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Abnormalities of the Offspring of Nucleo-cytoplasmic Hybrids between *Rana nigromaculata* and *Rana brevipoda*

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(With 17 Text-figures and 4 Plates)

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INTRODUCTION

Various kinds of diploid nucleo-cytoplasmic hybrids obtained from two different species of amphibians have been reported so far by many authors. Roughly speaking, two species which produce inviable hybrids at the late blastula or the early gastrula stage by ordinary crosses produce no nucleo-cytoplasmic hybrids which live beyond those stages, as found between *Rana pipiens* and *Rana catesbeiana* (BRIGGS and KING, 1952) or *Rana sylvatica* (MOORE, 1958, '60; HENNEN, 1963). On the other hand, there is such a combination as that between *Rana pipiens* and *Rana palustris*, in which reciprocal nucleo-cytoplasmic hybrids become abnormal and all die by the post-neurula stage (HENNEN, 1962, '65, '67, '72), differing from ordinary diploid hybrids which develop into normal adults (MOORE, 1941, '46, '49). Reciprocal nucleo-cytoplasmic hybrids between two species of African clawed toads, *Xenopus laevis* and *Xenopus tropicalis*, become abnormal and cease their development at the neurula or somewhat later stages, while these two species do not produce ordinary hybrids (GURDON, 1962).

A few viable reciprocal nucleo-cytoplasmic hybrids were obtained for the first

time by SAMBUICHI (1957, '60) from two species of Japanese pond frogs, *Rana nigromaculata* and *Rana brevipoda*, although they died immediately before or after the completion of metamorphosis. These two species give normal viable hybrids by ordinary crosses, although males are almost completely sterile. KAWAMURA and NISHIOKA (1963a) obtained 14 metamorphosed diploid nucleo-cytoplasmic hybrids by transplanting blastula nuclei of *Rana brevipoda* into enucleated *Rana nigromaculata* eggs as well as five ones by the reciprocal combination. Two females of the former and a female of the latter nucleo-cytoplasmic hybrids attained sexual maturity and produced their offspring by mating with a male of the nuclear or cytoplasmic species. However, it was found that the two females constructed of *nigromaculata* nuclei and *brevipoda* cytoplasm were remarkably inferior to the control *nigromaculata, brevipoda* or reciprocal ordinary hybrids in reproductive capacity. Diploid nucleo-cytoplasmic hybrids between two species of Japanese brown frogs, *Rana japonica* and *Rana ornativentris* were also obtained by KAWAMURA and NISHIOKA (1963b). A few males constructed of *japonica* nuclei and *ornativentris* cytoplasm produced offspring by mating with female *japonica*. Numerous males of these first-generation offspring were mated again with females of the nuclear species and produced second-generation offspring. From these mating experiments, it was clearly noticed that the males of both nucleo-cytoplasmic hybrids and first-generation offspring were generally inferior to the control *japonica* in reproductive capacity and that the viability in the first-generation offspring was scarcely improved in the second generation.

It was the first purpose of the present researches to obtain matured male nucleo-cytoplasmic hybrids from reciprocal combinations between *Rana nigromaculata* and *Rana brevipoda* and to examine their reproductive capacity. The second purpose was to examine the reproductive capacities of males and females of the first and second generations produced from the two female nucleo-cytoplasmic hybrids which were reported in a previous paper by KAWAMURA and NISHIOKA (1963a), and to make detailed observations on the various abnormalities found in the individuals of their next generations. The main results of these experiments and observations are described in this paper. Two preliminary reports have been made by KAWAMURA and NISHIOKA (1965) and NISHIOKA (1971a, b).

MATERIAL AND METHODS

In this research two Japanese pond frog species, *Rana nigromaculata* HALLOWELL and *R. brevipoda* ITO, were used. The former was the strain collected from the suburbs of Hiroshima, while the latter was that from the suburbs of Okayama. The nucleo-cytoplasmic hybrids used for producing their offspring were obtained in the breeding seasons of the years 1961, 1962 and 1963. Concerning the production and life histories of the nucleo-cytoplasmic hybrids obtained in 1961 and 1962, there is a report by KAWAMURA and NISHIOKA (1963a). Additional nucleo-cytoplasmic hybrids were produced in 1963b by the same method. The experi-

mental process in this year was as follows.

Enucleated eggs of four female *brevipoda* were transplanted with *nigromaculata* blastula nuclei. A similar transplantation experiment was also performed between enucleated eggs of four female *nigromaculata* and blastula nuclei of *brevipoda*. As one of the control series, enucleated eggs of the four female *nigromaculata* were transplanted with *nigromaculata* nuclei. The results of these procedures are presented in Table 1. The identification of the ploidy of each tadpole was made by the tail-tip method, as reported in the previous paper (KAWAMURA and NISHIOKA, 1963a). The control series was always higher than the experimental in the percentages of normally cleaved eggs, normal embryos and hatched tadpoles to the treated eggs. While there were 15(5.0%) or 11(5.5%) diploid tadpoles in the experimental series, there were 21(8.4%) in the control. Both kinds of tadpoles were mostly raised to their sexual maturity for the purpose of producing their next generations.

TABLE 1
Synthesis of nucleo-cytoplasmic hybrids between two species of Japanese pond frogs by the nuclear transplantation method in 1963

Combination / Results	Enucleated egg, (B) + Blastula nucleus, NN	Enucleated egg, (N) + Blastula nucleus, NN	Enucleated egg, (N) + Blastula nucleus, BB
No. of females	4	4	4
No. of treated eggs	300	250	200
No. of normally cleaved eggs	192(64.0%)	175(70.0%)	94(47.0%)
No. of normal blastulae	94(31.3%)	90(36.0%)	81(40.5%)
No. of normal gastrulae	65(21.7%)	72(28.8%)	45(22.5%)
No. of normal neurulae	34(11.3%)	45(18.0%)	37(18.5%)
No. of tail-bud embryos	26(8.7%)	38(15.2%)	35(17.5%)
No. of hatched tadpoles	20(6.7%)	31(12.4%)	32(16.0%)
No. of analysed tadpoles	18(6.0%)	25(10.0%)	20(10.0%)
Diploid	15(5.0%)	21(8.4%)	11(5.5%)
Mosaics	3(1.0%)	4(1.6%)	9(4.5%)

All the frogs used for producing first-, second- and third-generation offsprings in the years 1965 and 1966 were those which had been reared in our laboratory since their egg stage. The second-generation offspring were obtained in 1965 from the first-generation offspring of nucleo-cytoplasmic hybrids by brother and sister mating or by mating with *Rana nigromaculata* or *brevipoda*. The production and life histories of these first-generation offspring have been already reported in the previous paper (1963a, cf. p. 76).

In the breeding season of the year 1966, numerous tadpoles of the three generations were produced. From each of two groups of each generation, 40 feeding tadpoles of nearly normal appearance were picked out at random and examined in terms of their karyotypes. Metaphase chromosomes were obtained in clipped tail-tips by the squash method after the tadpoles had been kept in 0.005% colchicine solution for about 18 hours. The number of chromosomes in each tadpole was determined by counting them in more than ten mitoses, in about twenty in most cases. However, the karyotype was determined by analysing

the chromosomes of more than three good mitoses.

The description of developmental stages follows those of *Rana pipiens* established by SHUMWAY (1940) and TAYLOR and KOLLROS (1946) for convenience' sake.

The following abbreviations are used in the present paper.

N ——————A set of *Rana nigromaculata* chromosomes
(N) ——————*Rana nigromaculata* cytoplasm
B ——————A set of *Rana brevipoda* chromosomes
(B) ——————*Rana brevipoda* cytoplasm

Various kinds of frogs are shown by different combinations of these four abbreviations as follows.

(N)NN ———*Rana nigromaculata*
(B)BB ———*Rana brevipoda*
(N)+NN ——A control frog developed from an enucleated *nigromaculata* egg transplanted with a blastula nucleus of *nigromaculata*
(N)BB ———A nucleo-cytoplasmic hybrid developed from an enucleated *nigromaculata* egg transplanted with a blastula nucleus of *brevipoda*
(B)NN ———A nucleo-cytoplasmic hybrid developed from an enucleated *brevipoda* egg transplanted with a blastula nucleus of *nigromaculata*

Each of nuclear transfers and the control frogs is shown with the individual number together with the abbreviations of its kind and the year of its production, such as (N)BB. 62N1T1, (N)+NN. 63N1T1 or (N)NN. 61.1 for example. The first-generation offspring produced from a female nucleo-cytoplasmic hybrid mated with a male *nigromaculata* or *brevipoda* are shown by combination of the parenthesized abbreviation of the former and N or B of the latter, like {(B)NN, No. 61B12T2}N. The offspring of the reciprocal cross are shown by placing N or B in front of the parenthesized abbreviation. The second-generation offspring produced from a female of this first generation mated with a male *nigromaculata* are shown by adding a square mark, like {(B)NN. No. 61B12T2}N^2.

OBSERVATION

A. Experiments performed in 1965

I. Male and female parents

In order to produce the first- and second-generation offspring of nucleo-cytoplasmic hybrids as well as the controls, 49 frogs were used as male and female parents in various kinds of matings. They are shown in Tables 2~5 by dividing into 13 groups which are mostly arranged in accordance with the groups of mating experiments presented in Tables 6 and 7. The males were 2~4 years old, while the females were 3 years old. All of them were those which had been reared since the egg stage in the laboratory. Their body lengths were measured immediately after they were used for the mating experiments.

TABLE 2
Testes of control male parents used in 1965

Kind	Individual no.	Age (year)	Body length (mm.)	Size of the testes		Inner structure
				Left(mm.)	Right (mm.)	
i	(N)NN. 61.1	4	70.5	4.0×2.0	4.5×2.5	Type 1
	(N)NN. 63.2	2	55.0	2.5×1.0	2.5×1.0	Type 1
	(N)NN. 62.3	3	56.0	4.0×1.5	4.0×2.0	Type 1
	(N)NN. 62.4	3	57.5	4.0×2.5	4.0×2.5	Type 1
ii	(B)BB. 63.1	2	45.0	3.0×2.5	3.0×2.5	Type 1
	(B)BB. 62.2	3	47.0	2.5×2.5	2.5×2.5	Type 1
iii	(N)+NN. 63N1T1	2	57.5	5.0×2.0	5.0×2.5	Type 1
	(N)+NN. 63N2T2	2	60.0	5.5×2.0	5.0×2.0	Type 1
	(N)+NN. 63N2T3	2	50.5	3.0×1.5	3.0×1.0	Type 1
	(N)+NN. 63N3T4	2	53.0	3.5×2.0	3.5×1.5	Type 1

(N)+NN — Nucleus-exchanged *nigromaculata*

1. Testes of male parents

One of the testes of each nucleo-cytoplasmic hybrid or first-generation offspring produced from two female nucleo-cytoplasmic hybrids was preserved for observation of its inner structure. Besides, the testes of *Rana nigromaculata*, *Rana brevipoda* and nucleus-exchanged *nigromaculata* were preserved for histological observations. These testes were utilized as the controls.

a. Males of two species

Four male *Rana nigromaculata*, two male *Rana brevipoda* and four male nucleus-exchanged *Rana nigromaculata* were used as parents in the control or experimental series of the mating experiments performed in 1965. The ages, body lengths of these male frogs as well as the sizes of their testes are presented in Table 2.

i) *Rana nigromaculata* Nos. 61.1, 63.2 and 62.3~4

These four males were 2, 3 and 4 years old and 55~70.5 mm. in body length. They were sexually quite matured. Their testes were ellipsoid in shape; the width was nearly equal to half of the length, being 3.7 mm.×1.9 mm. on the average. They were quite normal in inner structure and their seminal tubules were filled with bundles of normally shaped spermatozoa. Morphologically abnormal spermatozoa were scarcely found. Such a type of testes that were filled with normal spermatozoa was called Type 1 (Plate I, 1).

ii) *Rana brevipoda* Nos. 63.1 and 62.2

These were two and three years old and 45 mm. and 47 mm. in body length, respectively. Their testes were rather spherical, differing from those of the above *nigromaculata*, although they were quite the same as the latter in inner structure (Type 1). The seminal tubules were filled with bundles of normally shaped spermatozoa.

iii) Nucleus-exchanged *nigromaculata*, (N)+NN, Nos. 63N1T1, 63N2T2~3, and 63N3T4

These frogs were those which had been produced from enucleated *nigromaculata*

eggs transplanted with blastula nuclei of the same species. All the four males were two years old and 55.3 mm. in the mean body length. Accordingly, they seemed quite normal in growth as compared with the above *nigromaculata*. Their testes were well developed and of *nigromaculata* type in shape, the width being nearly equal to half of the length, being 4.2 mm. × 1.8 mm. on the average. They were quite normal in inner structure, that is, of Type 1; their seminal tubules were filled with bundles of normally shaped spermatozoa (Plate I, 2).

b. Male nucleo-cytoplasmic hybrids

The male nucleo-cytoplasmic hybrids used in the experiments of the year 1965 were 17 in total number. Nine of them consisted of *nigromaculata* cytoplasm and *brevipoda* nuclei, (N)BB, while the other eight consisted of *brevipoda* cytoplasm and *nigromaculata* nuclei, (B)NN. The sizes and inner structures of their testes are presented in Table 3, together with their ages and body lengths.

TABLE 3
Testes of male parents, (B)NN and (N)BB, used in 1965

Kind	Individual no.	Age (year)	Body length (mm.)	Size of the testes		Inner structure
				Left (mm.)	Right (mm.)	
i	(N)BB. 62N1T1	3	40.5	3.0×2.5	3.0×2.0	Type 1
	(N)BB. 62N2T1	3	41.0	2.5×2.0	2.5×2.5	Type 3
	(N)BB. 62N3T1	3	37.5	2.5×2.5	2.5×2.5	Type 1
	(N)BB. 62N4T1	3	40.0	2.0×2.0	2.0×1.5	Type 2
	(N)BB. 63N1T1	2	35.5	2.5×2.0	3.0×2.5	Type 1
	(N)BB. 63N1T2	2	32.0	2.5×2.0	2.5×2.0	Type 2
	(N)BB. 63N3T3	2	35.0	3.0×2.5	3.0×2.5	Type 2
	(N)BB. 63N3T4	2	33.5	2.0×2.0	2.0×2.0	Type 3
	(N)BB. 63N4T5	2	34.0	2.5×2.0	2.5×2.5	Type 3
ii	(B)NN. 63B1T1	2	63.5	5.0×2.0	5.0×2.5	Type 1
	(B)NN. 63B1T2	2	61.0	4.5×2.0	4.0×2.0	Type 1
	(B)NN. 63B3T3	2	50.0	3.0×2.5	3.0×2.5	Type 1
	(B)NN. 63B4T4	2	45.0	2.0×2.0	2.0×2.0	Type 1
	(B)NN. 63B2T1	2	57.5	4.5×2.0	5.0×2.5	Type 1
	(B)NN. 63B2T2	2	60.0	4.0×2.5	4.5×3.0	Type 1
	(B)NN. 63B2T3	2	55.3	5.0×3.0	5.0×3.0	Type 1
	(B)NN. 63B2T4	2	57.0	4.5×2.5	4.5×2.5	Type 1

i) Nucleo-cytoplasmic hybrids constructed of *nigromaculata* cytoplasm and *brevipoda* nuclei, (N)BB, Nos. 62N1~4T1, 63N1T1~2, 63N3T3~4 and 63N4T5

Four of the nine males were three years old and 37.5~41.0 mm. in body length, while the other five were two years old and 32.0~35.5 mm. These males had grown very slowly and were distinctly smaller than the other kind of nucleo-cytoplasmic hybrids as well as the three kinds of control males. Their testes were nearly the same as those of the *brevipoda* controls in shape and size, being 2.5 mm. × 2.2 mm. on the average. Three of the males, (N)BB, Nos. 62N1T1, 62N3T1 and 63N1T1 had testes which were so similar to those of the control

nigromaculata and *brevipoda* in inner structure that they were classified as Type 1. Their seminal tubules were filled with bundles of normally shaped spermatozoa, although the latter were somewhat fewer than those of the control males, and, moreover, there were a few deformed spermatozoa.

The testes of three other males, (N)BB, Nos. 62N4T1, 63N1T2 and 63N3T3 had a small number of bundles of normally shaped spermatozoa, which were found here and there in the seminal tubules. Around these bundles there were numerous pycnotic nuclei. Such a structure of testis was classified as Type 2 (Plate I, 3).

