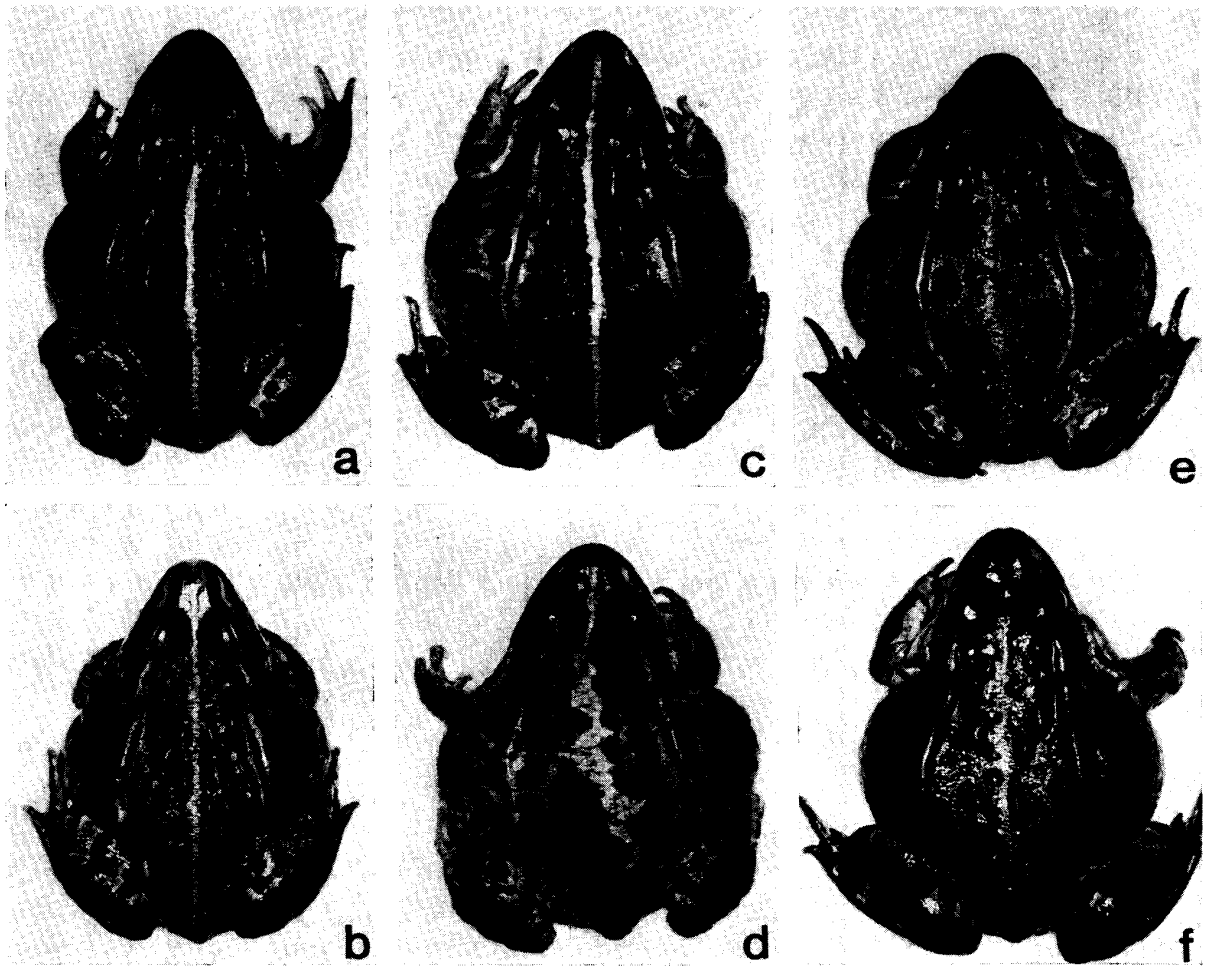


Fig. 8. Two-year-old allotriploids and amphidiploids between *Rana nigromaculata* and *Rana brevipoda*. ×0.6

a. Female (N)NNB allotriploid produced from (N)NN♀, No. 1 × (B)BB♂, No. 1 by refrigeration of the egg

b. Female (B)BBN allotriploid produced from (B)BB♀, No. 1 × (N)NN♂, No. 1 by refrigeration of the egg

c, d. Female and male (B)BNN allotriploids produced from (B)BBNN♀, No. 1 × (N)NN♂, No. 6

e, f. Female and male (B)BBNN amphidiploids produced from (B)BBNN♀, No. 1 × (B)BBNN♂, No. 1

No. 1, those of diploid *Rana nigromaculata* obtained in 1975 from a control mating, (N)NN♀, No. 1 × (N)NN♂, No. 1, and those of diploid *Rana brevipoda* obtained in 1975 from a control mating, (B)BB♀, No. 1 × (B)BB♂, No. 1. The results are presented in Table 8.

Except dominant characters such as the median stripe on the back and the white undersurfaces in *Rana nigromaculata*, the amphidiploids were intermediate between the diploid *Rana nigromaculata* and *Rana brevipoda* in various external characters. On the other hand, (B)BNN and (N)NNB allotriploids were intermediate between the amphidiploids and the diploid *Rana nigromaculata*, while (B)BBN allotriploids were intermidiate between the amphidiploids and the diploid *Rana brevipoda* (Fig. 8).

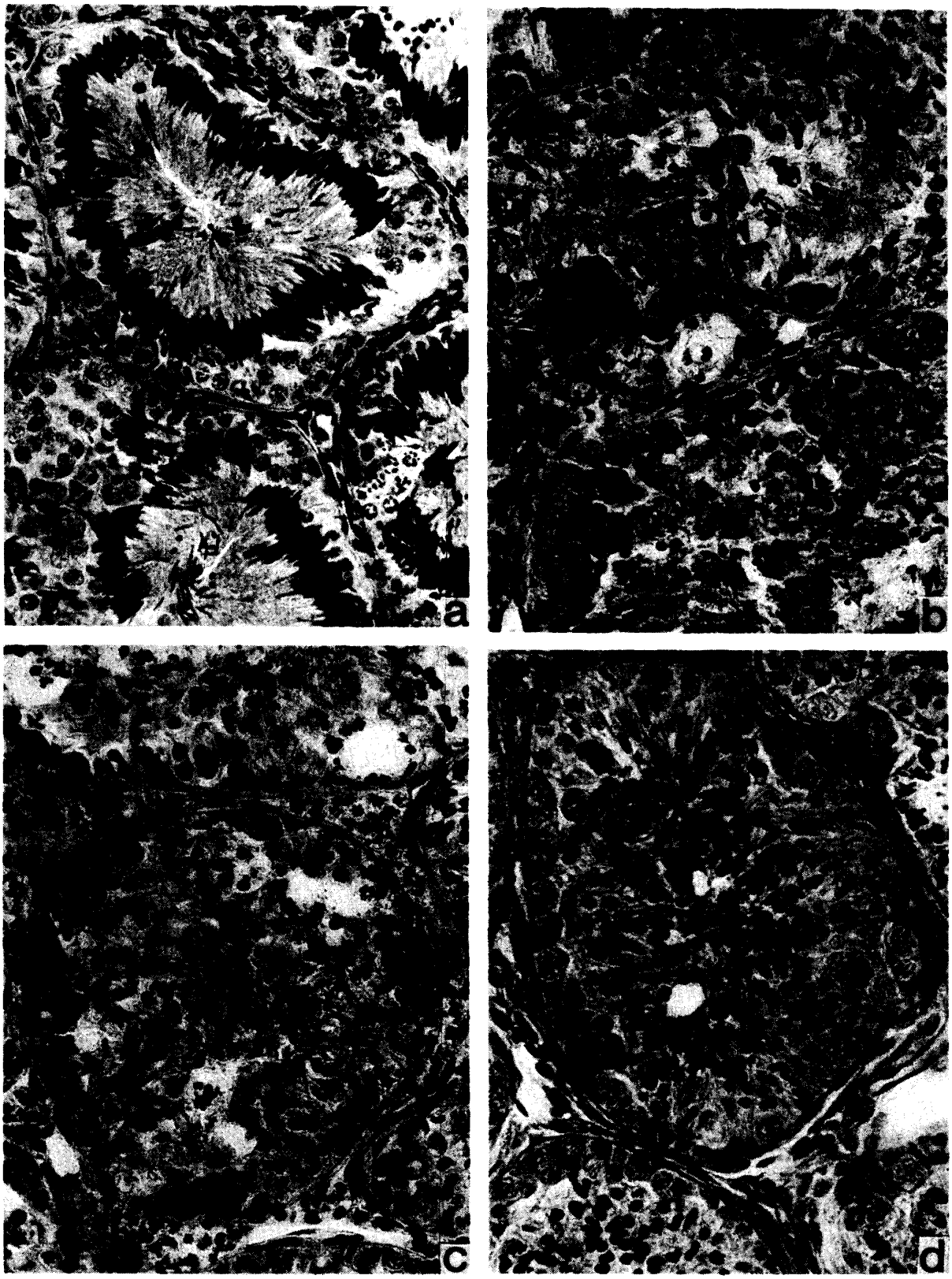
4. Inner structure of testes

The inner structure of testes was observed in three mature amphidiploid males, (B)BBNN 82♂, Nos. 1~3 obtained in 1982 from a mating between an amphidiploid female and an amphidiploid male. Although these amphidiploids were 6 months old and only three months after metamorphosis, they were already sexually matured. Their testes were compared with those of two diploid male *Rana nigromaculata* and two diploid male *Rana brevipoda* obtained in 1975 from control matings, those of two (B)BBB autotriploid males and two (N)NNN autotriploid males obtained in 1975 from a diploid female *Rana brevipoda* (No. 1) and a diploid female *Rana nigromaculata* (No. 1), respectively, by refrigeration of eggs after inseminating with sperm of a diploid male of the own species and those of two (B)BBN allotriploid males and two (N)NNB allotriploid males obtained in 1975 from a diploid female *Rana brevipoda* (No. 1) and a diploid female *Rana nigromaculata* (No. 1), respectively, by refrigeration of eggs after inseminating with sperm of a diploid male of the foreign species. They were also compared with those of three (B)BNN allotriploid males obtained in 1982 from a amphidiploid female, (B)BBNN 80♀, No. 1, by mating with a diploid male *Rana nigromaculata*, (N)NN♂, No. 6.