The remaining three nucleo-cytoplasmic hybrids, (N)BB, Nos. 62N2T1, 63N3T4 and 63N4T5 had a few normally shaped spermatozoa distributed sparsely among numerous, deformed spermatozoa and pycnotic nuclei in their testes. The structure of testes was classified as Type 3 (Plate I, 4). The fertilization rates of the eggs of normal female *brevipoda* with sperm of these males were 21.4~22.7%.

ii) Nucleo-cytoplasmic hybrids constructed of *brevipoda* cytoplasm and *nigromaculata* nuclei, (B)NN, Nos. 63B1T1~2, 63B3T3, 63B4T4 and 63B2T1~4

The eight males of this kind of nucleo-cytoplasmic hybrids were two years old. They were 45~63.5 mm. in body length, being much larger than the above described three-year-old males of the reciprocal combination. Two of them were rather superior in growth to the non-treated or nucleus-exchanged *R. nigromaculata* which were used as the control males. Six males had testes of the *nigromaculata* type, in which the width was nearly equal to half of the length, while the testes of the other two were of the *brevipoda* type, the width being approximately equal to the length. All these testes were nearly the same in size as those of the control males and quite normal in inner structure (Type 1). The seminal tubules were filled with bundles of normally shaped spermatozoa, just as those of the control *nigromaculata* were.

c. Male first-generation offspring of female nucleo-cytoplasmic hybrids, (B)NN

The testes of 15 matured males produced from two female nucleo-cytoplasmic hybrids by mating with male *nigromaculata* or *brevipoda* were observed, as shown in Table 4. All of them were three years old. Nine were obtained from a female nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 mated with a wild male *nigromaculata*, three from the same female mated with a wild male *brevipoda*, and the remaining three from another female of the same kind, (B)NN, No. 61B13T3 mated with the above male *nigromaculata*. Their body lengths, the sizes of testes and the types of inner structures of the latter are presented in the table. Three kinds of males were 47.5~60 mm., 41.5~50 mm. and 45.5~51 mm. in body length; there were no remarkable differences among them, differing from the relation between the two kinds of nucleo-cytoplasmic hybrids.

i) Nine offspring of a female nucleo-cytoplasmic hybrid No. 61B12T2 mated with a male *nigromaculata*

The testes of one (No. 2) of the nine males were of the *nigromaculata* type,

TABLE 4
Testes of male parents used for mating experiments performed in 1965

Kind	Individual no.	Age (year)	Body length (mm.)	Size of the testes		Inner structure
				Left (mm.)	Right (mm.)	
i	{(B)NN. 61B12T2}N.1	3	49.5	3.5×2.0	3.0×1.5	Type 1
	{(B)NN. 61B12T2}N.2	3	49.0	3.0×1.5	3.0×1.5	Type 2
	{(B)NN. 61B12T2}N.3	3	53.0	3.5×2.5	3.0×2.5	Type 1
	{(B)NN. 61B12T2}N.4	3	55.5	3.5×3.0	3.5×2.5	Type 1
	{(B)NN. 61B12T2}N.5	3	60.0	4.0×3.5	3.5×3.0	Type 1
	{(B)NN. 61B12T2}N.6	3	53.5	3.5×2.5	3.5×2.5	Type 2
	{(B)NN. 61B12T2}N.7	3	47.5	3.5×2.0	3.5×2.0	Type 3
	{(B)NN. 61B12T2}N.8	3	50.5	3.5×2.0	3.5×2.0	Type 2
	{(B)NN. 61B12T2}N.9	3	54.0	3.5×2.5	3.5×2.5	Type 3
ii	{(B)NN. 61B12T2}B.1	3	50.0	2.5×1.5	2.5×1.5	Type 3
	{(B)NN. 61B12T2}B.2	3	41.5	3.0×2.5	3.0×2.5	Type 2
	{(B)NN. 61B12T2}B.3	3	45.0	2.5×2.5	2.5×2.0	Type 3
iii	{(B)NN. 61B13T3}N.1	3	51.0	3.0×2.5	3.0×2.5	Type 3
	{(B)NN. 61B13T3}N.2	3	50.5	3.0×2.0	3.5×2.5	Type 2
	{(B)NN. 61B13T3}N.3	3	45.5	3.5×2.0	3.5×2.5	Type 3

{(B)NN. 61B12T2}N — Offspring of a female (B)NN. 61B12T2 mated with a male (N)NN
{(B)NN. 61B12T2}B — Offspring of a female (B)NN. 61B12T2 mated with a male (B)BB
{(B)NN, 61B13T3}N — Offspring of a female (B)NN. 61B13T3 mated with a male (N)NN

the width being equal to half of the length, while those of another male No. 5 were rather of the *brevipoda* type. The other seven males were intermediate between the two types in the shape of testes. The sizes of testes of the nine males were 3.4 mm. × 2.3 mm. on the average.

The testes of four males were of Type 1 (Plate II, 5) in inner structure, that is, they were quite normal and their seminal tubules were filled with bundles of normally shaped spermatozoa. The testes of three other males were of Type 2 (Plate II, 8). In these testes, there were a small number of coarse bundles of normally shaped spermatozoa and numerous abnormal spermatozoa and pycnotic nuclei. The remaining two males had testes of Type 3, in which there were a few normally shaped spermatozoa, distributed sparsely among numerous abnormal spermatozoa and pycnotic nuclei.

ii) Three offspring of a female nucleo-cytoplasmic hybrid No. 61B12T2 mated with a male *brevipoda*

The testes of two of the three males were rather of the *brevipoda* type in shape, while those of the other male were intermediate between the two types. Concerning the inner structure of testes, two males were of Type 3 and the other was of Type 2 (Plate II, 6).

iii) Three offspring of a female nucleo-cytoplasmic hybrid No. 61B13T3 mated with a male *nigromaculata*

The testes of one of the three males were near the *brevipoda* type in shape, while those of the other two were intermediate between the two types. In inner structure, the testes of two males were of Type 3 (Plate II, 7). The seminal tubules of these testes were occupied with somewhat large, deformed spermatozoa and pycnotic

nuclei, and normally shaped spermatozoa were scarcely found anywhere. The other male was of Type 2; spermatozoa were remarkably fewer than those in the testes of male *nigromaculata* or *brevipoda* stated above.

2. Eggs of female parents

In the mating experiments performed in the breeding season of the year 1965, 20 females of *Rana nigromaculata*, *Rana brevipoda* and the first-generation offspring of female nucleo-cytoplasmic hybrids mated with male *nigromaculata* or *brevipoda* were used as parents. The production, life histories and external characters of these female nucleo-cytoplasmic hybrids as well as their first-generation offspring have already been reported (KAWAMURA and NISHIOKA, 1963a).

After ovulation was accelerated by an injection of frog pituitaries, eggs were taken out of the oviducts of each female. An approximate number of total eggs obtained from each female was counted, and the diameters of 50 eggs picked out at random were measured to obtain the mean value. The number of eggs and the measurements of their diameters are presented together with their ages and body lengths in Table 5.

TABLE 5
Eggs of female parents used for mating experiments performed in 1965

Kind	Individual no.	Age (year)	Body length (mm.)	No. of eggs	Mean diameter of 50 eggs (mm.)
a, i	(N)NN. 62.1	3	55.0	1875	2.1±0.02
	(N)NN. 62.2	3	54.5	900	2.0±0.02
	(N)NN. 62.3	3	54.5	912	2.0±0.02
	(N)NN. 62.4	3	53.0	1792	1.8±0.02
	(N)NN. 62.5	3	52.5	895	1.7±0.01
	(N)NN. 62.6	3	55.0	1079	1.8±0.01
	(N)NN. 62.7	3	52.5	1275	1.8±0.02
	(N)NN. 62.8	3	55.5	1102	1.9±0.02
a, ii	(B)BB. 62.1	3	49.0	1512	1.5±0.01
	(B)BB. 62.2	3	47.5	1400	1.5±0.01
	(B)BB. 62.3	3	46.0	1312	1.5±0.02
	(B)BB. 62.4	3	45.5	1019	1.4±0.01
	(B)BB. 62.5	3	44.5	1229	1.4±0.02
	(B)BB. 62.6	3	42.0	1375	1.5±0.02
	(B)BB. 62.7	3	41.5	1002	1.5±0.02
b, i	{(B)NN. 61B12T2}N.1	3	65.5	1366	2.2±0.06
	{(B)NN. 61B12T2}N.2	3	47.0	612	2.0±0.03
b, ii	{(B)NN. 61B12T2}B.1	3	57.5	672	2.0±0.05
	{(B)NN. 61B12T2}B.2	3	50.0	476	2.0±0.04
b, iii	{(B)NN. 61B13T3}N.1	3	55.0	545	2.0±0.03

{(B)NN. 61B12T2}N — Offspring of a female (B)NN. 61B12T2 mated with a male (N)NN
{(B)NN. 61B12T2} B — Offspring of a female (B)NN. 61B12T2 mated with a male (B)BB
{(B)NN. 61B13T3}N — Offspring of a female (B)NN. 61B13T3 mated with a male (N)NN

a. Females of two species

i) *Rana nigromaculata* Nos. 62.1~8

The eight females were 52.5～55.5 mm. in body length. Form each of them about 895～1875 eggs were obtained. These eggs were about 1.7～2.1 mm. in diameter; larger females generally seemed to lay larger eggs.

ii) *Rana brevipoda* Nos. 62.1～7

These seven females were 41.5～49.0 mm. in body length, being distinctly smaller than the female *nigromaculata*, without regard to the same age. An approximate number of eggs obtained from each of them was 1002～1512, being larger for the body size than that from each of the female *nigromaculata*. However, the *brevipoda* eggs were distinctly smaller in size than the *nigromaculata*. They were about 1.4～1.5 mm. in diameter.

b. Female first-generation offspring of female nucleo-cytoplasmic hybrids, (B)NN

i) Two offspring of a female No. 61B12T2 mated with a male *nigromaculata*

The two females were 65.5 mm. and 47.0 mm. in body length, 1366 and 612 in an approximate number of total eggs and 2.2 mm. and 2.0 mm. in the diameter of eggs, respectively. Although there was a large difference in growth between the two females, they seemed very similar to each other and to the female *nigromaculata* in the number and size of eggs, except that the eggs were remarkably ununiform in size.

ii) Two offspring of a female No. 61B12T2 mated with a male *brevipoda*

These females were similar to the female *nigromaculata* in body length and egg size. However, the numbers of their eggs were distinctly smaller than those of the female *nigromaculata*. Moreover, there was a remarkable difference in the size of eggs.

iii) An offspring of a female No. 61B13T3 mated with a male *nigromaculata*

This female was very similar to the fore-going females Nos. (61B12T2)B.1～2 in body length and egg size as well as in the number of eggs.

II. Mating experiments

In the breeding season of the year 1965, the first- and second-generation offspring were produced from nucleo-cytoplasmic hybrids and their first-generation offspring by brother and sister mating or mating with the control *nigromaculata* or *brevipoda*.

1. Viability of the first-generation offspring of male nucleo-cytoplasmic hybrids, (N)BB and (B)NN

As the female parents of crosses, five *nigromaculata* and four *brevipoda* were used. These females were those which had been reared since their egg stage till sexual maturity for 3 years in our laboratory. The eggs of each of the five *nigromaculata* were divided into fifteen groups. The eggs of three groups were inseminated with sperms of three male *nigromaculata* as a part of the control series. One of these males was 4 years old, while the other two were 2 or 3 years old. Four other groups were used for insemination with sperms of four 2-year-old males

TABLE 6

Viability of first-generation offspring of male

Series	Parents		No. of eggs	No. of cleaved eggs		No. of gastrulae	
	Female no.	Male no.		Normal	Abnormal	Normal	Abnormal
I (Cont.)	(N)NN. 62.1～5	(N)NN. 61.1	116	115 (99.1%)	0	115 (9.1%)	0
		(N)NN. 63.2	90	89 (98.9%)	0	89 (98.9%)	0
	(B)BB. 62.1～4	(B)BB. 63.1	72	70 (97.2%)	0	70 (97.2%)	0
II (Cont.)	(N)NN. 62.1～5	(N)+NN. 63N1T1	137	133 (97.1%)	2 (1.5%)	133 (97.1%)	0
		(N)+NN. 63N2T2	167	162 (97.0%)	5 (3.0%)	162 (97.0%)	0
		(N)+NN. 63N2T3	218	176 (80.7%)	28 (12.8%)	171 (78.4%)	5 (2.3%)
		(N)+NN. 63N3T4	146	142 (97.3%)	0	142 (97.3%)	0
III	(B)BB. 62.1～4	(N)BB. 62N1T1	325	175 (53.8%)	14 (4.3%)	175 (53.8%)	0
		(N)BB. 62N2T1	541	116 (21.4%)	35 (6.5%)	116 (21.4%)	0
		(N)BB. 62N3T1	354	284 (80.2%)	5 (1.4%)	281 (79.4%)	3 (0.8%)
		(N)BB. 62N4T1	429	270 (62.9%)	7 (1.6%)	270 (62.9%)	0
IV	(N)NN. 62.1～5	(B)NN. 63B1T1	179	148 (82.7%)	0	148 (82.7%)	0
		(B)NN. 63B1T2	209	189 (90.4%)	0	182 (87.1%)	7 (3.3%)
		(B)NN. 63B3T3	303	231 (76.2%)	15 (5.0%)	217 (71.6%)	14 (4.6%)
		(B)NN. 63B4T4	349	259 (74.2%)	16 (4.6%)	251 (71.9%)	8 (2.3%)
V	(B)BB. 62.1～4	(B)NN. 63B1T1	252	202 (80.2%)	0	199 (79.0%)	3 (1.2%)
		(B)NN. 63B1T2	362	310 (85.6%)	0	310 (85.6%)	0
		(B)NN. 63B3T3	526	490 (93.2%)	24 (4.6%)	425 (80.8%)	65 (12.4%)
VI (Cont.)	(N)NN. 62.1～5	(N)NN. 62.3	245	214 (87.3%)	0	213 (86.9%)	1 (0.4%)
	(B)BB. 62.1～4	(B)BB. 62.2	313	312 (99.7%)	0	310 (99.0%)	2 (0.6%)
VII	(N)NN. 62.1～5	(B)NN. 63B2T1	201	169 (84.1%)	14 (7.0%)	169 (84.1%)	0
		(B)NN. 63B2T2	227	204 (89.9%)	4 (1.8%)	188 (82.8%)	16 (7.0%)
		(B)NN. 63B2T3	234	164 (70.1%)	19 (8.1%)	162 (69.2%)	1 (0.4%)
		(B)NN. 63B2T4	217	155 (71.4%)	36 (16.6%)	133 (61.3%)	22 (10.1%)

nucleo-cytoplasmic hybrids, (N)BB and (B)NN

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of metamorphosed frogs	
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
115 (99.1%)	0	115 (99.1%)	0	115 (99.1%)	0	113 (97.4%)	0
89 (98.9%)	0	89 (98.9%)	0	89 (98.9%)	0	89 (98.9%)	0
70 (97.2%)	0	70 (97.2%)	0	70 (97.2%)	0	68 (94.4%)	0
133 (97.1%)	0	133 (97.1%)	0	133 (97.1%)	0	100 (73.0%)	0
162 (97.0%)	0	162 (97.0%)	0	162 (97.0%)	0	131 (78.4%)	0
164 (75.2%)	7 (3.2%)	143 (65.6%)	21 (9.6%)	143 (65.6%)	0	126 (57.8%)	0
142 (97.3%)	0	142 (97.3%)	0	133 (91.1%)	9 (6.2%)	77 (52.7%)	0
172 (52.9%)	3 (0.9%)	165 (50.8%)	7 (2.2%)	67 (20.6%)	98 (30.2%)	52 (16.0%)	0
116 (21.4%)	0	116 (21.4%)	0	109 (20.1%)	7 (1.3%)	84 (15.5%)	0
275 (77.7%)	6 (1.7%)	249 (70.3%)	26 (7.3%)	31 (8.8%)	218 (61.6%)	0	0
270 (62.9%)	0	253 (59.0%)	17 (4.0%)	21 (4.9%)	232 (54.1%)	0	0
148 (82.7%)	0	148 (82.7%)	0	143 (79.9%)	5 (2.8%)	97 (54.2%)	9* (5.0%)
170 (81.3%)	12 (5.7%)	153 (73.2%)	17 (8.1%)	67 (32.1%)	86 (41.2%)	67 (32.1%)	0
183 (60.4%)	34 (11.2%)	162 (53.5%)	21 (6.9%)	20 (6.6%)	142 (46.9%)	0	0
245 (70.2%)	6 (1.7%)	240 (68.8%)	5 (1.4%)	18 (5.2%)	222 (63.6%)	0	0
182 (72.2%)	17 (6.7%)	177 (70.2%)	5 (2.0%)	174 (69.1%)	3 (1.2%)	150 (59.5%)	12* (4.8%)
301 (83.2%)	9 (2.5%)	295 (81.5%)	6 (1.7%)	147 (40.6%)	148 (40.9%)	139 (38.4%)	0
314 (59.3%)	112 (21.3%)	224 (42.6%)	90 (17.1%)	0	224 (42.6%)	0	0
213 (86.9%)	0	211 (86.1%)	0	208 (84.9%)	3 (1.2%)	206 (84.1%)	0
307 (98.1%)	3 (1.0%)	307 (98.1%)	0	302 (96.5%)	5 (1.6%)	264 (84.3%)	0
162 (80.6%)	7 (3.5%)	159 (79.1%)	3 (1.5%)	159 (79.1%)	0	101 (50.2%)	20 (10.0%)
161 (70.9%)	27 (11.9%)	151 (66.5%)	10 (4.4%)	104 (45.8%)	47 (20.7%)	103 (45.4%)	0
160 (68.4%)	2 (0.9%)	58 (24.8%)	102 (43.6%)	6 (2.6%)	52 (22.2%)	6 (2.6%)	0
71 (32.7%)	62 (28.6%)	67 (30.9%)	4 (1.8%)	7 (3.2%)	60 (27.6%)	7 (3.2%)	0

Continued

Series	Parents		No. of eggs	No. of cleaved eggs		No. of gastrulae	
	Female no.	Male no.		Normal	Abnormal	Normal	Abnormal
VIII	(B)BB. 62.1～4	(B)NN. 63B2T1	243	194 (79.8%)	35 (14.4%)	190 (78.2%)	4 (1.6%)
		(B)NN. 63B2T2	256	218 (85.2%)	10 (3.9%)	172 (67.2%)	46 (18.0%)
		(B)NN. 63B2T3	270	246 (91.1%)	11 (4.1%)	213 (78.9%)	21 (7.8%)
		(B)NN. 63B2T4	272	247 (90.8%)	6 (2.2%)	243 (89.3%)	4 (1.5%)
IX	(B)BB. 62.1～4	(N)BB. 63N1T1	164	131 (79.9%)	14 (8.5%)	127 (77.4%)	4 (2.4%)
		(N)BB. 63N1T2	201	129 (64.2%)	35 (17.4%)	115 (57.2%)	11 (5.5%)
		(N)BB. 63N3T3	134	72 (53.7%)	0	69 (51.5%)	3 (2.2%)
		(N)BB. 63N3T4	172	39 (22.7%)	0	36 (20.9%)	3 (1.7%)
		(N)BB. 63N4T5	156	35 (22.4%)	0	35 (22.4%)	0

of nucleus-exchanged *nigromaculata* as the other part of the control. The remaining eight groups were inseminated with sperms of four 2-year-old nucleo-cytoplasmic hybrids, (B)NN.

The eggs of each of the four *brevipoda* were divided into 18 groups. As the control series, the eggs of two group were inseminated with sperms of two 2- or 3-year-old male *brevipoda*. Nine other groups were inseminated with sperms of four 3-year-old and five 2-year-old nucleo-cytoplasmic hybrids, (N)BB. The other seven groups were used for mating with seven male nucleo-cytoplasmic hybrids, (B)NN. The latter were the same individuals with seven of the eight used for mating with female *nigromaculata*. The results of experiments are presented in Table 6.

Series I (Control A). Offspring of non-treated *Rana nigromaculata* and *Rana brevipoda*

Mating 1. *Rana nigromaculata*, (N)NN, Nos. 62.1～5♀ × (N)NN, No. 61.1♂

The rate of cleaved eggs obtained from each of the five females was about 99%. All the 115 cleaved eggs developed normally and became tadpoles. They completed their metamorphosis except two which died of under-development. Four frogs died one to two weeks after metamorphosis. Of the remaining 109 frogs, 56 were preserved for histological observation of their gonads and the others were reared. Forty-one frogs attained their sexual maturity.

Mating 2. *Rana nigromaculata*, (N)NN, Nos. 62.1～5♀ × (N)NN, No. 63.2♂

All the 89 cleaved eggs developed normally passing through the hatching and metamorphosing stages. Of these frogs which were morphologically quite normal, 79 were preserved for histological observation 17 to 25 days after their metamorphosis. The remaining ten frogs attained their sexual maturity.