The seminiferous tubules of the testes of diploid male *Rana nigromaculata* and *Rana brevipoda* were filled with compact bundles of spermatozoa and masses of first or second spermatocytes. Along their inner walls, there were primary and secondary spermatogonia. In contrast, no normal spermatozoa were found in the testes of the autotriploid *Rana nigromaculata* and *Rana brevipoda*. In their seminiferous tubules, abnormally shaped spermatozoa of various sizes and pycnotic

Fig. 9. Cross-sections of the testes of triploid male *Rana nigromaculata* and *Rana brevipoda*, an allotriploid male between the two species and the control diploid. ×260

a. Control diploid *Rana nigromaculata* produced from (N)NN♀, No. 1 × (N)NN♂, No. 1
b. Triploid *Rana nigromaculata* produced from (N)NN♀, No. 1 × (N)NN♂, No. 1 by refrigeration of the egg
c. Triploid *Rana brevipoda* produced from (B)BB♀, No. 1 × (B)BB♂, No. 1 by refrigeration of the egg
d. (B)BNN allotriploid produced from (B)BBNN♀, No. 1 × (N)NN♂, No. 6


a
b
c
d

nculei were sparsely distributed. Besides, first spermatocytes and primary and secondary spermatogonia were found in the peripheral parts of seminiferous tubules. The primary spermatogonia appeared somewhat larger than those of the diploid male *Rana nigromaculata* and *Rana brevipoda*. Allotriploids (B)BBN and (N)NNB obtained from eggs by refrigeration after inseminating with sperm of the foreign species as well as (B)BNN allotriploids obtained from an amphidiploid female by mating with a diploid male *Rana nigromaculata* were very similar to the above two kinds of autotriploids in inner structure of the testes (Fig. 9). Their testes contained no normal spermatozoa. In the seminiferous tubules, abnormally shaped spermatozoa of various sizes and pycnotic nuclei were sparsely distributed. In the peripheral parts of seminiferous tubules, there were many primary and secondary spermatogonia. The primary spermatogonia appeared somewhat larger than those of the diploid males, as found in the autotriploid males.

The testes of the three amphidiploid males were completely normal and very similar to those of the diploid male *Rana nigromaculata* and *Rana brevipoda*, except that the germ cells at various differentiation stages were remarkably larger. The seminiferous tubules were filled with compact bundles of normally shaped spermatozoa which were distinctly larger than those of the diploid male *Rana nigromaculata* and *Rana brevipoda* (Fig. 10).