Mating 3. *Rana brevipoda*, (B)BB, Nos. 62.1～4♀ × (B)BB, No. 63.1♂

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of metamorphosed frogs	
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
181	9	180	1	170	10	126	25
(74.5%)	(3.7%)	(74.1%)	(0.4%)	(70.0%)	(4.1%)	(51.9%)	(10.3%)
168	4	165	3	102	63	92	0
(65.6%)	(1.6%)	(64.5%)	(1.2%)	(39.8%)	(24.6%)	(35.6%)	
201	12	98	103	0	98	0	0
(74.4%)	(4.4%)	(36.3%)	(38.1%)		(36.3%)		
173	70	51	122	0	51	0	0
(63.6%)	(25.7%)	(18.8%)	(44.9%)		(18.8%)		
90	37	86	4	16	70	0	0
(54.9%)	(22.6%)	(52.4%)	(2.4%)	(9.8%)	(42.7%)		
101	14	40	61	12	28	0	0
(50.2%)	(7.0%)	(19.9%)	(30.3%)	(6.0%)	(13.9%)		
48	21	36	12	32	4	16	0
(35.8%)	(15.7%)	(26.9%)	(9.0%)	(23.9%)	(3.0%)	(11.9%)	
35	1	34	1	33	1	17	0
(20.3%)	(0.6%)	(19.8%)	(0.6%)	(19.2%)	(0.6%)	(9.9%)	
35	0	35	0	31	4	14	0
(22.4%)		(22.4%)		(19.9%)	(2.6%)	(9.0%)	

All the 70 cleaved eggs developed normally until the hatching stage. During the tadpole stage, two died of under-development. The other 68 tadpoles completed their metamorphosis. Fifty-four of them were preserved for histological observation 16 to 25 days after metamorphosis and 14 were reared continuously. Ten of the latter attained their sexual maturity.

Series II (Control B). Offspring of four male nucleus-exchanged *nigromaculata*, (N)+NN

Mating 1. *Rana nigromaculata*, (N)NN, Nos. 62.1～5♀ × (N)+NN, No. 63N1T1♂

All the 133 normally cleaved eggs developed normally until the tadpole stage V. After this stage, 33 tadpoles became under-developed and thin, and eventually died before the metamorphosing stage. The other 100 tadpoles completed their metamorphosis, although 20 of them died within one month thereafter. Forty-six frogs were preserved for histological observation 15～28 days after metamorphosis. Fourteen of the remaining 34 frogs attained their sexual maturity.

Mating 2. *Rana nigromaculata*, (N)NN, Nos. 62.1～5♀ × (N)+NN, No. 63N2T2♂

All the normally cleaved eggs, 162 in number, developed normally until the completion of their metamorphosis, except 31 which died at the tadpole stage. The 131 metamorphosed frogs were all externally normal. Fifteen to 28 days after metamorphosis, 113 frogs were preserved for histological observation. Seventeen of the remaining 18 frogs attained their sexual maturity.

Mating 3. *Rana nigromaculata*, (N)NN, Nos. 62.1～5♀ × (N)+NN, No. 63N2T3♂

Comparatively many. eggs (12.8%) cleaved abnormally. Of 176 normally

cleaved eggs, 50 died of various types of abnormalities at the embryonal and tadpole stages. The remaining 126 tadpoles completed their metamorphosis. All these frogs were externally normal. Fifteen to 25 days after metamorphosis 94 frogs were preserved for histological observation. Twenty-four of the remaining 32 frogs attained their sexual maturity.

Mating 4. *Rana nigromaculata*, (N)NN, Nos. 62.1～5♀ × (N)+NN, No. 63N3T4♂

Only nine of 142 cleaved eggs showed various types of abnormalities at the hatching stage. However, 56 of the remaining tadpoles gradually became underdeveloped since the stage V and eventually died before metamorphosis. All the other 77 tadpoles completed normally their metamorphosis. Fourteen to 30 days after the metamorphosis 50 frogs were preserved for histological observation and the other 27 were reared. Twenty-five of these frogs attained their sexual maturity.

Series III. Offspring between four female *brevipoda* and four male nucleo-cytoplasmic hybrids, (N)BB

Mating 1. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (N)BB, No. 62N1T1♂

Although there were 175 normally cleaved eggs, 105 became edematous and died at the stages from the tail-bud to the hatching (Fig. 1). Fifteen of 67 nor-

Fig. 1. Edematous embryos and tadpoles among the first-generation offspring of a male nucleo-cytoplasmic hybrid consisting of *Rana nigromaculata* cytoplasm and *Rana brevipoda* nuclei. ×3

a, Edematous embryos at the tail-bud stage among the first-generation offspring produced by mating, (B)BB, Nos. 62.1～4 ♀ × (N)BB, No. 62N1T1 ♂.

b, Ascitic tadpoles at the hatching stage among the same first-generation offspring as (a).

mally hatched tadpoles became edematous and died during the period from the stage X to metamorphosis, while 52 tadpoles completed their metamorphosis. Within 30 days 32 frogs were preserved for histological observation. The others were reared and eventually 19 frogs attained their sexual maturity.

Mating 2. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (N)BB, No. 62N2T1♂

Abnormally cleaved eggs were comparatively numerous; there were 35, while 116 eggs cleaved normally. All the latter grew into normal embryos, except for seven which became edematous at the hatching stage. Twenty-five tadpoles died of edema at the stages V～XII. Of 84 normally metamorphosed frogs 49 were preserved for histological observation and 34 attained their sexual maturity.

Mating 3. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (N)BB, No. 62N3T1♂

Most of 284 normally cleaved eggs showed edema or some other abnormalities and died before the hatching stage. Only 31 embryos hatched normally. Moreover, all the tadpoles became edematous and died by the metamorphosing stage (Fig. 2).

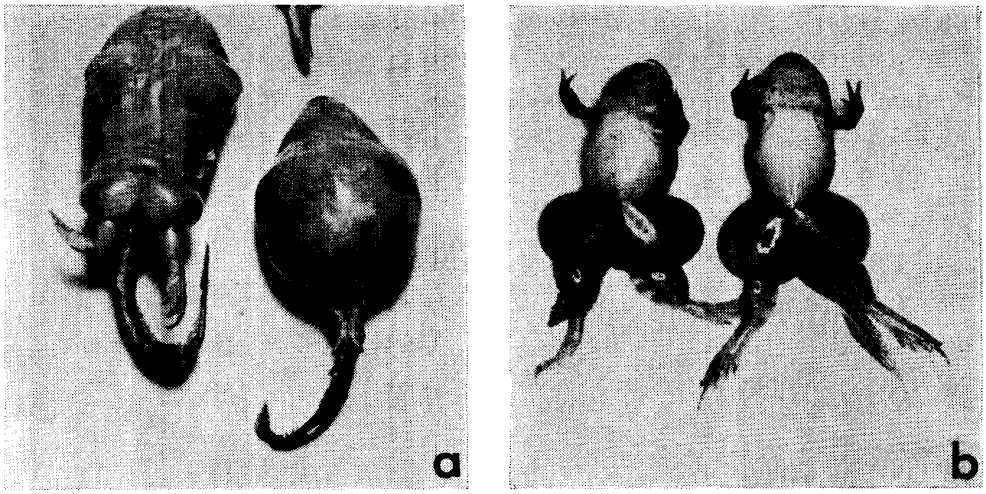

Fig. 2 Edematous tadpoles and frogs among the first-generation offspring of a male nucleo-cytoplasmic hybrid consisting of *Rana nigromaculata* cytoplasm and *Rana brevipoda* nuclei. ×1

a, Edematous tadpoles among the first-generation offspring produced by mating, (B)BB, Nos. 62.1～4 ♀ × (N)BB, No. 62N3T1 ♂.

b, Edematous frogs at the metamorphosing stage among the same first-generation offspring as (a).

Mating 4. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (N)BB, No. 62N4T1♂

All the 270 normally cleaved eggs became edematous and died at the tail-bud, hatching and feeding stages.

Series IV. Offspring between five female *nigromaculata* and four male nucleo-cytoplasmic hybrids, (B)NN

Mating 1. First-generation offspring from (N)NN, Nos. 62.1～5♀ × (B)NN, No. 63B1T1♂

Of 148 normally cleaved eggs, 5 and 37 became edematous and died at the hatching and feeding stages, respectively; the remaining 106 became completely metamorphosed frogs. Nine of them had ill-developed hind legs which could not support their body and died before long. The other 97 frogs were normal. Within 14 days after metamorphosis 82 frogs were preserved. Fourteen of the remaining 15 which were reared continuously attained their sexual maturity.

Mating 2. First-generation offspring from (N)NN, Nos. 62.1～5♀ × (B)NN, No. 63B1T2♂

Most of 189 normally cleaved eggs became edematous and died during the period from the gastrula stage to the hatching, while 67 embryos hatched normally. All of the latter continued normally their growth and completed metamorphosis. All these frogs were of normal appearance. Eighteen of them were reared until their sexual maturity, while 49 were preserved within one month after metamorphosis.

Mating 3. First-generation offspring from (N)NN, Nos. 62.1～5♀ × (B)NN, No. 63B3T3♂

Although 231 (76.2%) eggs cleaved normally, most of them revealed various types of abnormalities at various stages and mostly died at the hatching stage. All the normally hatched tadpoles which were only twenty in number became under-developed and died without attaining the metamorphosing stage.

Mating 4. First-generation offspring from (N)NN, Nos. 62.1～5♀ × (B)NN, No. 63B4T4♂

Although there were 259 (74.2%) normally cleaved eggs, only 18 embryos hatched normally and the remaining ones died of various types of abnormalities: 222 (63.6%) died at the hatching stage. All the 18 tadpoles became abnormal and died, too. There were no tadpoles which attained the metamorphosing stage.

Series V. Offspring between four female *brevipoda* and three male nucleo-cytoplasmic hybrids, (B)NN

Mating 1. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (B)NN, No. 63B1T1♂

Forty of 202 normally cleaved eggs died of various types of abnormalities at the embryonal and tadpole stages and the remaining 162 attained the stage of completion of metamorphosis. However, twelve of the latter died soon after, as their hind legs could not give support to their body, owing to ill-development of these hind legs. Of the 150 frogs, 83 were preserved 14 to 28 days after their metamorphosis and the other 67 were continuously reared; 63 of the latter attained their sexual maturity.

Mating 2. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (B)NN, No. 63B1T2♂

Of normally cleaved 310 eggs, 171 became edematous and died at various stages:

Nine at the neurula stage, six at the tail-bud, 148 at the hatching and eight at the feeding. The remaining 139 tadpoles completed their metamorphosis. Within one month after metamorphosis, 19 frogs died and 89 were preserved for histological observation. The other 31 frogs were continuously reared; they all attained their sexual maturity, except for one frog.

Mating 3. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (B)NN, No. 63B3T3♂

Although 490 (93.2%) eggs cleaved normally, all of them died by the hatching stage.

Series VI (Control). Offspring of non-treated *Rana nigromaculata* or *brevipoda*

Mating 1. (N)NN, Nos. 62.1～5♀ × (N)NN, No. 62.3♂

The rate of normal cleavages of 245 eggs obtained from the five females was 87.3%. There were no abnormally cleaved eggs. All but one cleaved egg developed into normal neurulae. While two embryos died at the tail-bud stage, 208 embryos hatched normally, and 206 (84.1%) completed their metamorphosis.

Mating 2. (B)BB, Nos. 62.1～4♀ × (B)BB, No. 62.2♂

All but one egg obtained from the four females cleaved normally. Of these cleaved eggs two and three embryos died at the gastrula and neurula stages, respectively. Three hundred and two embryos hatched normally and 264 (84.3%) tadpoles completed their metamorphosis.

Series VII. Offspring between five female *nigromaculata* and four male nucleo-cytoplasmic hybrids, (B)NN

Mating 1. First-generation offspring from (N)NN, Nos. 62.1～5♀ × (B)NN, No. 63B2T1♂

Out of 201 eggs, 169 (84.1%) cleaved normally and 14 (7%) died abnormally.

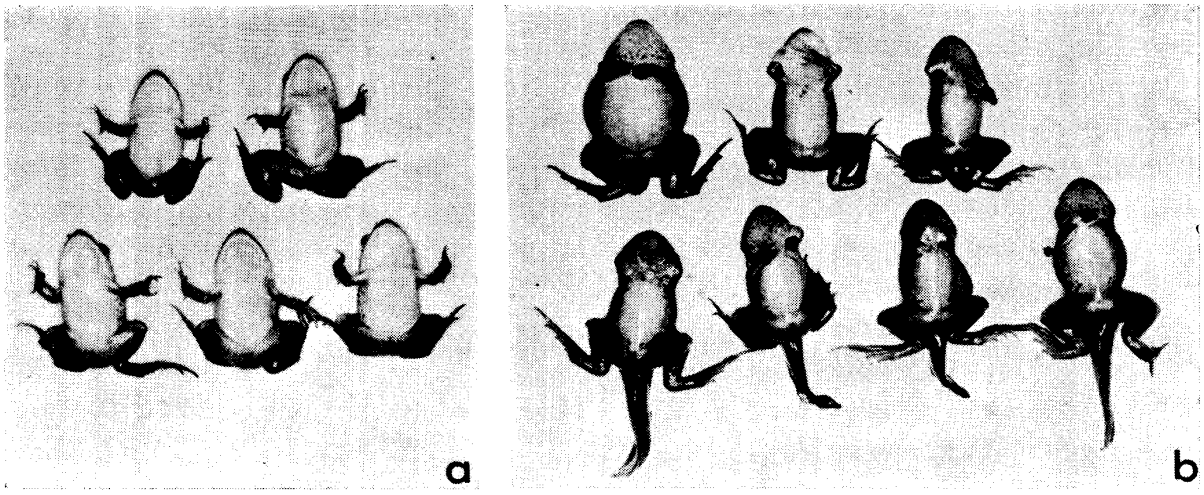

Fig. 3 Ill-developed forelegs of first-generation offspring of a nucleo-cytoplasmic hybrid consisting *Rana brevipoda* cytoplasm and *Rana nigromaculata* nuclei. ×1

a, Ventral views of normal frogs among the control *Rana nigromaculata*.

b, Ventral views of abnormal frogs with ill-developed forelegs among the first-generation offspring produced by mating, (N)NN, Nos. 62. 1～5 ♀ × (B)NN, No. 63B2T1 ♂.

Seven and three cleaved eggs died at the neurula and the tail-bud stage, respectively. All the remaining embryos hatched, although many individuals died at the tadpole stages. Eventually, 101 (50.2%) tadpoles became normal metamorphosed frogs, while 20 (10.0%) became abnormal ones with ill-developed forelegs which were too small and slender to support their body (Fig. 3).

Mating 2. First-generation offspring from (N)NN, Nos. 62.1～5♀ × (B)NN, No. 63B2T2♂

Although normally cleaved eggs were numerous (89.9%), about half of them became abnormal at the embryonal stages and 104 (45.8%) embryos hatched normally. All but one tadpole grew normally and completed their metamorphosis.

Mating 3. First-generation offspring from (N)NN, Nos. 62.1～5♀ × (B)NN, No. 63B2T3♂

Normally cleaved eggs were 70.1% of total eggs. They developed normally into neurulae except four eggs. However, the majority of embryos became abnormal and died at the tail-bud stage. Only six embryos hatched normally and became swimming tadpoles. All of them completed their metamorphosis.

Mating 4. First-generation offspring from (N)NN, Nos. 62.1～5♀ × (B)NN, No. 63B2T4♂

Out of 217 eggs obtained from the five females, 155 (71.4%) cleaved normally, while 36 (16.6%) did abnormally. At the gastrula and neurula stages, 22 and 62 normally cleaved eggs became abnormal, respectively. Although there were 67 normal tail-bud embryos, only seven hatched normally. The latter completed their metamorphosis.

Series VIII. Offspring between four female *brevipoda* and four male nucleo-cytoplasmic hybrids, (B)NN

Mating 1. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (B)NN, No. 63B2T1♂

Although 229 out of 243 eggs obtained from the three females cleaved, 35 (14.4%) did abnormally. Except for a small number of eggs, the normally cleaved eggs developed into normal tail-bud embryos; 170 (70.0%) of the latter hatched normally. While 126 (51.9%) became normal metamorphosed frogs, 25 (10.3%) became abnormal ones with ill-developed forelegs which were too small and slender to support their body.

Mating 2. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (B)NN, No. 63B2T2♂

Normally cleaved eggs were 85.2%. However, 46 of them died at the gastrula stage. At the hatching stage, 102 (39.8%) embryos were normal, while 63 abnormal. Ninety-two (35.9%) eventually completed their metamorphosis.

Mating 3. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (B)NN, No. 63B2T3♂

Out of 270 eggs, 246 (91.1%) cleaved normally and 201 became normal neurulae. However, most of the latter became abnormal at the tail-bud stage and

there were no normal embryos at the hatching stage.

Mating 4. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (B)NN, No. 63B2T4♂

This male was very similar to the above one No. 63B2T3, in reproductive capacity. Although 247 (90.8%) of 272 eggs obtained from the four females cleaved normally, only 51 developed into normal tail-bud embryos and there were no normal embryos at the hatching stage.

Series IX. Offspring between four female *brevipoda* and five male nucleo-cytoplasmic hybrids, (N)BB

Mating 1. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (N)BB, No. 63N1T1♂

Out of 164 eggs obtained from the four females, 131 (79.9%) cleaved normally. Although there were 86 normal embryos at the tail-bud stage, only 16 (9.8%) hatched normally. All of the latter became abnormal and died at the tadpole stage.

Mating 2. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (N)BB, No. 63N1T2♂

The rates of normal and abnormal cleavages were 64.2 and 17.4%, respectively. At the tail-bud stage, there were 40 normal and 61 abnormal embryos. At the hatching stage, 12 embryos were normal, while 28 abnormal. There were no metamorphosed frogs.

Mating 3. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (N)BB, No. 63N3T3♂

Normally cleaved eggs were 72 (53.7%) in number. Most of them became abnormal by the tail-bud stage and 32 embryos (23.9%) hatched normally. Sixteen tadpoles completed their metamorphosis.

Mating 4. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (N)BB, No. 63N3T4♂

Thirty-nine (22.7%) of 172 eggs cleaved normally. Of these normally cleaved eggs, 33 hatched normally and eventually 17 tadpoles became normal metamorphosed frogs.