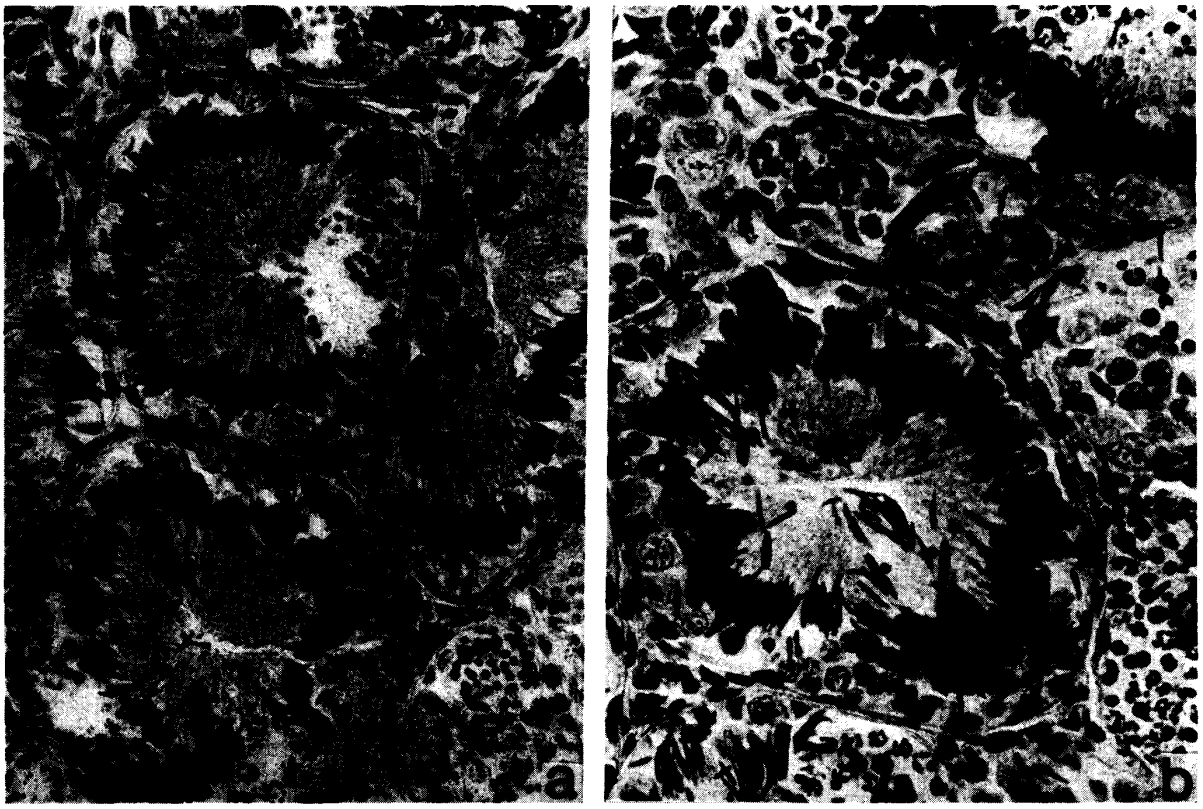


Fig. 10. Cross-sections of the testes of a male amphidiploid and the control diploid. ×300
a. Control diploid *Rana nigromaculata* produced from (N)NN♀, No. 2 × (N)NN♂, No. 2
b. (B)BBNN amphidiploid produced from (B)BBNN♀, No. 1 × (B)BBNN♂, No. 1

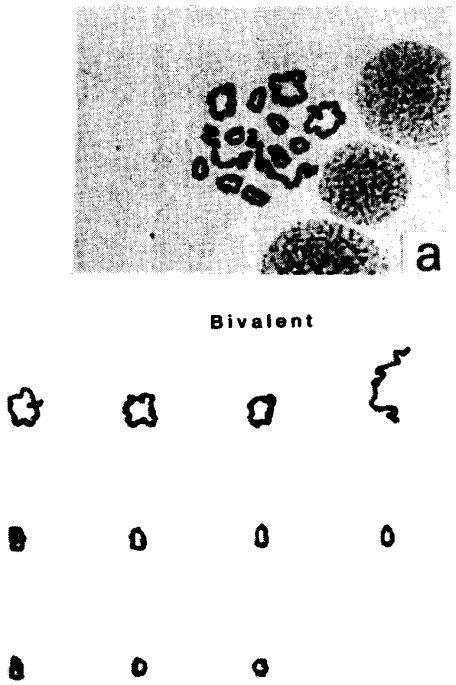


Fig. 11. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 5 large and 8 small bivalents in a diploid male *Rana nigromaculata*. ×560

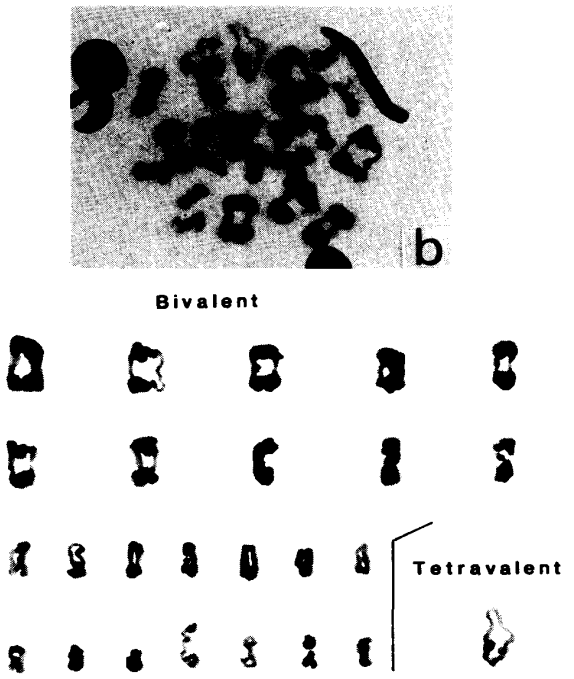


Fig. 12. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 10 large and 14 small bivalents and one tetravalent in a male amphidiploid produced from (B)BBNN♀, No. 1 × (B)BBNN♂, No. 1. ×560

Chromosomes of the first meiotic division were prepared by the method of SCHMID, OLERT and KLETT (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. 11, 12).

5. Electrophoretic patterns

Five kinds of frogs, diploid *Rana nigromaculata*, diploid *Rana brevipoda*, diploid (B)BN and (N)NB hybrids obtained from reciprocal matings between *Rana brevipoda* and *Rana nigromaculata*, and (B)BBNN amphidiploids were biochemically compared with one another by electrophoretic method. Albumin (Ab) and protein-C from blood serum, hemoglobin (Hb) from erythrocytes and 12 kinds of enzymes extracted from skeletal muscles, that is, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), α-glycerophosphate dehydrogenase (α-GDH), isocitrate dehydrogenase (IDH), superoxide dismutase (SOD), aspartate aminotransferase (AAT), creatine kinase (CK), pyruvate kinase (PK), adenylate kinase (AK), phosphoglucomutase (PGM), glucose-phosphate isomerase (GPI) and glutamate dehydrogenase (GaDH) were analyzed by starch-gel electrophoresis (NISHIOKA, UEDA and SUMIDA, 1981).