Mating 5. First-generation offspring from (B)BB, Nos. 62.1～4♀ × (N)BB, No. 63N4T5♂

The rate of cleavages was 22.4% of eggs obtained from the four females. All the normally cleaved eggs became normal tail-bud embryos. Thirty-one of the latter hatched normally and 14 tadpoles completed their metamorphosis.

2. Viability of the second-generation offspring of female nucleo-cytoplasmic hybrids, (B)NN

Second-generation offspring were produced from first-generation offspring by brother and sister mating or by mating with *nigromaculata* or *brevipoda*. The first-generation offspring were those which had been obtained from female nucleo-cytoplasmic hybrids, (B)NN, by mating with a male *nigromaculata* or *brevipoda*.

TABLE 7
Viabilty of second-generation off-

Series	Parents		No. of eggs	No. of cleaved eggs	
	Female no.	Male no.		Normal	Abnorm.
I	(N)NN.62.6～8	(N)NN.62.3	143	140 (97.9%)	0
		(N)NN.62.4	134	133 (99.3%)	0
II	(N)NN.62.6～8	{(B)NN.61B12T2}N. 1	512	474 (92.6%)	5 (1.0%)
		{(B)NN.61B12T2}N. 2	173	148 (85.5%)	25 (14.5%)
		{(B)NN.61B12T2}N. 3	177	169 (95.5%)	0
		{(B)NN.61B12T2}N. 4	160	139 (86.9%)	0
		{(B)NN.61B12T2}N. 5	469	452 (96.4%)	0
		{(B)NN.61B12T2}N. 6	192	183 (95.3%)	0
		{(B)NN.61B12T2}N. 7	102	12 (11.8%)	4 (3.9%)
		{(B)NN.61B12T2}N. 8	177	111 (62.7%)	3 (1.7%)
		{(B)NN.61B12T2}N. 9	172	11 (6.4%)	6 (3.5%)
III	(N)NN.62.6～8	{(B)NN.61B13T3}N. 1	111	9 (8.1%)	5 (4.5%)
		{(B)NN.61B13T3}N. 2	141	61 (43.3%)	12 (8.5%)
		{(B)NN.61B13T3}N. 3	142	1 (0.7%)	12 (8.5%)
IV	{(B)NN.61B12T2}N.1	(N)NN.62.3	322	320 (99.4%)	2 (0.6%)
		{(B)NN.61B12T2}N. 7	284	15 (5.3%)	4 (1.4%)
		{(B)NN.61B12T2}N. 8	254	56 (22.0%)	4 (1.6%)
		{(B)NN.61B12T2}N. 9	204	28 (13.7%)	0
V	{(B)NN.61B12T2}N.2	(N)NN.62.3	287	282 (98.3%)	5 (1.7%)
		{(B)NN.61B12T2}N. 7	84	3 (3.6%)	29 (34.5%)
		{(B)NN.61B12T2}N. 8	148	31 (20.9%)	15 (10.1%)
		{(B)NN.61B12T2}N. 9	74	3 (4.1%)	20 (27.0%)
VI	{(B)NN.61B13T3}N.1	(N)NN.62.4	150	130 (86.7%)	17 (11.3%)
		{(B)NN.61B13T3}N. 1	71	14 (19.7%)	26 (36.6%)
		{(B)NN.61B13T3}N. 2	115	21 (18.3%)	36 (31.3%)
		{(B)NN.61B13T3}N. 3	146	27 (18.5%)	16 (11.0%)
VII	(B)BB.62.5～7	(B)BB.62.2	196	175 (89.3%)	0
		{(B)NN.61B12T2}N. 7	138	2 (1.4%)	23 (16.7%)
		{(B)NN.61B12T2}N. 8	147	79 (53.7%)	3 (2.0%)
		{(B)NN.61B12T2}N. 9	254	7 (2.8%)	12 (4.7%)

spring of female nucleo-cytoplasmic hybrids, (B)NN

No. of gastrulae		No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of metamorphosed frogs	
Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.
140 (97.9%)	0	140 (97.9%)	0	140 (97.9%)	0	140 (97.9%)	0	140 (97.9%)	0
133 (99.3%)	0	133 (99.3%)	0	133 (99.3%)	0	133 (99.3%)	0	133 (99.3%)	0
462 (90.2%)	12 (2.3%)	451 (88.1%)	11 (2.1%)	426 (83.2%)	25 (4.9%)	0	426 (83.2%)	0	0
145 (83.8%)	3 (1.7%)	145 (83.8%)	0	145 (83.8%)	0	0	145 (83.8%)	0	0
164 (92.7%)	5 (2.8%)	164 (92.7%)	0	161 (91.0%)	3 (1.7%)	161 (91.0%)	0	118 (66.7%)	0
139 (86.9%)	0	139 (86.9%)	0	136 (85.0%)	3 (1.9%)	136 (85.0%)	0	120 (75.0%)	0
450 (96.0%)	2 (0.4%)	447 (95.3%)	3 (0.6%)	445 (94.9%)	2 (0.4%)	0	445 (94.9%)	0	0
183 (95.3%)	0	180 (93.8%)	3 (1.6%)	172 (89.6%)	8 (4.2%)	0	172 (89.6%)	0	0
12 (11.8%)	0	12 (11.8%)	0	12 (11.8%)	0	11 (10.8%)	1 (1.0%)	8 (7.8%)	0
111 (62.7%)	0	111 (62.7%)	0	111 (62.7%)	0	111 (62.7%)	0	100 (56.5%)	0
11 (6.4%)	0	11 (6.4%)	0	11 (6.4%)	0	11 (6.4%)	0	11 (6.4%)	0
9 (8.1%)	0	9 (8.1%)	0	9 (8.1%)	0	9 (8.1%)	0	4 (3.6%)	0
61 (43.3%)	0	61 (43.3%)	0	61 (43.3%)	0	61 (43.3%)	0	57 (40.4%)	0
1 (0.7%)	0	1 (0.7%)	0	1 (0.7%)	0	1 (0.7%)	0	1 (0.7%)	0
320 (99.4%)	0	316 (98.1%)	4 (1.2%)	309 (96.0%)	7 (2.2%)	301 (93.5%)	8 (2.5%)	222 (68.9%)	0
15 (5.3%)	0	15 (5.3%)	0	15 (5.3%)	0	15 (5.3%)	0	13 (4.6%)	0
56 (22.1%)	0	56 (22.1%)	0	56 (22.1%)	0	56 (22.1%)	0	55 (21.7%)	0
28 (13.7%)	0	28 (13.7%)	0	28 (13.7%)	0	25 (12.3%)	3 (1.5%)	24 (11.8%)	0
282 (98.2%)	0	277 (96.5%)	5 (1.7%)	247 (86.1%)	30 (10.5%)	232 (80.8%)	15 (5.2%)	163 (56.8%)	0
3 (3.6%)	0	3 (3.6%)	0	3 (3.6%)	0	3 (3.6%)	0	3 (3.6%)	0
31 (20.9%)	0	31 (20.9%)	0	29 (19.6%)	2 (1.4%)	20 (13.5%)	9 (6.1%)	16 (10.8%)	0
3 (4.1%)	0	3 (4.1%)	0	3 (4.1%)	0	3 (4.1%)	0	2 (2.7%)	0
127 (84.7%)	1 (0.7%)	127 (84.7%)	0	127 (84.7%)	0	124 (82.7%)	3 (2.0%)	105 (70.0%)	0
14 (19.7%)	0	14 (19.7%)	0	14 (19.7%)	0	14 (19.7%)	0	7 (9.9%)	0
19 (16.5%)	2 (1.7%)	19 (16.5%)	0	19 (16.5%)	0	19 (16.5%)	0	18 (15.7%)	0
26 (17.8%)	1 (0.7%)	26 (17.8%)	0	26 (17.8%)	0	21 (14.4%)	5 (3.4%)	21 (14.4%)	0
175 (89.3%)	0	175 (89.3%)	0	175 (89.3%)	0	172 (87.8%)	3 (1.5%)	170 (86.7%)	0
1 (0.7%)	0	1 (0.7%)	0	1 (0.7%)	0	1 (0.7%)	0	1 (0.7%)	0
77 (52.4%)	2 (1.4%)	77 (52.4%)	0	76 (51.7%)	1 (0.7%)	43 (29.3%)	33 (22.5%)	37 (25.2%)	0
6 (2.4%)	1 (0.4%)	6 (2.4%)	0	5 (2.0%)	1 (0.4%)	5 (2.0%)	0	3 (1.2%)	0

Continued

Series	Parents		No. of eggs	No. of cleaved eggs	
	Female no.	Male no.		Normal	Abnorm.
VIII	(B)BB.62.5~7	(B)BB.62.2	158	147 (93.0%)	0
		{(B)NN.61B13T3}N. 1	120	1 (0.8%)	14 (11.7%)
		{(B)NN.61B13T3}N. 2	119	63 (52.9%)	4 (3.4%)
		{(B)NN.61B13T3}N. 3	160	3 (1.9%)	27 (16.9%)
IX	{(B)NN.61B12T2}N.1	(B)BB.62.2	302	243 (80.5%)	0
X	{(B)NN.61B12T2}B.1	(N)NN.62.4	251	246 (98.0%)	5 (2.0%)
		(B)BB.62.2	206	202 (98.1%)	2 (1.0%)
		{(B)NN.61B12T2}B. 1	65	34 (52.3%)	10 (15.4%)
		{(B)NN.61B12T2}B. 2	138	115 (83.3%)	4 (2.9%)
XI	{(B)NN.61B12T2}B.2	(N)NN.62.4	179	174 (97.2%)	3 (1.7%)
		(B)BB.62.2	215	170 (79.1%)	19 (8.8%)
		{(B)NN.61B12T2}B. 3	62	13 (21.0%)	4 (6.5%)
XII	(N)NN.62.6~8	(N)NN.62.4	193	184 (95.3%)	0
		{(B)NN.61B12T2}B. 1	108	3 (2.8%)	0
		{(B)NN.61B12T2}B. 2	154	38 (24.7%)	11 (7.1%)
		{(B)NN.61B12T2}B. 3	130	0	0
XIII	(B)BB.62.5~7	(B)BB.62.2	235	211 (89.8%)	0
		{(B)NN.61B12T2}B. 1	122	1 (0.8%)	19 (15.6%)
		{(B)NN.61B12T2}B. 2	150	96 (64.0%)	15 (10.0%)
		{(B)NN.61B12T2}B. 3	138	3 (2.2%)	12 (8.7%)

In order to produce the second-generation offspring, the following 3-year-old frogs were used: three female first-generation offspring which had been produced from female nucleo-cytoplasmic hybrids, (B)NN, and male *nigromaculata*; two female first-generation offspring which had been produced from a female nucleo-cytoplasmic hybrid, (B)NN, and a male *brevipoda*; three female *nigromaculata*; three female *brevipoda*; twelve male first-generation offspring of female nucleo-cytoplasmic hybrids, (B)NN, and a male *nigromaculata*; three male first-generation offspring of a female nucleo-cytoplasmic hybrid, (B)NN, and a male *brevipoda*; two male *nigromaculata*; a male *brevipoda*.

The eggs of each of the three *nigromaculata* females were divided into 18 groups. Fifteen of these groups were inseminated with sperms of 15 male first-generation offspring, while the other three were used as the control by insemination with sperms of the two male *nigromaculata*. On the other hand, the eggs of each of the three *brevipoda* females were divided into 12 groups. Nine of the latter were insemi-

No. of gastrulae		No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of metamorphosed frogs	
Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.
147 (93.0%)	0	147 (93.0%)	0	147 (93.0%)	0	145 (91.8%)	2 (1.3%)	141 (89.2%)	0
1 (0.8%)	0	1 (0.8%)	0	1 (0.8%)	0	1 (0.8%)	0	0	0
62 (52.1%)	0	62 (52.1%)	0	62 (52.1%)	0	55 (46.2%)	7 (5.9%)	52 (43.7%)	0
3 (1.9%)	0	3 (1.9%)	0	3 (1.9%)	0	3 (1.9%)	0	2 (1.3%)	0
243 (80.5%)	0	241 (79.8%)	2 (0.7%)	237 (78.5%)	4 (1.3%)	223 (73.8%)	14 (4.6%)	180 (59.6%)	0
201 (80.1%)	25 (10.0%)	151 (60.2%)	50 (19.9%)	151 (60.2%)	0	145 (57.8%)	6 (2.4%)	112 (44.6%)	0
168 (81.6%)	34 (16.5%)	79 (38.3%)	89 (43.2%)	79 (38.3%)	0	75 (36.4%)	4 (1.9%)	53 (25.7%)	0
34 (52.3%)	0	33 (50.8%)	1 (1.5%)	33 (50.8%)	0	29 (44.6%)	4 (6.2%)	26 (40.0%)	0
115 (83.3%)	0	102 (73.9%)	13 (9.4%)	102 (73.9%)	0	61 (44.2%)	41 (29.7%)	36 (26.1%)	0
167 (93.3%)	7 (3.9%)	135 (75.4%)	32 (17.9%)	100 (55.9%)	35 (19.6%)	95 (53.1%)	5 (2.8%)	85 (47.5%)	0
161 (74.9%)	9 (4.2%)	88 (40.9%)	73 (34.0%)	76 (35.3%)	12 (5.6%)	70 (32.6%)	6 (2.8%)	63 (29.3%)	0
13 (21.0%)	0	12 (19.4%)	1 (1.6%)	11 (17.7%)	1 (1.6%)	11 (17.7%)	0	6 (9.7%)	0
184 (95.3%)	0	184 (95.3%)	0	184 (95.3%)	0	181 (93.8%)	3 (1.6%)	175 (90.7%)	0
3 (2.8%)	0	3 (2.8%)	0	3 (2.8%)	0	3 (2.8%)	0	2 (1.9%)	0
38 (24.7%)	0	38 (24.7%)	0	36 (23.4%)	2 (1.3%)	35 (22.7%)	1 (0.6%)	33 (21.4%)	0
0	0	0	0	0	0	0	0	0	0
211 (89.8%)	0	211 (89.8%)	0	207 (88.1%)	4 (1.7%)	202 (86.0%)	5 (2.1%)	186 (79.1%)	0
1 (0.8%)	0	1 (0.8%)	0	1 (0.8%)	0	1 (0.8%)	0	0	0
94 (62.7%)	2 (1.3%)	92 (61.3%)	2 (1.3%)	90 (60.0%)	2 (1.3%)	70 (46.7%)	20 (13.3%)	63 (42.0%)	0
2 (1.4%)	1 (0.7%)	2 (1.4%)	0	2 (1.4%)	0	1 (0.7%)	1 (0.7%)	0	0

nated with sperms of the nine male first-generation offspring, and the other three were inseminated with sperm of a male *brevipoda* as the control series.

The eggs of one of the three female first-generation offspring obtained by mating with a male *nigromaculata* were divided into five groups and inseminated with sperms of three brothers, a male *nigromaculata* and a male *brevipoda*. The eggs of another female which was a sister of the above female were divided into four groups and inseminated with sperms of the same brothers and *nigromaculata* as stated above. The eggs of the remaining female were divided into four groups and inseminated with sperms of three brothers and a male *nigromaculata*.

The eggs of one of the two female first-generation offspring obtained by mating with a male *brevipoda* were divided into four groups and inseminated with sperms of two brothers, a male *nigromaculata* and a male *brevipoda*. The eggs of the other female were divided into three groups and inseminated with sperm of a brother and those of the same *nigromaculata* and *brevipoda* as stated above.

The results of experiments are presented in Table 7.

Series I (Control). Offspring of *Rana nigromaculata* produced by brother and sister mating

Mating 1. *Rana nigromaculata* from Nos. 62.6～8♀ × No. 62.3♂

All the 140 cleaved eggs developed normally and became tadpoles which were mostly 79.5～80 mm. in maximum total length. All of them completed normally their metamorphosis. Twenty-two frogs were preserved for histological observation within 20 days after metamorphosis and 59 attained their sexual maturity.

Mating 2. *Rana nigromaculata* from Nos. 62.6～8♀ × No. 62.4♂

Of 134 eggs 133 cleaved and developed quite normally and became tadpoles which were mostly 79～81 mm. in maximum total length. All the tadpoles completed their metamorphosis. Ninety-one frogs were preserved 20 to 25 days after metamorphosis and the other 42 were reared until their sexual maturity.

Series II. Offspring between three female *nigromaculata* and nine male first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2

Mating 1. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B12T2}N, No. 1♂

Although 474(92.6%) eggs cleaved normally, all of them became edematous and died at various stages: 12 at the gastrula, 11 at the neurula, 25 at the tail-bud and the remaining 426 at the hatching.

Mating 2. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B12T2}N, No. 2♂

All the 148(85.5%) normally cleaved eggs died of edema by the hatching stage.

Mating 3. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B12T2}N, No. 3♂

Fifty-one of 169(95.5%) normally cleaved eggs became edematous and died at various stages: five at the gastrula, three at the tail-bud and 43 at the feeding. The remaining 118 developed normally and became tadpoles which were mostly 72.5～75 mm. in maximum total length. All of them completed their metamorphosis; 104 were preserved 14 to 25 days after metamorphosis and the other 14 were reared until their sexual maturity.

Mating 4. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B12T2}N, No. 4♂

Of 139(86.9%) normally cleaved eggs three and sixteen died of various types of abnormalities at the tail-bud and the feeding stage, respectively. The remaining 120 tadpoles were mostly 71.5～72.5 mm. in maximum total length and completed their metamorphosis. Fourteen to 25 days after metamorphosis 115 frogs were preserved and the others were continuously reared until their sexual maturity.

Mating 5. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B12T2}N, No. 5♂

Seven of 452(96.4%) normally cleaved eggs died of various abnormalities by the tail-bud stage, and all the others died simultaneously of edema.

Mating 6. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B12T2}N, No. 6♂

Although there were 183(95.3%) normally cleaved eggs, 11 died of various abnormalities at the neurula and tail-bud stages, and all others became simultaneously edematous and died at the hatching stage.

Mating 7. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B12T2}N, No. 7♂

Only twelve (11.8%) eggs cleaved normally. Moreover, four of them died of ill-development at the tadpole stages. But the other eight tadpoles developed very well and became 84.5～86 mm. in maximum total length. All these tadpoles completed normally their metamorphosis and attained sexual maturity.

Mating 8. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B12T2}N, No. 8♂

The normally cleaved eggs, 111(62.7%) in number, developed normally and became tadpoles. Although eleven of them died of ill-development at the tadpole stages V～X, the remaining 100 grew well and became mostly 83～84.5 mm. in maximum total length. These tadpoles completed their metamorphosis; 80 of them were preserved 14 to 23 days after metamorphosis and the other 20 died within one month.

Mating 9. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B12T2}N, No. 9♂

Although the rate of fertilization was very low, eleven (6.4%) normally cleaved eggs developed and became normal tadpoles which were 84.5～85.5 mm. in maximum total length. They completed their metamorphosis. Three of them died within one week after metamorphosis and the other eight frogs attained their sexual maturity.

Series III. Offspring between three female *nigromaculata* and three male first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No.61B13T3

Mating 1. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B13T3}N, No. 1♂

The rate of fertilization was very low. Only nine (8.1%) eggs cleaved normally and grew into tadpoles. Five of them died of edema at the stage XI. The growth of the other four tadpoles was especially good and became 90～90.5 mm. in maximum total length. They completed their metamorphosis and attained sexual maturity.

Mating 2. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B13T3}N, No. 2♂

There were 61(43.3%) normally cleaved eggs. While four of them died of edema during the period from the tadpole stages X to XII, the other 57 developed normally and grew into tadpoles which were mostly 85～87.5 mm. in maximum

total length. All these tadpoles completed their metamorphosis. Twelve of them were preserved 25 days after metamorphosis and the remaining 45 were continuously reared. Thirty-eight of the latter attained their sexual maturity.

Mating 3. Second-generation offspring from (N)NN, Nos. 62.6~8♀ × {(B)NN, No. 61B13T3}N, No. 3♂

Only one egg cleaved normally. This egg developed normally and grew into a tadpole, which was 80 mm. in maximum total length. Ten days after completion of metamorphosis this frog was preserved.

Series IV. Offspring between a female first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2, and a male *nigromaculata* or three brothers of the female

Mating 1. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 1♀ × (N)NN, No. 62.3♂

Normally cleaved eggs were 320(99.4%) in number. Nineteen of them died of various types of abnormalities at the stages from the neurula to the hatching. Fifty-two others died of ill-development which appeared since the tadpole stage V. Almost all these tadpoles were defective in development of teeth. Twenty-seven other tadpoles died of edema at the stages X to XII. The remaining 222 grew into normal tadpoles, which were mostly 83~87.5 mm. in maximum total length, and completed their metamorphosis. Ten of them died within 14 days and 153 were preserved 14 to 27 days after metamorphosis. Out of 59 frogs which were continuously reared, 51 attained their sexual maturity.

Mating 2. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 1♀ × {(B)NN, No. 61B12T2}N, No. 7♂

Only 15(5.3%) eggs cleaved normally. Two of them died of edema at the tadpole stages. The remaining 13 grew very well and became tadpoles which were 89~90.5 mm. in maximum total length. These tadpoles made their metamorphosis quite normally. While eight of them were preserved ten days after metamorphosis, the other five were continuously reared and sexually matured.

Mating 3. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 1♀ × {(B)NN, No. 61B12T2}N, No. 8♂

All the 56(22.0%) normally cleaved eggs developed normally, except for one egg, which died at the feeding tadpole stage. The tadpoles were mostly 85.5~87.5 mm. in maximum total length. They completed normally their metamorphosis. Thirty of them were preserved within one month after metamorphosis and the other 25 were sexually matured.

Mating 4. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 1♀ × {(B)NN, No. 61B12T2}N, No. 9♂

There were 28(13.7%) normally cleaved eggs. Three of them died of abnormalities at the hatching stage and one died at the feeding tadpole stage. The other 24 developed normally and grew into normal tadpoles, which were mostly 80~81.5 mm. in maximum total length. All these tadpoles completed their metamorphosis. Six of them died within one month after metamorphsis and

18 attained their sexual maturity.

Series V. Offspring between another female first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and a male *nigromaculata* or three brothers of the female

Mating 1. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 2♀ × (N)NN, No. 62.3♂

Normally cleaved eggs were 282(98.3%) in number. While 50 eggs died of various types of abnormalities at various embryonal stages, the other 232 hatched normally. Sixty-nine of the latter became edematous and died at the tadpole stages X~XIII. The remaining 163 grew into normal tadpoles which were mostly 80~81.5 mm. in maximum total length, and completed their metamorphosis. Within one month after metamorphosis, 21 frogs died and 122 were preserved. The other 20 frogs were continuously reared; 18 of them attained their sexual maturity.

Mating 2. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 2♀ × {(B)NN, No. 61B12T2}N, No. 7♂

While 29(34.5%) eggs cleaved abnormally, only three (3.6%) did normally. Although the development of the latter was normal, the growth of tadpoles was not good. They were 75~77 mm. in maximum total length. After completion of metamorphosis they were somewhat feeble. Accordingly, they were preserved immediately after metamorphosis.

Mating 3. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 2♀ × {(B)NN, No. 61B12T2}N, No. 8♂

There were 31(20.9%) normally and 15(10.1%) abnormally cleaved eggs. Fifteen of the former died of edema at various stages: two at the tail-bud, nine at the hatching and four at the feeding tadpole stage. The remaining 16 became normal tadpoles which were mostly 80~81.5 mm. in maximum total length. These completed their metamorphosis. While three of them died within one month after metamorphosis, the others attained their sexual maturity, except for one frog.

Mating 4. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 2♀ × {(B)NN, No. 61B12T2}N, No. 9♂

Only three (4.1%) eggs cleaved normally, while there were 20(27%) abnormally cleaved eggs. One of the three eggs died at the feeding tadpole stage. The other two became normal tadpoles and then completed their metamorphosis. However, they died of ill-development five days after metamorphosis.

Series VI. Offspring between a female first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B13T3 and a male *nigromaculata* or three brothers of the female

Mating 1. Second-generation offspring from {(B)NN, No. 61B13T3}N, No. 1♀ × (N)NN, No. 62.4♂

There were 130(86.7%) normally cleaved eggs. Six of them died of various

types of abnormalities at various stages, while 124 hatched normally. Nineteen of the latter became edematous and died at the tadpole stages X~XVII. The other 105 tadpoles were mostly 80.5~82.5 mm. in maximum total length and completed their metamorphosis. Ninety-one frogs were preserved 14 to 28 days after metamorphosis and the other 14 were continuously reared. Ten of them attained their sexual maturity.

Mating 2. Second-generation offspring from {(B)NN, No. 61B13T3}N, No. 1♀ × {(B)NN, No. 61B13T3}N, No. 1♂

There were 14(19.7%) normally cleaved eggs, while 26(36.6%) did abnormally. The former developed normally and grew into tadpoles. However, seven of them became edematous and died at the stages XII~XVII. The remaining seven were about 86.5~87 mm. in maximum total length and made their metamorphosis quite normally. Four of them died ten days after metamorphosis. The other three were reared until their sexual maturity.

Mating 3. Second-generation offspring from {(B)NN, No. 61B13T3}N, No. 1♀ × {(B)NN, No. 61B13T3}N, No. 2♂

Twenty-one (18.3%) eggs cleaved normally, while 36(31.3%) did abnormally. Two of the former died at the gastrula and one at the feeding tadpole stage. The remaining 18 eggs developed normally and grew into normal tadpoles, which were mostly 83.5~85 mm. in maximum total length. Although they completed their metamorphosis, 14 died within one month after metamorphosis. The other four attained their sexual maturity.

Mating 4. Second-generation offspring from {(B)NN, No. 61B13T3}N, No. 1♀ × {(B)NN, No. 61B13T3}N, No. 3♂

Twenty-seven (18.5%) eggs cleaved normally. Six of them died of various types of abnormalities by the hatching stage. The other 21 developed normally and grew into normal tadpoles which were mostly 80.5~82.5 mm. in maximum total length. All these tadpoles completed their metamorphosis. Ten of them were killed and preserved 20 days after metamorphosis. While seven of the remaining 11 frogs died within one month after metamorphosis, four attained their sexual maturity.

Series VII. Offspring between three female *brevipoda* and a male *brevipoda* or three male first-generation offspring of the nucleo-cytoplasmic hybrids, (B)NN, No. 61B12T2

Mating 1. *Rana brevipoda*, (B)BB, No. 62.5~7♀ × (B)BB, No. 62.2♂

There were 175 normally cleaved eggs. Five of them died of various abnormalities; three at the hatching and two at the feeding tadpole stage. The other 170 eggs developed normally and completed their metamorphosis. Ninety-two frogs were preserved within one month after metamorphosis and the remaining 78 were continuously reared. Seventy-three of the latter attained their sexual maturity.

Mating 2. Second-generation offspring from (B)BB, Nos. 62.5~7♀ × {(B)NN, No. 61B12T2}N, No. 7♂

The rate of fertilization was extremely low. There were only two (1.4%) normally cleaved eggs, while 23(16.7%) did abnormally. One of the former two became a partial blastula. The other developed normally and completed its metamorphosis, although died two days after metamorphosis.

Mating 3. Second-generation offspring from (B)BB, Nos. 62.5～7♀ × {(B)NN, No. 61B12T2}N, No. 8♂

Thirty-seven of 79(53.7%) normally cleaved eggs developed normally and completed their metamorphosis. The others became edematous and died at various stages. Within one month after metamorphosis 13 frogs died and ten were preserved. The remaining 14 frogs were continuously reared, and 12 attained their sexual maturity.

Mating 4. Second-generation offspring from (B)BB, Nos. 62.5～7♀ × {(B)NN, No. 61B12T2}N, No. 9♂

The rate of fertilization was very low; only seven (2.8%) eggs cleaved normally. Four of them died of various abnormalities and three developed normally to the stage of completion of metamorphosis. However, these three frogs died within ten days after metamorphosis.

Series VIII. Offspring between three female *brevipoda* and a male *brevipoda* or three male first-generation offspring of the nucleo-cytoplasmic hybrids, (B)NN, No. 61B13T3

Mating 1. *Rana brevipoda*, (B)BB, Nos. 62.5～7♀ × (B)BB, No. 62.2♂

There were 147(93.0%) normally cleaved eggs. Two of them died at the hatching and four at the feeding tadpole stage. The other 141 eggs developed normally and completed their metamorphosis. Within one month after metamorphosis 102 frogs were preserved and the remaining 39 were continuously reared. Thirty-six of the latter attained their sexual maturity.

Mating 2. Second-generation offspring from (B)BB, Nos. 62.5～7♀ × {(B)NN, No. 61B13T3}N, No. 1♂

Only one (0.8%) egg cleaved normally. Although this egg developed normally by the hatching stage and became a feeding tadpole, the latter died of ill-development.

Mating 3. Second-generation offspring from (B)BB, Nos. 62.5～7♀ × {(B)NN, No. 61B13T3}N, No. 2♂

Sixty-three (52.9%) eggs cleaved normally. Seven and three of them became edematous and died at the hatching and the feeding tadpole stage, respectively. The remaining 52 developed normally and completed their metamorphosis. Thirty-four frogs were preserved within one month after metamorphosis and the other 18 were sexually matured.

Mating 4. Second-generation offspring from (B)BB, Nos. 62.5～7♀ × {(B)NN, No. 61B13T3}N, No. 3♂

There were only three (1.9%) normally cleaved eggs. They developed normally by the hatching stage and became feeding tadpoles. One of them died of ill-development and the other two completed their metamorphosis. Ten days

after metamorphosis these two frogs were preserved.

Series IX. Offspring between a female first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and a male *brevipoda*

Mating 1. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 1♀ × (B)BB, No. 62.2♂

Although there were 243(80.5%) normally cleaved eggs, 63 died of various types of abnormalities at various stages, that is, two at the neurula, four at the tail-bud, 14 at the hatching and 43 at the feeding tadpole stage. The other 180 eggs developed normally and became metamorphosed frogs. Within one month after metamorphosis 145 frogs were preserved and the remaining 35 were continuously reared. Thirty of the latter attained their sexual maturity.

Series X. Offspring between a femal first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and a male *nigromaculata*, a male *brevipoda* or two brothers of the female

Mating 1. Second-generation offspring from {(B)NN, No. 61B12T2}B, No. 1♀ × (N)NN, No. 62.4♂

There were 246(98.0%) normally cleaved eggs. Twenty at the blastula stage, 25 at the gastrula, 50 at the neurula and six at the hatching, died of various types of abnormalities, respectively. Although the remaining 145 hatched normally, 33 died of ill-development at the stages V~X. Such tadpoles had so defective teeth that they could not sufficiently take food. The other 112 tadpoles completed their metamorphosis. Ninety-eight were preserved within one month after metamorphosis and the remaining 14 were sexually matured.

Mating 2. Second-generation offspring from {(B)NN, No. 61B12T2}B, No. 1♀ × (B)BB, No. 62.2♂

There were 202(98.1%) normally cleaved eggs, but 34, 89 and four died of various types of abnormalities at the gastrula, the neurula and the hatching stage, respectively. Moreover, 22 tadpoles died of ill-development which occurred suddenly at the stages V~X. The remaining 53 tadpoles completed their metamorphosis. Forty-two were preserved within one month after metamorphosis and eleven were sexually matured.

Mating 3. Second-generation offspring from {(B)NN, No. 61B12T2}B, No. 1♀ × {(B)NN, No. 61B12T2}B, No. 1♂

Of 34(52.3%) normally cleaved eggs one and four died of various abnormalities at the neurula and the hatching stage, respectively. At the feeding tadpole stage three died of ill-development. The remaining 26 completed their metamorphosis. Within one month after metamorphosis eight frogs died and the others were continuously reared; 16 of the latter attained their sexual maturity.

Mating 4. Second-generation offspring from {(B)NN, No. 61B12T2}B, No. 1♀ × {(B)NN, No. 61B12T2}B, No. 2♂

There were 115(83.3%) normally cleaved eggs, of which 13 and 41 died of various types of abnormalities at the neurula and the hatching stage, respectively.

Twenty-five of the remaining hatched tadpoles died of ill-development at the stages V~X and 36 completed their metamorphosis. Within one month after metamorphosis 11 died and 25 were continuously reared. Twenty-two of the latter attained their sexual maturity.

Series XI. Offspring between another female first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and a male *nigromaculata*, a male *brevipoda* or a brother of the female

Mating 1. Second-generation offspring from {(B)NN, No. 61B12T2}B, No. 2♀ × (N)NN, No. 62.4♂

Of 174(97.2%) normally cleaved eggs, 89 died of various types of abnormalities at various stages, that is, seven at the gastrula, 32 at the neurula, 35 at the tail-bud, five at the hatching and ten at the feeding tadpole stage. The other 85 completed their metamorphosis, but 83 of them died within one month after metamorphosis and the remaining two died two months later.

Mating 2. Second-generation offspring from {(B)NN, No. 61B12T2}B, No. 2♀ × (B)BB, No. 62.2♂

Of 170(79.1%) normally cleaved eggs 107 died of various types of abnormalities at various stages: nine at the gastrula, 73 at the neurula, 12 at the tail-bud, six at the hatching and seven at the feeding tadpole stage. The other 63 tadpoles completed their metamorphsis. Sixteen of them died within one month after metamorphosis and the remaining 47 were continuously reared. Forty-five attained their sexual maturity.

Mating 3. Second-generation offspring from {(B)NN, No. 61B12T2}B, No. 2♀ × {(B)NN, No. 61B12T2}B, No. 3♂

The normally cleaved eggs were 13(21.0%) in number. One egg at each of the neurula and the tail-bud stage and five at the feeding tadpole stage became edematous and died. Six tadpoles completed their metamorphosis. Two of them died nine days after metamorphosis and four were sexually matured.

Series XII. Offspring between three female *nigromaculata* and a male *nigromaculata* or three male first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2

Mating 1. *Rana nigromaculata* from Nos. 62.6~8♀ × No. 62.4♂

There were 184(95.3%) normally cleaved eggs, of which three and six died of various types of abnormalities at the hatching and the feeding tadpole stage, respectively. The remaining tadpoles completed their metamorphosis. Two weeks after metamorphosis 120 frogs were preserved and the other 55 were continuously reared. Forty-one of the latter attained their sexual maturity.

Mating 2. Second-generation offspring from (N)NN, Nos. 62.6~8♀ × {(B)NN, No. 61B12T2}B, No. 1♂

Only three (2.8%) eggs cleaved normally. One of them died at the feeding tadpole stage. The other two became normal tadpoles and completed their metamorphosis. However, they died 24 and 27 days after metamorphosis.

Mating 3. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B12T2}B, No. 2♂

Thirty-eight (24.7%) eggs cleaved normally. Five of them died of various types of abnormalities at various stages: two at the tail-bud, one at the hatching and two at the feeding tadpole stage. Although the remaining 33 tadpoles completed their metamorphosis, all of them died within one month after metamorphosis.

Mating 4. Second-generation offspring from (N)NN, Nos. 62.6～8♀ × {(B)NN, No. 61B12T2}B, No. 3♂

No fertilization occurred.

Series XIII. Offspring between three female *brevipoda* and a male *brevipoda* or three male first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2

Mating 1. *Rana brevipoda* from Nos. 62.5～7♀ × No. 62.2♂

There were 211(89.8%) normally cleaved eggs, of which four and five died of various types of abnormalities at the tail-bud and the hatching stage, respectively. At the feeding tadpole stage 16 died of ill-development, while the other 186 completed their metamorphosis. Within one month after metamorphosis 80 frogs were preserved and the remainder were continuously reared. One hundred of the latter attained their sexual maturity.

Mating 2. Second-generation offspring from (B)BB, Nos. 62.5～7♀ × {(B)NN, No. 61B12T2}B, No. 1♂

Only one egg cleaved normally, while there were 19(15.6%) abnormally cleaved ones. The former died of ill-development at the feeding tadpole stage.

Mating 3. Second-generation offspring from (B)BB, Nos. 62.5～7♀ × {(B)NN, No. 61B12T2}B, No. 2♂

Although there were 96(64.0%) normally cleaved eggs, 26 of them died of various types of abnormalities at various stages; two at the gastrula, two at the neurula, two at the tail-bud and 20 at the hatching stage. At the feeding tadpole stage seven died of ill-development and the other 63 completed their metamorphosis. Within one month after metamorphosis 38 frogs were preserved and the remaining 25 were continuously reared. Twenty-one frogs attained their sexual maturity.

Mating 4. Second-generation offspring from (B)BB, Nos. 62.5～7♀ × {(B)NN, No. 61B12T2}B, No. 3♂

Only three (2.2%) eggs cleaved normally. All of them died at the gastrula, the hatching or the feeding tadpole stage.

III. Sex and gonads

The sex and the gonads of the first- and second-generation offspring of nucleo-cytoplasmic hybrids were observed at the juvenile and the matured frog stage, together with those of the control frogs. The results of observations are presented in Tables 8 and 9, by arranging them in the same order as that of the mating

experiments in Tables 6 and 7. The age of each frog at the time of landing from water and the body length immediately after metamorphosis are given in the tables, too. It should be noted that the matured frogs were those which were generally larger than the remainder at the stage immediately after metamorphosis. At this stage, they were selected from just metamorphosed frogs for the purpose of raising them till their sexual maturity. As females are mostly a little larger than males even at the youngest frog stage, although both sexes can not be distinguished from each other by appearences, it might be rather usual that females were unconsciously selected and reared to sexual maturity more frequently than males were.