It was found that the amphidiploids were almost completely equal to the diploid hybrids in electrophoretic pattern of each of the above proteins. They always showed the sum of the bands or the characteristic hybrid bands derived from the two parental species, *Rana nigromaculata* and *Rana brevipoda* (Fig. 13).

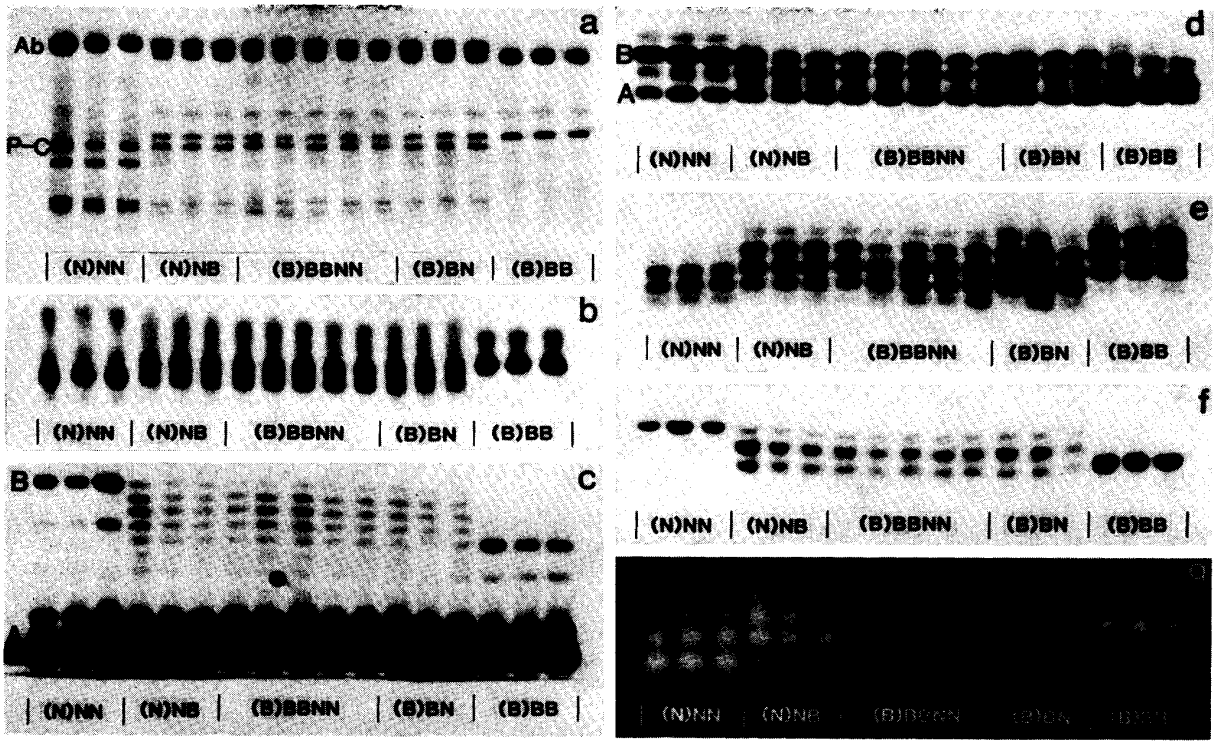


Fig. 13. Electrophoretic patterns of three blood proteins and five enzymes from reciprocal diploid hybrids and amphidiploids between *Rana nigromaculata* and *Rana brevipoda*, and the control diploids.

a. Serum albumin (Ab) and protein-C (P-C)
b. Hemoglobin (Hb)
c. Lactate dehydrogenase (LDH)
d. Malate dehydrogenase (MDH)
e. α-Glycerophosphate dehydrogenase (α-GDH)
f. Isocitrate dehydrogenase (IDH)
g. Superoxide dismutase (SOD)

DISCUSSION

HUMPHREY and FANKHAUSER (1949) reported in axolotls that triploid females occasionally spawned as many as several hundred eggs. When such triploid females were matcd with diploid males, a few viable tetraploids were produced by chance in certain spawnings, while the offspring were mostly hyperdiploids and non-viable. From females of such tetraploids, triploid offspring werc obtained by mating with diploid males. In the axolotl, polyploid males were completely or nearly sterile. FANKHAUSER and HUMPHREY (1950) examined the chromosome numbers in the offspring of triploid females mated with diploid males in the axolotl. According to their data, 2027 eggs from 18 spawnings were fertilized, 1315 grew into larvae at the tail-clipping stage and 445 were analyzed in terms of chromosomc number. The results indicated that 377 (84.7%) larvae were 29~41 (2n+1 to 3n−1), 48 (10.8%) were tetraploids (56±), three were pentaploids (70±) and two were heptaploids (98±), while no triploids were found. Some tetraploids of mixed axolotl and *Ambystoma tigrinum* ancestry reached the size typical for diploids. A considerable number of tetraploid females sexually matured and spawned fertile eggs by mating with diploid males. According to

HUMPHREY and FANKHAUSER (1956), tetraploid axolotls occurred with a frequency of 3 to 4% in the spawnings of triploid females mated with diploid males, probably as a result of failure of the meiosis in the egg. The spawnings of tetraploid females averaged 225 eggs, while those of triploid and diploid females laid 300 and over 650 eggs on the average, respectively. FANKHAUSER and HUMPHREY (1959) lastly reported that 105 (2.9%) unusual types of heteroploids, including haploids, hypo-diploids, tetraploids (the majority), pentaploids, hexaploids, heptaploids, and mosaics, appeared in addition to aneuploids with chromosome numbers between diploid and triploid among 3636 embryos obtained from $3n \times 2n$ matings.

GALLIEN and BEETSCHEN (1959) and BEETSCHEN (1960) reported in *Pleurodeles waltl* that no viable individuals were obtained from matings between triploid males and diploid females. Most of the offspring were 25 ($2n+1$) to 32 ($3n-4$) in chromosome number, while the others were hypertriploids. Thereafter, BEETSCHEN (1962) obtained four tetraploid females from among 3179 eggs of five triploid females mated with diploid males. Two of these tetraploid females sexually matured at the age of 22 months and produced triploid or nearly triploid larvae by mating with diploid males. According to BEETSCHEN (1967), 14 (0.33%) tetraploid larvae were produced from among 4204 eggs of six triploid females mated with diploid males during the period from 1959 to 1966. Of these 14 tetraploid larvae, 11 (0.26%) became adults. Five mature tetraploid females of the latter laid 1365 fertilized eggs by mating with diploid males and produced 211 triploid adults including 185 females and 26 males.

FISCHBERG (1945, 1948) reported that mature triploid females obtained by refrigeration of inseminated eggs were sterile in *Triturus alpestris*, although their ovaries contained a few, considerably large oocytes distributed separately. A similar phenomenon was found in mature triploid females of *Cynops pyrrhogaster* (KAWAMURA, 1951) and of reciprocal allotriploids between *Cynops pyrrhogaster* and *Cynops ensicauda* (KAWAMURA, 1952). However, two triploid females, 7 and 8 years old, obtained in *Cynops pyrrhogaster* by KAWAMURA produced five offspring by mating with diploid males. These offspring were 25 ($2n+1$) to 31 in chromosome number and died at the age of $18 \sim 28$ days.

In *Rana*, HUMPHREY, BRIGGS and FANKHAUSER (1950) observed degeneration of young oocytes in the ovaries of triploid tadpoles of *Rana pipiens* and stated that such a degeneration was not believed to result from nutritional or hormonal deficiencies, or from a numerical inbalance of the sex-determining genes. It was considered to be a consequence of a physiological disturbance in the oocyte incident to the presence of the third set of chromosomes. Nine years before HUMPHREY, BRIGGS and FANKHAUSER reported on the ovaries of triploid tadpoles, KAWAMURA (1941a, b) described that mature triploid females of *Rana nigromaculata* had small and underdeveloped ovaries. In these ovaries, only a few growing auxocytes were contained together with many young oocytes and oogonia. No offspring were produced from triploid females by mating with diploid males. KAWAMURA presumed that the cause of underdevelopment of the ovaries in the triploid frogs could not be explained by other than abnormality of trophic

conditions common to both sexes, because the ovaries did not qualitatively differ from those of diploid females in inner structure, in addition to that the female was probably homogametic in this species. This presumption by KAWAMURA was confirmed in the present study. The trophic conditions of frogs reared in our laboratory were remarkably increased by changing their food from mosquitoes and domestic flies to two-spotted crickets, *Gryllus bimaculatus* DE GEER, since 1976.