Both *Rana nigromaculata* and *Rana brevipoda* are of semi-differentiated type in sex differentiation. At the stage immediately after metamorphosis, juvenile hermaphrodites are rarely found in these two species, although the sex differentiation in *brevipoda* occurs somewhat earlier than that in *nigromaculata*. The gonads of the first- or second-generation offspring of nucleo-cytoplasmic hybrids at the youngest frog stage were generally smaller than those of the control *nigromaculata* or *brevipoda*. Moreover, comparatively numerous juvenile hermaphrodites were found in a small number of mating groups. Accordingly, the sex of the young frogs at the stage within one month after metamorphosis was divided into the following six categories shown with signs on the basis of the inner structures of their gonads.

1. ♀$_N$(female with normal ovaries) The gonads are ovaries filled with growing auxocytes (Plate III, 9).

2. ♀$_U$(female with under-developed ovaries) The gonads are under-developed ovaries; no or a small number of growing auxocytes are found (Plate III, 10).

3. ⚥$_1$(hermaphrodite at the first phase) The gonads are partly of an ovarian structure, and partly of a testicular. In most cases rete cells of the medullary portions of the gonads are extremely increased in number and reveal a testicular structure, while the cortical portions which are partly left on the marginal areas of the gonads still have an ovarian structure (Plate III, 11 and 12). In a few cases the gonad on one side is a testis and that on the other side is an ovary (Plate IV, 13).

4. ⚥$_2$(hermaphrodite at the second phase) The gonads are of a testicular structure as a whole. Although rete cells are distributed all over the gonads, oocytes are found in the inner portion as small isolated groups (Plate IV, 14).

5. ♂$_R$(male with rudimentary testes) The gonads are rudimentary testes. These testes are especially small, owing to their ill-development or paucity of germ cells (Plate IV, 16).

6. ♂$_N$(male with normal testes) The gonads are typical testes. Germ cells are almost completely surrounded by rete cells. There are no cortical portions and ovarian cavities. A few isolated or grouped oocytes are sometimes found in the testes (Plate IV, 15).

Most of sexually matured males and females of each mating groups were used

for producing their next generation. While a testis of each male was used for insemination experiments, the other testis was fixed and sectioned for histological examinations. According to the inner structure, each testis was identified as that of Type 1, 2, or 3 by the same standard as already stated in the case of male nucleo-cytoplasmic hybrids.

1. Sex of the first-generation offspring of male nucleo-cytoplasmic hybrids, (N)BB and (B)NN

As already described in the part of mating experiments, there were two (I and II) control and three (III~V) experimental series. In the control series, there were three and four mating groups, while in the experimental series, there were four, four and three mating groups, respectively.

Series I (Control A). Offspring of non-treated frogs

In the three mating groups, 94.4~98.9% of the total number of eggs developed

TABLE 8

Sex of first-generation offspring of male nucleo-cytoplasmic

Series	Parents		Age at the time of landing (days)	No. of metamorphosed frogs	Body length immediately after metamorphosis (mm.)	Sex of frogs within one metamor-			
	Female no.	Male no.					♀N	♀U	♂1
I	(N)NN.62.1~5	(N)NN.61.1	75~84 (79.0)	113	23.5±0.07	60	31	0	0
		(N)NN.63.2	76~84 (78.9)	89	23.0±0.08	79	39	2	0
	(B)BB.62.1~4	(B)BB.63.1	77~97 (83.5)	68	19.7±0.04	58	29	1	0
II	(N)NN.62.1~5	(N)+NN.63N1T1	75~89 (78.5)	100	25.1±0.09	86	43	0	0
		(N)+NN.63N2T2	75~89 (78.6)	131	22.3±0.10	113	66	0	2
		(N)+NN.63N2T3	75~89 (80.0)	126	20.4±0.15	94	51	1	0
		(N)+NN.63N3T4	76~90 (79.0)	77	21.2±0.09	50	37	0	3
III	(B)BB.62.1~4	(N)BB.62N1T1	65~80 (70.7)	52	19.6±0.07	32	17	0	0
		(N)BB.62N2T1	66~84 (73.7)	84	19.5±0.09	49	25	0	0
IV	(N)NN.62.1~5	(B)NN.63B1T1	70~82 (73.8)	106	22.8±0.14	91	47	0	0
		(B)NN.63B1T2	62~75 (68.4)	67	24.3±0.15	49	19	14	2
V	(B)BB.62.1~4	(B)NN.63B1T1	62~78 (67.5)	162	25.1±0,11	95	48	0	0
		(B)NN.63B1T2	63~80 (68.7)	139	26.7±0.16	108	44	0	5

into normal, metamorphosed frogs. While the *nigromaculata* eggs attained the stage of landing after 79 days on the average and the young frogs were about 23 mm. in body length, the *brevipoda* eggs attained the same stage after 83.5 days and the frogs were about 20 mm. Among 139 young *nigromaculata* frogs examined within one month after metamorphosis, there were 65 males with normal testes, 2 hermaphrodites with gonads transforming from ovaries to testes, two females with under-developed ovaries and 70 females with normal ovaries. On the other hand, among 58 young *brevipoda* frogs, there were 27 males with normal testes, a hermaphrodite with transforming gonads, 29 females with normal ovaries and a female with under-developed ovaries.

The sex of matured frogs in this control series was observed on 51 *nigromaculata* and 10 *brevipoda*. There were 27 females and 24 males among the former, while 6 females and 4 males among the latter. The testes of 15 male *nigromaculata* and a male *brevipoda* were examined histologically and found to be quite normal, that is, of Type 1. The seminal tubules were filled with bundles of normal

hybrids, (N)BB and (B)NN, at the juvenile or matured frog stage

dead or killed month after phosis				Sex of matured frogs			Structure of testes				Sex of all frogs examined		
							No. of observed males	Type of testes					
♀2	♂R	♂N	♂(%)*	No. of frogs	♀	♂		1	2	3	Total	♀	♂(%)*
0	0	29	(48)	41	22	19	14	14	0	0	101	53	48 (48)
2	0	36	(48)	10	5	5	1	1	0	0	89	46	43 (48)
1	0	27	(48)	10	6	4	1	1	0	0	68	36	32 (47)
0	0	43	(50)	14	12	2	2	2	0	0	100	55	45 (45)
5	0	40	(42)	17	13	4	3	3	0	0	130	79	51 (39)
1	0	41	(45)	24	19	5	4	4	0	0	118	71	47 (40)
0	0	10	(26)	25	20	5	2	2	0	0	75	57	18 (24)
0	0	15	(47)	19	11	8	6	4	1	1	51	28	23 (45)
0	0	24	(49)	34	16	18	17	9	5	3	83	41	42 (51)
0	0	44	(48)	14	11	3	2	2	0	0	105	58	47 (45)
2	0	12	(33)	18	10	8	3	2	0	1	67	43	24 (36)
0	0	47	(49)	63	30	33	30	0	2	28	158	78	80 (51)
0	0	59	(59)	30	12	18	13	0	2	11	138	56	82 (59)

*……Including hermaphrodites

TABLE 9

Sex of second-generation offspring of female nucleo-

Series	Parents		Age at the time of landing (days)	No. of metamorphosed frogs	Body length immediately after metamorphosis (mm.)	Total
	Female no.	Male no.				
I	(N)NN.62.6~8	(N)NN.62.3	59~65 (61.3)	140	24.2±0.07	22
		(N)NN.62.4	59~62 (60.5)	133	23.0±0.04	81
II	(N)NN.62.6~8	{(B)NN.61B12T2}N.3	74~89 (82.0)	118	21.3±0.03	104
		{(B)NN.61B12T2}N.4	74~89 (79.3)	120	21.0±0.04	115
		{(B)NN.61B12T2}N.7	51~59 (56.4)	8	25.0±0.14	0
		{(B)NN.61B12T2}N.8	62~72 (65.6)	100	23.5±0.09	99
		{(B)NN.61B12T2}N.9	51~59 (55.0)	11	27.3±0.05	3
III	(N)NN.62.6~8	{(B)NN.61B13T3}N.1	53~57 (54.8)	4	30.4±0.16	0
		{(B)NN.61B13T3}N.2	60~67 (63.3)	57	29.0±0.05	12
		{(B)NN.61B13T3}N.3	67	1	20.0	1
IV	{(B)NN.61B12T2}N.1	(N)NN.62.3	60~73 (63.9)	222	25.3±0.07	163
		{(B)NN.61B12T2}N.7	53~58 (54.9)	13	32.2±0.72	8
		{(B)NN.61B12T2}N.8	59~68 (62.1)	55	27.0±0.14	30
		{(B)NN.61B12T2}N.9	60~69 (63.0)	24	26.4±0.12	6
V	{(B)NN.61B12T2}N.2	(N)NN.62.3	60~71 (64.1)	163	23.3±0.06	143
		{(B)NN.61B12T2}N.7	64~67 (65.6)	3	19.1~21.5	3
		{(B)NN.61B12T2}N.8	61~76 (66.0)	16	24.5±0.15	3
		{(B)NN.61B12T2}N.9	67~69 (68.0)	2	18.0~19.5	2
VI	{(B)NN.61B13T3}N.1	(N)NN.62.4	59~72 (63.0)	105	24.1±0.04	91
		{(B)NN.61B13T3}N.1	57~61 (59.4)	7	26.3±0.21	4
		{(B)NN.61B13T3}N.2	68~78 (71.4)	18	25.0±0.09	14
		{(B)NN.61B13T3}N.3	60~69 (64.0)	21	23.5±0.09	17

cytoplasmic hybrids, (B)NN, at the juvenile or matured frog stage

Sex of frogs dead or killed within one month after metamorphosis							Sex of matured frogs			Structure of testes				Sex of all frogs examined		
										No. of observed males	Type of testes					
♀N	♀U	♀1	♀2	♂R	♂N	♂(%)*	No. of frogs	♀	♂		1	2	3	Total	♀	♂ (%)*
11	1	0	1	0	9	(45)	59	31	28	23	23	0	0	81	43	38 (47)
42	1	0	1	0	37	(47)	42	20	22	17	17	0	0	123	63	60 (49)
54	2	0	2	0	46	(46)	14	11	3	2	2	0	0	118	67	51 (43)
65	0	0	3	0	47	(43)	5	5	0	0	0	0	0	120	70	50 (42)
0	0	0	0	0	0		8	4	4	4	2	1	1	8	4	4 (50)
97	0	0	0	0	2	(2)	0	0	0	0	0	0	0	99	97	2 (2)
1	0	0	0	0	2	(67)	8	6	2	0	0	0	0	11	7	4 (36)
0	0	0	0	0	0		4	2	2	1	0	1	0	4	2	2 (50)
12	0	0	0	0	0	(0)	38	24	14	13	8	3	2	50	36	14 (28)
1	0	0	0	0	0	(0)	0	0	0	0	0	0	0	1	1	0 (0)
81	0	3	1	1	77	(50)	51	35	16	15	11	3	1	214	116	98 (46)
5	0	0	0	0	3	(38)	5	3	2	2	1	0	1	13	8	5 (38)
28	1	0	0	0	1	(3)	25	24	1	1	0	1	0	55	53	2 (4)
2	0	0	0	0	4	(67)	18	11	7	7	4	1	2	24	13	11 (46)
56	3	2	4	0	78	(59)	18	8	10	4	2	2	0	161	67	94 (58)
0	1	0	1	0	1	(67)	0	0	0	0	0	0	0	3	1	2 (67)
3	0	0	0	0	0	(0)	12	10	2	1	0	1	0	15	13	2 (13)
0	0	0	1	0	1	(100)	0	0	0	0	0	0	0	2	0	2(100)
30	1	3	2	1	54	(66)	10	7	3	2	2	0	0	101	38	63 (62)
0	0	0	0	0	4	(100)	3	2	1	1	0	0	1	7	2	5 (71)
12	0	0	1	0	1	(14)	4	3	1	1	0	1	0	18	15	3 (17)
11	2	1	1	0	2	(24)	4	2	2	1	0	1	0	21	15	6 (29)

*……Including hermaphrodites

Continued

Series	Parents		Age at the time of landing (days)	No. of metamorphosed frogs	Body length immediately after metamorphosis (mm.)	Total
	Female no.	Male no.				
VII	(B)BB.62.5~7	(B)BB.62.2	78~101 (86.3)	170	19.5±0.04	92
		{(B)NN.61B12T2}N.7	65	1	18.0	1
		{(B)NN.61B12T2}N.8	68~78 (72.2)	37	22.3±0.06	23
		{(B)NN.61B12T2}N.9	64~67 (65.3)	3	18.5~19.5	3
VIII	(B)BB.62.5~7	(B)BB.62.2	78~99 (85.6)	141	20.4±0.03	102
		{(B)NN.61B13T3}N.2	67~81 (73.4)	52	22.5±0.04	34
		{(B)NN.61B13T3}N.3	65~66 (65.5)	2	21.5~22.0	2
IX	{(B)NN.61B12T2}N.1	(B)BB.62.2	62~80 (67.1)	180	24.0±0.03	145
X	{(B)NN.61B12T2}B.1	(N)NN.62.4	60~72 (64.0)	112	24.2±0.06	98
		(B)BB.62.2	60~81 (67.8)	53	22.0±0.12	42
		{(B)NN.61B12T2}B.1	61~68 (63.6)	26	23.4±0.10	8
		{(B)NN.61B12T2}B.2	64~77 (68.3)	36	22.4±0.13	11
XI	{(B)NN.61B12T2}B.2	(N)NN.62.4	60~69 (64.1)	85	24.5±0.11	83
		(B)BB.62.2	68~93 (76.0)	63	22.7±0.07	16
		{(B)NN.61B12T2}B.3	66~73 (70.7)	6	27.2±0.09	2
XII	(N)NN.62.6~8	(N)NN.62.4	59~62 (60.4)	175	23.3±0.03	120
		{(B)NN.61B12T2}B.1	65~67 (66.0)	2	18.0~18.5	2
		{(B)NN.61B12T2}B.2	60~66 (62.3)	33	23.1±0.08	33
XIII	(B)BB.62.5~7	(B)BB.62.2	77~101 (86.2)	186	19.0±0.08	80
		{(B)NN.61B12T2}B.2	69~83 (73.8)	63	21.2±0.09	38

spermatozoa.

Series II (Control B). Offspring of male nucleus-exchanged *nigromaculata* In the four mating groups, 52.7~78.4% of the total number of eggs developed

Sex of frogs dead or killed within one month after metamorphosis							Sex of matured frogs			Structure of testes				Sex of all frogs examined		
										No. of observed males	Type of testes					
♀N	♀U	♀1	♀2	♂R	♂N	♂(%)*	No. of frogs	♀	♂		1	2	3	Total	♀	♂ (%)*
38	6	0	0	0	48	(52)	73	36	37	34	34	0	0	165	80	85 (52)
1	0	0	0	0	0	(0)	0	0	0	0	0	0	0	1	1	0 (0)
10	12	0	0	0	1	(4)	12	10	2	2	0	1	1	35	32	3 (9)
3	0	0	0	0	0	(0)	0	0	0	0	0	0	0	3	3	0 (0)
52	0	0	0	0	50	(49)	36	20	16	11	11	0	0	138	72	66 (48)
13	8	3	3	0	7	(38)	18	14	4	3	0	2	1	52	35	17 (33)
1	0	0	0	0	1	(50)	0	0	0	0	0	0	0	2	1	1 (50)
08	18	0	1	0	18	(13)	30	23	7	6	0	3	3	175	149	26 (15)
16	19	0	0	0	63	(64)	14	7	7	5	2	2	1	112	42	70 (63)
1	9	2	2	2	26	(76)	11	3	8	5	3	1	1	53	13	40 (75)
2	0	0	0	0	6	(75)	16	6	10	9	4	2	3	24	8	16 (67)
0	3	0	1	0	7	(73)	22	8	14	12	4	5	3	33	11	22 (67)
12	9	2	0	0	60	(75)	0	0	0	0	0	0	0	83	21	62 (75)
6	1	0	0	0	9	(56)	45	21	24	21	10	6	5	61	28	33 (54)
1	0	0	0	0	1	(50)	4	3	1	1	0	1	0	6	4	2 (33)
63	0	0	0	0	57	(48)	41	21	20	17	17	0	0	161	84	77 (48)
2	0	0	0	0	0	(0)	0	0	0	0	0	0	0	2	2	0 (0)
18	0	0	0	0	15	(45)	0	0	0	0	0	0	0	33	18	15 (45)
38	0	0	0	0	42	(53)	100	48	52	47	47	0	0	180	86	94 (52)
29	9	0	0	0	0	(0)	25	16	9	7	5	1	1	63	54	9 (14)

into metamorphosed frogs. The frogs of each of these mating groups reached the time of landing 79~80 days on the average after their development. They were 20.4~25.1 mm. on the average in body length immediately after metamorphosis. Accordingly, the offspring of the male nucleus-exchanged *nigro-*

maculata were nearly the same in ages at the time of landing and in body lengths immediately after metamorphosis as the offspring of non-treated *nigromaculata* were.

Among 343 frogs which were examined in terms of sex within one month after metamorphosis there were 134 males with normal testes, 11 juvenile hermaphrodites with gonads transforming from ovaries to testes and 198 females with normal or under-developed ovaries. The males were just or nearly equal in number to the females in three of the four mating groups, when hermaphrodites were added to males. In one mating group the females were more in number to the males.

Eighty frogs of the four mating groups were reared to sexual maturity. Among these frogs there were 64 females and 16 males. The testes of 11 males were examined histologically and found to be quite normal (Type 1).

Series III. Offspring between male nucleo-cytoplasmic hybrids, (N)BB, and female *brevipoda*

From two of the four mating groups 136 metamorphosed frogs were obtained. The landing of the tadpoles of the two groups occurred at the ages of 70.7 and 73.7 days on the average, considerably earlier than that of the control *brevipoda*. The young frogs were about 20 mm. in body length immediately after metamorphosis, quite the same as those of the control *brevipoda*. Among 81 young frogs which died or were killed within one month after metamorphosis, there were 39 males with normal testes and 42 females with normal ovaries. The remaining frogs were reared and most of them attained sexual maturity.

Among 53 matured frogs of the two mating groups there were 26 males and 27 females. The testes of 23 males were examined histologically. As a result, it was found that they were not always of normal structures. Besides 13 males with Type 1 testes, there were 6 with Type 2 and 4 with Type 3 testes.