In the present study, many autotetraploids and allotetraploids including amphidiploids were produced from auto- and allotriploid females by mating with diploid males in *Rana nigromaculata* and *Rana brevipoda*. In the matings between female autotriploids and diploid males of *Rana nigromaculata*, 104 (3.4%) of 3044 eggs became feeding tadpoles. Of 97 analyzed tadpoles, 12 (12.4%) were autotetraploids, while 11, 17 and 57 of the others were diploids, triploids and aneuploids or mosaics, respectively. In the same kind of matings in *Rana brevipoda*, 199 (4.7%) of 4208 eggs obtained in 1977 and 1978 became feeding tadpoles. Of 192 analyzed tadpoles, 28 (14.6%) were autotetraploids, while 25, 29 and 110 of the others were diploids, triploids and aneuploids or mosaics, respectively. In contrast, only 70 (0.4%) of 18099 eggs obtained in 1980 became feeding tadpoles. Of 63 analyzed tadpoles, 14, 24 and 25 were diploids, triploids and aneuploids between diploid and triploid, respectively. No tetraploids were produced in this year. In the matings between (N)NNB allotriploid females and diploid male *Rana nigromaculata*, 82 (2.1%) of 3873 eggs became feeding tadpoles, of which 32 (39.0%), 2, 16 and 32 were allotetraploids, diploids, triploids and aneuploids or mosaics, respectively. When the maternal allotriploids were mated with diploid male *Rana brevipoda*, 64 (1.3%) of 4895 became feeding tadpoles, of which 21 (32.8%), 2, 8 and 33 were amphidiploids, diploids, triploids and aneuploids or others, respectively. In the matings between (B)BBN allotriploid females and diploid male *Rana nigromaculata*, 176 (6.2%) of 2837 eggs obtained in 1977 and 1978 became feeding tadpoles. Of 176 analyzed tadpoles, 70 (39.8%), 6, 15 and 85 were amphidiploids, diploids, triploids and aneuploids or mosaics, respectively. In the same kind of matings, 230 (3.6%) of 6473 obtained in 1980 became feeding tadpoles. Of 188 analyzed tadpoles, 19 (10.1%), 61, 31 and 77 were amphidiploids, diploids, triploids and aneuploids or mosaics, respectively. When (B)BBN allotriploid females were mated with diploid male *Rana brevipoda*, 86 (3.4%) of 2538 eggs became feeding tadpoles, of which 27 (31.4%), 2, 11 and 46 were allotetraploids, diploids, triploids and aneuploids or mosaics, respectively.

The two kinds of autotriploid females were somewhat higher than the two kinds of allotriploid females in fertilization rate; the former were 82.8% and 91.4% on the average except the case of *Rana brevipoda* eggs obtained in 1980, which were 48.7~77.5%. When the percentages of normally feeding tadpoles to normally cleaved eggs were calculated, they were 4.1% and 5.2% in the matings of autotriploid female *Rana nigromaculata* and *Rana brevipoda*, respectively. On the other hand, they were 4.1% and 2.1% in the matings of (N)NNB allotriploid

females with diploid male *Rana nigromaculata* and *Rana brevipoda*, respectively. They were also 8.2% and 7.0% in the matings in 1977 and 1978 between (B)BBN allotriploid females and diploid male *Rana nigromaculata* and *Rana brevipoda*, respectively, and 4.6% in those between the same females and diploid male *Rana nigromaculata* in 1980. If these percentages are compared with that of triploid female axolotls reported by FANKHAUSER and HUMPHREY (1950), they are extremely small, as 1315 (64.9%) of 2027 fertilized axolotl eggs became larvae.

The percentages of auto- or allotetraploids to analyzed tadpoles in the present study were 12.4% and 14.6% in the matings of autotriploid female *Rana nigromaculata* and *Rana brevipoda*, respectively, except the matings with *Rana brevipoda* performed in 1980. In the matings of (N)NNB allotriploid females with diploid male *Rana nigromaculata* and *Rana brevipoda*, they were 39.0% and 32.8%, respectively, while in those of (B)BBN allotriploid females with diploid male *Rana nigromaculata* and *Rana brevipoda*, they were 39.8% and 31.4%, respectively, in 1977 and 1978. In the matings in 1980 between (B)BBN allotriploid females and diploid male *Rana nigromaculata*, it was 10.1%. It seems evident that the two kinds of allotriploids produce much more tetraploids than the two kinds of autotriploids. Even in the experiments performed in 1980, the (B)BBN allotriploid females produced tetraploids corresponding to 0.4% of fertilized eggs, while no tetraploids were produced from 5043 fertilized eggs of autotriploid female *Rana brevipoda*.

The rates of tetraploids to normally cleaved eggs of the maternal triploids were similar to those obtained in the axolotl and *Pleurodeles waltl*. In the axolotl, the rate of tetraploids was described as 48 (10.8%) of 2027 fertilized eggs by FANKHAUSER and HUMPHREY (1950), as 3~4% of spawned eggs by HUMPHREY and FANKHAUSER (1956) and as the majority of 105 (2.9%) of 3636 fertilized eggs by FANKHAUSER and HUMPHREY (1959). In *Pleurodeles waltl*, BEETSCHEN (1967) reported that 14 (0.33%) of 4204 fertilized eggs from triploid females mated with diploid males became tetraploid larvae. In the present study, the percentages of tetraploids to normally cleaved eggs were as follows. In the matings of autotriploid female *Rana nigromaculata* and *Rana brevipoda*, 0.48% and 0.73% became autotetraploids, respectively. On the other hand, 1.59% and 0.70% became allotetraploids in the matings of (N)NNB allotriploid females with diploid male *Rana nigromaculata* and *Rana brevipoda*, respectively, while 3.25% and 2.18% became allotetraploids in the matings in 1977 and 1978 between (B)BBN allotriploid females and diploid male *Rana nigromaculata* and *Rana brevipoda*, respectively. In 1980, 0.38% became allotetraploids in the matings between (B)BBN allotriploid females and diploid male *Rana nigromaculata*.