Series IV. Offspring between male nucleo-cytoplasmic hybrids, (B)NN, and female *nigromaculata*

There were 106 and 67 metamorphosed frogs in two of the four mating groups, respectively. The former were 73.8 days old at the time of landing, and 22.8 mm. in body length immediately after metamorphosis on the average, while the latter were 68.4 days old and 24.3 mm. Accordingly, the frogs of the second mating group were especially more rapid in development and growth than the control non-treated and nucleus-exchanged *nigromaculata*.

Among 91 young frogs of the first mating group, there were 44 males with normal testes and 47 females with normal ovaries, while there were 4 hermaphrodites besides 12 males with normal testes and 33 females with normal or under-developed ovaries among 49 frogs of the second mating group.

Thirty-two frogs attained sexual maturity; 11 were males and 21 were females. While four of five males had normal testes (Type 1), the remainder had those of Type 3 in inner structure.

Series V. Offspring between male nucleo-cytoplasmic hybrids, (B)NN, and female *brevipoda*

Numerous metamorphosed frogs were obtained from two of the three mating groups. The frogs of each group were 67.5 or 68.7 days old at the time of landing, and 25.1 or 26.7 mm. in body length immediately after metamorphosis, on the average. These figures show that they were most rapid in development and growth among those of the five series.

Among 93 matured frogs there were 51 males and 42 females. Of 43 males 39 had testes of Type 3 and the other four had those of Type 2.

2. Sex of the second-generation offspring of female nucleo-cytoplasmic hybrids, (B)NN

This part of researches consisted of 13 series which corresponded with those of mating experiments described above.

Series I (Control). Offspring of *Rana nigromaculata* produced by brother and sister mating

In the two mating groups, 140 and 133 metamorphosed frogs were, on the average, 61.3 and 60.5 days old at the time of landing, and 24.2 and 23.0 mm. in body length immediately after metamorphosis, respectively. Among 103 young frogs observed within one month after metamorphosis, there were 46 males with normal testes, 2 hermaphrodites with transforming gonads from ovaries to testes and 55 females with normal or under-developed ovaries.

Among 101 matured frogs there were 50 males and 51 females. All the testes of 40 males were quite normal (Type 1) in inner structure.

Series II. Second-generation offspring between male first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and female *nigromaculata*

From five of the 9 mating groups 8～120 metamorphosed frogs were obtained. The frogs of each mating group were, on the average, 55.0～82.0 days old at the time of landing, and 21.0～27.3 mm. in body length immediately after metamorphosis. As to the sex, 321 frogs of four mating groups were examined. As a result, it was found that 97 of 99 frogs of one mating group with the male, No. {(B)NN. 61B12T2}N, No .8, were females with normal ovaries and the other two were males with normal testes, while there was no great difference in number between males and females in each of the other three mating groups. In the latter groups there were 95 males with normal testes, 5 juvenile hermaphrodites and 122 females with normal or under-developed ovaries.

Among 35 matured frogs of four mating groups there were 9 males and 26 females. Of the six males whose testes were fixed and sectioned in order to observe their inner structures, four were of Type 1 and the other two were of Type 2 and Type 3.

Series III. Second-generation offspring between male first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B13T3 and female *nigromaculata*

There were 62 metamorphosed frogs in the three mating groups. The frogs of two mating groups were, on the average, 54.8 and 63.3 days old at the time of landing and 30.4 and 29.0 mm. in body length. All the 12 frogs of one mating group in which the gonads were observed were females with normal ovaries. Thirty-eight frogs of this mating group and four of another mating group were reared to their sexual maturity. There were 14 males and 24 females in the former group, while two males and two females in the latter. Of the 14 males whose testes were examined, eight were of Type 1, four of Type 2 and two of Type 3 in the inner structures of their testes.

Series IV. Second-generation offspring between a female first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and a male *nigromaculata* or brothers of the female

In the mating with a male *nigromaculata* there were 222 metamorphosed frogs, which were, on the average, 63.9 days old at the time of landing and 25.3 mm. in body length. Among 163 young frogs there were 78 males with normal or rudimentary testes, four juvenile hermaphrodites and 81 females with normal ovaries. Among 51matured frogs there were 16 males and 35 females. The testes of 15 males were examined and it was found that there were 11 with Type 1, three with Type 2 and one with Type 3 testis.

There were 92 metamorphosed frogs in the three matings between a female and three male first-generation offspring of No. (B)NN. 61B12T2. The frogs of the three matings were, on the average, 54.9, 62.1 and 63.0 days old at the time of landing and 32.2, 27.0 and 26.4 mm. in body length, respectively. Among 44 young frogs whose gonads were examined there were 8 males with normal testes and 36 females with normal or under-developed ovaries. It was remarkable that 29 of these females and only one male were produced by mating with the male, {(B)NN, No. 61B12T2}N, No. 8.

Forty-eight frogs of the three mating groups attained sexual maturity; there were 10 males and 38 females, although one male and 24 females were produced by the male, {(B)NN, No. 61B12T2}N, No. 8. The testes of 10 males were examined and it was found that there were five with Type 1, two with Type 2 and three with Type 3 testes.

Series V. Second-generation offspring between another female first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and a male *nigromaculata* or brothers of the female

In the mating with a male *nigromaculata,* there were 163 metamorphosed frogs which were, on the average, 64.1 days old at the time of landing and 23.3 mm. in body length. Among 143 young frogs there were 78 males with normal

testes, 6 juvenile hermaphrodites and 59 females with normal or under-developed ovaries. Of 18 matured frogs, 10 were males and 8 were females. Among four males whose testes were sectioned and observed, there were two with Type 1 and two with Type 2 testes.

In the three matings between a female and three male first-generation offspring of the nucleo-cytoplasmic hybrid, there were 21 metamorphosed frogs. However, only three and two frogs were produced in two mating groups. These five frogs were distinctly small in body length, that is, 18.0~21.5 mm., although they were, on the average, 65.6 and 68.0 days old at the time of landing, respectively. Two of them were males with normal testes and two others were juvenile hermaphrodites and the rest was a female with under-developed ovaries. The 16 frogs of the remaining mating group with the male, {(B)NN, Nos. 61B12T2}N, No. 8, were, on the average, 66.0 days old at the time of landing and 24.5 mm. in body length. Three of them were females with normal ovaries. Twelve of the remaining 13 frogs of the above mating attained sexual maturity. Two of them were males and 10 were females. The testes of one of these males were of Type 2.

Series VI. Second-generation offspring between a female first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B13T3 and a male *nigromaculata* or brothers of the female

There were 105 metamorphosed frogs in the mating with a male *nigromaculata*. They were, on the average, 63.0 days old at the time of landing and 24.1 mm. in body length. Among 91 young frogs there were 54 males with normal testes, a male with rudimentary testes, 5 juvenile hermaphrodites and 31 females with normal or under-developed ovaries. Ten frogs of this mating attained sexual maturity. Three of them were males and seven were females. Two of the three males had testes of Type 1.

In the three matings between a female and three male first-generation offspring of the female nucleo-cytoplasmic hybrid, there were 46 metamorphosed frogs, which were, on the average, 59.4, 71.4 and 64.0 days old at the time of landing and 26.3, 25.0 and 23.5 mm. in body length, respectively. While four frogs produced by one mating were males with normal testes, 31 frogs from the other matings consisted of three males with normal testes, three juvenile hermaphrodites and 25 females with normal or under-developed ovaries. Eleven frogs attained sexual maturity in these three matings; four were males and seven were females. Of three males whose testes were examined, two were of Type 2 and the other was of Type 3.

Series VII. Second-generation offspring between female *brevipoda* and male first-generation offspring of the nucleo-cytoplasmic hybrids, (B)NN, No. 61B12T2

From the matings of male and female *brevipoda*, 170 metamorphosed frogs were obtained as the control of this series. They were, on the average, 86.3 days old at the time of landing and 19.5 mm. in body length immediately after meta-

morphosis. Among 92 young frogs whose gonads were examined, there were 48 males with normal testes and 44 females with normal or under-developed ovaries. Of 73 matured frogs, 37 were males and 36 females. The testes of 34 males were examined and it was found that all of them were of Type 1.

In the matings with three male first-generation offspring of the female nucleo-cytoplasmic hybrid, there were 41 metamorphosed frogs. Thirty-seven of them belonged to one mating group with the male, {(B)NN, No. 61B12T2}N. No. 8, and were, on the average, 72.2 days old at the time of landing and 22.3 mm. in body length. Among 23 young frogs whose gonads were examined, there were a male with normal testes and 22 females with normal or under-developed ovaries. The four frogs of the other two mating groups were about 65 days old at the time of landing and 18~19 mm. in body length. All of them were females with normal ovaries. Among 12 matured frogs produced from the male, {(B)NN, No. 61B12T2}N. No. 8, there were two males and ten females. The testes of these two males were of Types 2 and 3.

Series VIII. Second-generation offspring between female *brevipoda* and male first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B13T3

In the mating between three female and a male *brevipoda*, there were 141 metamorphosed frogs which were, on the average, 85.6 days old at the time of landing and 20.4 mm. in body length. Among 102 young frogs, there were 50 males with normal testes and 52 females with normal ovaries. Among 36 matured frogs, there were 16 males and 20 females. Eleven males examined had testes of Type 1.

In two of the three matings with a male first-generation offspring of the nucleo-cytoplasmic hybrid, there were 52 and 2 metamorphosed frogs, respectively. The former frogs were, on the average, 73.4 days old at the time of landing and 22.5 mm. in body length. Among 34 young frogs examined there were 7 males with normal testes, 6 juvenile hermaphrodites and 21 females with normal or under-developed ovaries. The two frogs from the other mating were about 65.5 days old at the time of landing and 22 mm. in body length. They were a male and a female with normal gonads. Among 18 matured frogs obtained by one of the three matings, there were 4 males and 14 females. Two of 3 males examined had testes of Type 2 and the other was of Type 3.

Series IX. Second-generation offspring between a female first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and a male *brevipoda*

There were 180 metamorphosed frogs which were, on the average, 67.1 days old at the time of landing and 24.0 mm. in body length. Among 145 young frogs there were 18 males with normal testes, a juvenile hermaphrodite and 126 females with normal or under-developed ovaries. Thirty matured frogs consisted of 7 males and 23 females. Of 6 frogs examined, three had testes of

Type 2 and the other three had those of Type 3.

Series X. Second-generation offspring between a female first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and a male *nigromaculata*, a male *brevipoda* or brothers of the female

The 112 metamorphosed frogs obtained from the mating with a male *nigromaculata* were, on the average, 64.0 days old at the time of landing and 24.2 mm. in body length. Among 98 young frogs, there were 63 males with normal testes and 35 females with normal or under-developed ovaries. In contrast with this, there were 7 males and 7 females among 14 matured frogs. While two of five males examined had testes of Type 1, two others were of Type 2 and the remainder was of Type 3.

In the mating with a male *brevipoda* there were 53 metamorphosed frogs which were, on the average, 67.8 days old at the time of landing and 22.0 mm. in body length. Among 42 young frogs there were 26 males with normal testes, two with rudimentary testes, 4 juvenile hermaphrodites and 10 females with normal or under-developed ovaries. Among 11 matured frogs there were eight males and three females. Three of five males examined had testes of Type 1. The testes of another were of Type 2 and those of the remainder were of Type 3.

From the two matings between two male and a female first-generation offspring of the female nucleo-cytoplasmic hybrids, 62 metamorphosed frogs were obtained. They were, on the average, 63.6 and 68.3 days old at the time of landing and 23.4 and 22.4 mm. in body length. Among 19 young frogs there were 13 males with normal testes, a juvenile hermaphrodite and five females with normal or under-developed ovaries. Among 38 matured frogs there were 24 males and 14 females. The testes of 21 males were examined and it was found that eight were of Type 1, seven of Type 2 and six of Type 3.

Series XI. Second-generation offspring between another female of the first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and a male *nigromaculata*, a male *brevipoda* or a brother of the female

The 85 metamorphosed frogs obtained from the mating with a male *nigromaculata* were, on the average, 64.1 days old at the time of landing and 24.5 mm. in body length. Among 83 young frogs, there were 60 males with normal testes, 2 juvenile hermaphrodites and 21 females with normal or under-developed ovaries.

The 63 metamorphosed frogs in the mating with a male *brevipoda* were, on the average, 76.0 days old at the time of landing and 22.7 mm. in body length. Among 16 young frogs there were nine males with normal testes and seven females with normal or under-developed ovaries.

The six metamorphosed frogs obtained from a mating between a female and a male first-generation offspring of the nucleo-cytoplasmic hybrid were, on the average, 70.7 days old at the time of landing and 27.2 mm. in body length.

Two frogs examined consisted of a male with normal testes and a female with normal ovaries. The remaining four frogs attained sexual maturity; there were a male and three females. A male examined had testes of Type 2.

Series XII. Second-generation offspring between female *nigromaculata* and male first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2

The 175 metamorphosed frogs produced from the mating between three female and a male *nigromaculata* were, on the average, 60.4 days old at the time of landing and 23.3 mm. in body length. Among 120 young frogs there were 57 males with normal testes and 63 females with normal ovaries. The 41 matured frogs consisted of 20 males and 21 females. The testes of 17 males examined were all of Type 1.

From the two mating groups between female *nigromaculata* and male first-generation offspring of the nucleo-cytoplasmic hybrid, 35 metamorphosed frogs were obtained. The frogs of each mating group were, on the average, 66.0 or 62.3 days old at the time of landing and about 18 or 23 mm. in body length. Fifteen of them were males with normal testes and 20 were females with normal ovaries.

Series XIII. Second-generation offspring between female *brevipoda* and male first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2

The 186 metamorphosed frogs obtained from matings between three female and a male *brevipoda* were, on the average, 86.2 days old at the time of landing and 19.0 mm. in body length. Among 80 young frogs there were 42 males with normal testes and 38 females with normal ovaries. The 100 matured frogs consisted of 52 males and 48 females. All the 47 males examined had testes of Type 1.

From one of the three mating groups, 63 metamorphosed frogs were obtained. They were, on the average, 73.8 days old at the time of landing and 21.2 mm. in body length. All the 38 young frogs examined were females with normal or under-developed ovaries. Among 25 matured frogs there were 9 males and 16 females. While five of seven males examined had testes of Type 1, another was of Type 2 and the remaining one of Type 3.

B. Experiments performed in 1966

I. Male and female parents

In the season of the year 1966, 12 male and 10 female frogs were used as parents for producing the first-, second- and third-generation offspring of nucleo-cytoplasmic hybrids, (B)NN, and the controls.

1. Testes of male parents

Among the 12 males there were four *nigromaculata,* two *brevipoda,* a nucleus-

exchanged *nigromaculata*, a male first-generation offspring of a male nucleus-exchanged *nigromaculata*, two male nucleo-cytoplasmic hybrids, (B)NN, and two male second-generation offspring of a female nucleo-cytoplasmic hybrid, (B)NN. The former seven males were all 3 years old, while three and two of the other five were one and three years old, respectively. The body length and the size of testes of each male are presented in Table 10. All the males were quite normal in the inner structures of their testes; the seminal tubules were filled with bundles of normally shaped spermatozoa. Any remarkable difference was hardly found in these structures between the control *nigromaculata* or *brevipoda* and the other kinds of frogs.

TABLE 10

Testes of male parents used for mating experiments performed in 1966

Kind	Individual no.	Age (year)	Body length (mm.)	Size of the testes		Inner structure
				Left (mm.)	Right (mm.)	
i	(N)NN.63.1	3	50.5	3.5×2.0	3.5×1.5	Type 1
	(N)NN.63.2	3	50.0	3.0×1.5	3.0×2.0	Type 1
	(N)NN.63.3	3	57.5	4.0×2.0	4.0×2.0	Type 1
	(N)NN.63.4	3	55.5	3.5×1.5	4.0×2.0	Type 1
ii	(B)BB.63.1	3	40.0	3.0×2.5	3.0×2.5	Type 1
	(B)BB.63.2	3	42.5	3.0×2.5	3.0×2.5	Type 1
iii	(N)+NN.63N4T7	3	45.0	3.5×1.0	3.5×2.0	Type 1
	N{(N)+NN.63N2T2}	1	45.0	4.0×2.0	3.5×2.0	Type 1
iv	(B)NN.63B4T6	3	41.0	3.0×2.5	3.0×2.5	Type 1
	(B)NN.63B4T7	3	37.0	2.5×2.0	2.5×2.0	Type 1
v	{(B)NN.61B12T2}N².1	1	46.0	4.0×2.0	3.5×2.5	Type 1
	{(B)NN.61B12T2}N².2	1	45.5	3.5×2.0	3.5×2.0	Type 1

N{(N)+NN.63N2T2} ——— Offspring of a female *nigromaculata* (N)NN mated with a male nucleus-exchanaged *nigromaculata*, (N)+NN.63N2T2

{(B)NN.61B12T2}N² ——— Offspring of a female first-generation offspring, {(B)NN.61B12T2}N, mated with a control *nigromaculata* (N)NN

The four male *nigromaculata* were 50.0~57.5 mm. in body length. Their testes were 3.0~4.0 mm. in length and 1.5~2.0 mm. in width. On the other hand, the two *brevipoda* were 40.0 and 42.5 mm. in body length, and 3.0 mm.× 2.5 mm. in the size of testes.

Both the 3-year-old male nucleus-exchanged *nigromaculata* and the one-year-old male first-generation offspring of a male nucleus-exchanged *nigromaculata* were 45.0 mm. in body length. Accordingly, the former male was inferior in growth to the control *nigromaculata* of the same age. The left testis of this male was also more slender than the testes of the latter. However, the right testis was nearly the same as those in size and shape. The testes of the other male were quite similar to those of the control *nigromaculata*, in spite of the age of one year. They were 3.5 mm. and 4.0 mm. in length and 2.0 mm. in width.

The two male nucleo-cytoplasmic hybrids constructed of *nigromaculata* nuclei and *brevipoda* cytoplasm were 41 and 37 mm. in body length and 3.0 mm.× 2.5 mm. and 2.5 mm.×2.0 mm. in the size of testes, respectively.

The two one-year-old male second-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2, were about 46 mm. in body length. Their testes were 3.5 mm. and 4.0 mm. in length and 2.0 mm. and 2.5 mm. in width, being the same in size and shape as those of the one-year-old male of the first-generation offspring of a male nucleus-exchanged *nigromaculata*.