The mechanisms to produce offspring with various chromosome numbers including tetraploid, triploid, diploid and aneuploid from triploid females by mating with diploid males are not always evident for the time being. They would be clarified to a large extent by observing mitosis in oogonia and meiosis in the oocytes of auto- and allotriploid females minutely.

SUMMARY

1. Autotetraploids and amphidiploids were produced from eggs of auto- and allotriploid females, respectively, by inseminating with sperm of diploid male *Rana nigromaculata* or *Rana brevipoda*. The auto- and allotriploids were those which had been produced by refrigerating eggs of diploid females of the two species after insemination with sperm of the own and foreign species, respectively.

2. Of 3044 eggs of five triploid females mated in 1977 and 1978 with two diploid males in *Rana nigromaculata*, 2521 (82.8%) cleaved normally and 104 (3.4%) became feeding tadpoles. Of 97 of these feeding tadpoles, 12 (12.4%) were tetraploids, 11 were diploids, 17 were triploids and the remaining 57 were aneuploids and mosaics.

3. Of 4208 eggs of six triploid females mated in 1977 and 1978 with three diploid males in *Rana brevipoda*, 3845 (91.4%) cleaved normally and 199 (4.7%) became feeding tadpoles. Of 192 of these tadpoles, 28 (14.6%) were tetraploids, 25 were diploids, 29 were triploids and 110 were aneuploids and mosaics. In 1980, 5043 (27.9%) of 18099 eggs of 14 triploid females mated with four diploid males in *Rana brevipoda* cleaved normally and only 70 (0.4%) became feeding tadpoles. None of 63 of these tadpoles was tetraploid, while 14 were diploids, 24 were triploids and the remaining 25 were hyperdiploids or hypotriploids.

4. Of 3873 eggs of four (N)NNB allotriploid females mated in 1977 and 1978 with two diploid male *Rana nigromaculata*, 2010 (51.9%) cleaved normally and 82 (2.1%) became feeding tadpoles. Of these tadpoles, 32 (39.0%) were tetraploids, two were diploids, 16 were triploids and the remaining 32 were aneuploids and mosaics. Of 4895 eggs of the same four (N)NNB allotriploid females mated with three diploid male *Rana brevipoda*, 2986 (61.0%) cleaved normally and 64 (1.3%) became feeding tadpoles. Of these tadpoles, 21 (32.8%) were tetraploids, two were diploids, eight were triploids and the remaining 33 were aneuploids and mosaics.

5. In 1977 and 1978, 2152 (75.9%) of 2837 eggs of four (B)BBN allotriploid females mated with two diploid male *Rana nigromaculata* cleaved normally and 176 (6.2%) became feeding tadpoles. Of these tadpoles, 70 (39.8%) were tetraploids, six were diploids, 15 were triploids and the remaining 85 were aneuploids and mosaics. Of 2538 eggs of the same (B)BBN allotriploid females mated with three diploid male *Rana brevipoda*, 1236 (48.7%) cleaved normally and 86 (3.4%) became feeding tadpoles. Of these tadpoles, 27 (31.4%) were tetraploids, two were diploids, 11 were triploids and the remaining 46 were aneuploids and mosaics.

In 1980, 5014 (77.5%) of 6473 eggs of seven (B)BBN allotriploid females mated with three diploid male *Rana nigromaculata* cleaved normally and 230 (3.6%) became feeding tadpoles. Of 188 of these tadpoles, 19 (10.1%) were tetraploids, 61 were diploids, 31 were triploids and the remaining 77 were aneuploids and mosaics.

6. The sex of metamorphosed tetraploids produced from auto- or allotriploids females by mating with diploid male *Rana nigromaculata* or *Rana brevipoda* were as follows.

a. Three autotetraploids produced from (N)NNN♀ × (N)NN♂ were males.
b. Of six allotetraploids produced from (N)NNB♀ × (N)NN♂, one was a female and five were males.
c. Of 11 allotetraploids produced from (N)NNB♀ × (B)BB♂, two were females and nine were males.
d. Of 24 allotetraploids produced from (B)BBN♀ × (N)NN♂, three were females and 21 were males.
e. Of seven allotetraploids produced from (B)BBN♀ × (B)BB♂, one was a female and six were males.

In total, 44 (86.3%) of 51 tetraploids were males.

7. Of 857 eggs of two amphidiploid females mated with two amphidiploid males, 576 (67.2%) cleaved normally, 294 (34.3%) became feeding tadpoles and 267 (31.2%) completed metamorphosis. A preliminary observation on the chromosomes of these individuals showed that they were tetraploids, that is, amphidiploids with a few exceptions.

8. When the external characters of these amphidiploids were observed at the sexually mature stage, they were intermediate between those of the two parental species, except a few dominant characters. The testes of mature amphidiploid males were completely normal in inner structure. The electrophoretic patterns of serum proteins, hemoglobin and enzymes extracted from the skeletal muscles of these amphidiploids were the same as those of the hybrids between the two species.

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