2. Eggs of female parents

Four *nigromaculata*, two *brevipoda*, two nucleo-cytoplasmic hybrids and two frogs of the first-generation offspring of a female nucleo-cytoplasmic hybrid were utilized as female parents. While the last two females were 4 years old, all the others were 3 years old. The body length, the approximate number of total eggs and the mean diameter of 50 eggs of each female are presented in Table 11.

TABLE 11

Eggs of female parents used for mating experiments performed in 1966

Kind	Individual no.	Age (year)	Body length (mm.)	No. of eggs	Mean diameter of 50 eggs (mm.)
i	(N)NN.63.1	3	54.0	915	2.0±0.01
	(N)NN.63.2	3	55.0	1437	2.1±0.01
	(N)NN.63.3	3	55.5	1520	2.0±0.01
	(N)NN.63.4	3	56.0	1064	2.1±0.01
ii	(B)BB.63.1	3	45.0	1932	1.5±0.01
	(B)BB.63.2	3	46.0	1625	1.4±0.01
iii	(B)NN.63B4T5	3	57.5	874	1.8±0.05
	(B)NN.63B4T6	3	56.5	916	1.7±0.04
iv	{(B)NN.61B12T2}N.3	4	65.0	974	2.1±0.03
	{(B)NN.61B12T2}N.4	4	56.5	1275	1.8±0.03

Each of the four *nigromaculata* was 54.0~56.0 mm. in body length and about 915~1520, 1234 on the average, in the number of total eggs. The mean diameter of 50 eggs picked out at random was 2.0~2.1 mm. In contrast with these females, each of the two *brevipoda* was 45.0 mm. and 46.0 mm. in body length and about 1625 and 1932 in the number of total eggs. The mean diameter of 50 eggs in each female was 1.4 or 1.5 mm.

The two female nucleo-cytoplasmic hybrids, Nos. (B)NN.63B4T5 and (B)NN. 63B4T6, were larger in body length and smaller in the number of total eggs than the control *nigromaculata*, when compared with the averages in the latter. The mean diameter of 50 eggs in each of the two females was somewhat smaller than those of the control *nigromaculata* for their body lengths, although clearly larger than those of the control *brevipoda*. The eggs were remarkably ununiform in size.

The two females of the first-generation offspring of a female nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2. mated with a male *nigromaculata* seemed to be nearly the same with or rather smaller than the above two female nucleo-cytoplasmic hybrids in the number of total eggs as well as the mean diameter of 50 eggs, if their larger body lengths were taken into consideration. Although they were

4 years old and 65.0 mm. and 56.5 mm. in body length, their eggs were 974 and 1275 in approximate number and 2.1 mm. and 1.8 mm. in mean diameter.

II. Mating experiments

In order to obtain the first-generation offspring, the two 3-year-old female nucleo-cytoplasmic hybrids, (B)NN, were mated with male *nigromaculata* and *brevipoda*. The second-generation offspring were obtained from the two 4-year-old females of the first-generation offspring of a nucleo-cytoplasmic hybrid by mating with male *nigromaculata* and *brevipoda*. The third-generation offspring were obtained from the two 1-year-old male second-generation offspring of a nucleo-cytoplasmic hybrid by mating with female *nigromaculata*. The four male and four female *nigromaculata* as well as the two male and two female *brevipoda* were used in the above experiments and control series. Besides them, the male 3-year-old nucleus-exchanged *nigromaculata* and the 1-year-old male first-generation offspring produced by mating of a male nucleus-exchanged and a female non-treated *nigromaculata* were used in the control series. The results of experiments were presented in Table 12.

Series I (Control A). Offspring of female and male non-treated *nigromaculata*

Four females Nos. 1～4 and four males Nos. 1～4 were mated as four control groups. These females and males were those which had developed from *nigromaculata* eggs fertilized artificially in 1963 and had been raised in our laboratory. The rate of fertilization was very high in each group: more than 97 percent of the total number of eggs cleaved normally. Nearly all the cleaved eggs developed normally, grew into tadpoles and completed their metamorphosis. However, a few eggs died of various types of abnormalities at various stages of development.

Series II (Control B). Offspring of female and male non-treated *brevipoda*

Two control groups were made by the use of two females Nos. 1～2 and two males Nos. 1～2 of the first-generation offspring which had been obtained from normal female and male *brevipoda* collected from the field in 1963. The rate of fertilization was very high in each group, although it was slightly lower than that in *nigromaculata*. All the normally cleaved eggs developed into normal metamorphosed frogs.

Series III (Control C). Offspring of male nucleus-exchanged *nigromaculata*

These males were those which had developed from enucleated *nigromaculata* eggs by transplanting blastula nuclei of the same species.

Mating 1. *Rana nigromaculata* from (N)NN, No. 63.1♀ × (N)+NN, No. 63N4T7♂

The nucleus-exchanged male, (N)+NN, No. 63N4T7 was mated with a female *nigromaculata* which had been raised since the egg stage in our laboratory. All the eggs cleaved normally. Five of them died of various types of abnor-

TABLE 12

Viability of first-, second- and third-generation offspring of

Series	Parents		No. of eggs	No. of cleaved eggs		No. of neurulae	
	Female no.	Male no.		Normal	Abnormal	Normal	Abnormal
I	(N)NN.63.1	(N)NN.63.1	45	45 (100%)	0	45 (100%)	0
	(N)NN.63.2	(N)NN.63.2	74	72 (97.3%)	1 (1.4%)	72 (97.3%)	0
	(N)NN.63.3	(N)NN.63.3	60	59 (98.3%)	0	59 (98.3%)	0
	(N)NN.63.4	(N)NN.63.4	50	50 (100%)	0	50 (100%)	0
II	(B)BB.63.1	(B)BB.63.1	50	41 (82.0%)	0	41 (82.0%)	0
	(B)BB.63.2	(B)BB.63.2	60	59 (98.3%)	0	59 (98.3%)	0
III	(N)NN.63.1	(N)+NN.63N4T7	145	145 (100%)	0	144 (99.3%)	1 (0.7%)
	(N)NN.63.2	N{(N)+NN.63N2T2}	150	141 (94.0%)	2 (1.3%)	141 (94.0%)	0
IV	(N)NN.63.1	(B)NN.63B4T6	159	146 (91.8%)	0	134 (84.3%)	5 (3.1%)
		(B)NN.63B4T7	163	156 (95.7%)	0	156 (95.7%)	0
	(B)BB.63.2	(B)NN.63B4T6	171	168 (98.2%)	4 (2.3%)	156 (91.2%)	7 (4.1%)
		(B)NN.63B4T7	180	176 (97.8%)	0	171 (95.0%)	3 (1.7%)
V	(B)NN.63B4T5	(N)NN.63.1	89	83 (93.3%)	0	80 (89.9%)	3 (3.4%)
		(B)BB.63.1	120	118 (98.3%)	2 (1.7%)	102 (85.0%)	16 (13.3%)
	(B)NN.63B4T6	(N)NN.63.2	140	140 (100%)	0	140 (100%)	0
		(B)BB.63.2	135	133 (98.5%)	0	132 (97.8%)	1 (0.7%)
VI	{(B)NN.61B12T2}N.3	(N)NN.63.1	154	151 (98.1%)	0	151 (98.1%)	0
		(B)BB.63.1	160	155 (96.9%)	2 (1.3%)	153 (95.6%)	2 (1.3%)
	{(B)NN.61B12T2}N.4	(N)NN.63.2	170	165 (97.1%)	2 (1.2%)	164 (96.5%)	1 (0.6%)
		(B)BB.63.2	161	160 (99.4%)	0	160 (99.4%)	0
VII	(N)NN.63.3	{(B)NN.61B12T2}N².1	154	135 (87.7%)	3 (1.9%)	130 (84.4%)	0
	(N)NN.63.4	{(B)NN.61B12T2}N².2	160	140 (87.5%)	3 (1.9%)	134 (83.8%)	3 (1.9%)

malities at the neurula, the hatching and the feeding tadpole stage. The others developed normally into normal tadpoles and completed their metamorphosis. Within one month after metamorphosis 21 frogs died and 109 were preserved for histological observations. The remaining ten were reared continuously until

female nucleo-cytoplasmic hybrids and the controls

No. of tail-bud embryos		No. of hatched tadpoles		No. of tadpoles at X stage		No. of metamorphosed frogs	
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
44	1	41	3	41	0	40	0
(97.8%)	(2.2%)	(91.1%)	(6.7%)	(91.1%)		(88.9%)	
70	2	66	4	66	0	66	0
(94.6%)	(2.7%)	(89.2%)	(5.4%)	(89.2%)		(89.2%)	
57	2	52	5	50	2	48	0
(95.0%)	(3.3%)	(86.7%)	(8.3%)	(83.3%)	(3.3%)	(80.0%)	
50	0	50	0	48	2	44	0
(100%)		(100%)		(96.0%)	(4.0%)	(88.0%)	
41	0	41	0	41	0	41	0
(82.0%)		(82.0%)		(82.0%)		(82.0%)	
59	0	59	0	59	0	59	0
(98.3%)		(98.3%)		(98.3%)		(98.3%)	
144	0	142	2	140	2	140	0
(99.3%)		(97.9%)	(1.4%)	(96.6%)	(1.4%)	(96.6%)	
141	0	140	1	140	0	140	0
(94.0%)		(93.3%)	(0.7%)	(93.3%)		(93.3%)	
131	3	127	4	108	19	83	0
(82.4%)	(1.9%)	(79.9%)	(2.5%)	(67.9%)	(11.9%)	(52.2%)	
156	0	156	0	126	30	121	0
(95.7%)		(95.7%)		(77.3%)	(18.4%)	(74.2%)	
125	31	106	19	99	7	99	0
(73.1%)	(18.1%)	(62.0%)	(11.1%)	(57.9%)	(4.1%)	(57.9%)	
171	0	169	2	152	17	148	0
(95.0%)		(93.9%)	(1.1%)	(84.4%)	(9.4%)	(82.2%)	
77	3	71	6	64	7	47	3
(86.5%)	(3.4%)	(79.8%)	(6.7%)	(71.9%)	(7.9%)	(52.8%)	(3.4%)
89	13	74	15	74	0	70	0
(74.2%)	(10.8%)	(61.7%)	(12.5%)	(61.7%)		(58.3%)	
137	3	135	2	116	16	104	4
(97.9%)	(2.1%)	(96.4%)	(1.4%)	(82.9%)	(11.4%)	(74.3%)	(2.9%)
130	2	127	3	120	7	112	5
(96.3%)	(1.5%)	(94.1%)	(2.2%)	(88.9%)	(5.2%)	(83.0%)	(3.7%)
149	2	145	4	140	5	136	4
(96.8%)	(1.3%)	(94.2%)	(2.6%)	(90.9%)	(3.2%)	(88.3%)	(2.6%)
153	0	151	2	150	1	145	3
(95.6%)		(94.4%)	(1.3%)	(93.8%)	(0.6%)	(90.6%)	(1.9%)
160	4	160	0	155	5	150	2
(94.1%)	(2.4%)	(94.1%)		(91.2%)	(2.9%)	(88.2%)	(1.2%)
154	6	150	4	147	3	140	3
(95.7%)	(3.7%)	(93.2%)	(2.5%)	(91.3%)	(1.9%)	(87.0%)	(1.9%)
121	9	116	5	116	0	103	13
(78.6%)	(5.8%)	(75.3%)	(3.2%)	(75.3%)		(66.9%)	(8.4%)
122	12	110	12	107	3	101	6
(76.3%)	(7.5%)	(68.8%)	(7.5%)	(66.9%)	(1.9%)	(63.1%)	(3.8%)

their sexual maturity.

Mating 2. *Rana nigromaculata* from (N)NN, No. 63.2♀ × N{(N)+NN, No. 63N2T2}♂

The first-generation offspring were obtained by mating the nucleus-exchanged

male No. 63N2T2 with a female *nigromaculata* which had been raised since the egg stage in our laboratory. The second-generation offspring were produced by mating a male first-generation offspring with a female *nigromaculata* raised in our laboratory. Out of 141 normally cleaved eggs, 140 developed normally and completed their metamorphosis. Within one month after metamorphosis 120 frogs were picked out at random and preserved. The other 20 frogs were reared continuously. After two years, 15 of them were alive and sexually matured.

Series IV. Offspring between a female *nigromaculata* or *brevipoda* and two male nucleo-cytoplasmic hybrids, (B)NN

The male nucleo-cytoplasmic hybrids were those which were obtained in the breeding season of 1963 by transplanting blastula nuclei of *nigromaculata* into enucleated *brevipoda* eggs. The female *nigromaculata* and *brevipoda* were two of the 3-year-old frogs which were used in the three control series.

Mating 1. First-generation offspring from (N)NN, No. 63.1♀ × (B)NN, No. 63B4T6♂

Out of 159 eggs, 146(91.8%) cleaved normally. At the hatching stage, there were 127 normal and 4 abnormal embryos. Although there were 108 normal tadpoles at the stage X, 83(52.2%) completed their metamorphosis.

Mating 2. First-generation offspring from (N)NN, No. 63.1♀ × (B)NN, No. 63.B4T7♂

The rate of normal cleavages was 95.7%. All the 156 normally cleaved eggs developed normally and hatched. At the stage X, 126 tadpoles were normally growing, while the other 30 were abnormal and eventually died. Five tadpoles became abnormal afterwards and died by the stage of metamorphosis, while 121 completed their metamorphosis.

Mating 3. First-generation offspring from (B)BB, No. 63.2♀ × (B)NN, No. 63B4T6♂

Out of 171 eggs, 168(98.2%) cleaved normally and 4 did abnormally. A considerable number of normally cleaved eggs became abnormal and died at the neurula, tail-bud and hatching stages; 106 embryos hatched normally. Seven tadpoles were abnormal at the stage X, while the other 99(57.9%) grew normally and completed their metamorphosis.

Mating 4. First-generation offspring from (B)BB, No. 63.2♀ × (B)NN, No. 63B4T7♂

Normally cleaved eggs were 97.8% of the total number of eggs. Seven of them became abnormal by the hatching stage, while 169 hatched normally. At the stage X, there were 152 normal and 17 abnormal tadpoles. Normal metamorphosed frogs were 148(82.2%) in number.

Series V. Offspring between two female nucleo-cytoplasmic hybrids, (B)NN, and male *nigromaculata* or *brevipoda*

The female nucleo-cytoplasmic hybrids were those which were produced in the breeding season of 1963 by transplanting blastula nuclei of *nigromaculata*

into enucleated *brevipoda* eggs. They were mated with the control male *nigromaculata* or *brevipoda*, which had been raised since the egg stage for 3 years in the laboratory.

Mating 1. First-generation offspring from (B)NN, No. 63B4T5♀ × (N)NN, No. 63.1♂

Thirty-three of 83 normally cleaved eggs became edematous and died at various stages: three at the neurula, three at the tail-bud, six at the hatching, seven at the stages V~X and 14 at the stages XIII~XX. The other 50 completed their metamorphosis, although three of them had abnormal hind legs. Within one month after metamorphosis 13 frogs died and 37 were killed and preserved.

Mating 2. First-generation offspring from (B)NN, No. 63B4T5♀ × (B)BB, No. 63.1♂

Out of 118 normally cleaved eggs, 48 became edematous and died at various stages: 16 at the neurula, 13 at the tail-bud, 15 at the hatching and four at the stage XVII. The other 70 developed normally into metamorphosed frogs. Within one month after metamorphosis 21 frogs died and 49 were preserved.

Mating 3. First-generation offspring from (B)NN, No. 63B4T6♀ × (N)NN, No. 63.2♂

All the 140 eggs cleaved normally and developed into late neurulae. However, 32 died of edema at various stages: three at the tail-bud, two at the hatching and 27 at the feeding tadpole stage. The other 108 developed normally and completed their metamorphosis. Four of these frogs had abnormal hind legs, while the remaining 104 were externally quite normal. Within one month after metamorphosis 18 frogs died and 60 were preserved. The other 30 frogs were continuously reared. While six of them died within one year, the other 24 were alive and sexually matured in two years.

Mating 4. First-generation offspring from (B)NN, No. 63B4T6♀ × (B)BB, No. 63.2♂

Out of 135 eggs, 133 cleaved normally. Sixteen of the latter died of edema or other kinds of abnormalities at various stages: one at the neurula, two at the tail-bud, three at the hatching, ten at the feeding tadpole stage, while the other 117 developed normally and completed their metamorphosis. Five of the latter had abnormal hind legs, while 112 frogs were externally quite normal. Within one month after metamorphosis 26 died and 61 were preserved. The remaining 30 frogs were continuously reared. However, two of them died within one year and another died within two years. Seventeen frogs were alive and sexually matured in two years.

Series VI. Offspring between two female first-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and male *nigromaculata* or *brevipoda*

The female nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2, was that which had been obtained in the breeding season of the year 1961 by transplanting a blastula nucleus into an enucleated *brevipoda* egg. The first-generation offspring

of this female was produced by mating with a male *nigromaculata*. Two females of the first generation were mated with male *nigromaculata* or *brevipoda* in order to produce the second-generation offspring.

Mating 1. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 3♀ × (N)NN, No. 63.1♂

While eleven of 151 normally cleaved eggs became abnormal and died at the tail-bud, the hatching and the feeding tadpole stage, all the other 140 developed and completed their metamorphosis. But four of them had abnormal hind legs. Within one month after metamorphosis 21 frogs died and 79 were killed and preserved. The remaining 40 frogs were reared continuously. After two years, 33 of the latter were alive and sexually matured. There were 10 females and 23 males among them.

Mating 2. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 3♀ × (B)BB, No. 63.1♂

Out of 155 normally cleaved eggs 148 developed normally into metamorphosed frogs. The other seven died of various types of abnormalities at various stages: two at the neurula, two at the hatching and three at the feeding tadpole stage. Three metamorphosed frogs had abnormal hind legs. Within one month after metamorphosis 16 frogs died and 102 were preserved. The remaining 30 frogs were reared continuously. After two years, there were 21 matured frogs alive. They consisted of 6 females and 15 males.

Mating 3. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 4♀ × (N)NN, No. 63.2♂

One and four of 165 normally cleaved eggs became abnormal at the neurula and the tail-bud stage, respectively. While eight tadpoles became abnormal and died, the other 152 completed their metamorphosis, although two had abnormal hind legs. Within one month after metamorphosis 24 frogs died and 98 were preserved for histological observations. The remaining 30 frogs were reared. After two years, 17 of them were alive and sexually matured. There were 5 females and 12 males.

Mating 4. Second-generation offspring from {(B)NN, No. 61B12T2}N, No. 4♀ × (B)BB, No. 63.2♂

Seventeen of 160 normally cleaved eggs died of various types of abnormalities at various stages: six at the tail-bud, four at the hatching and seven at the feeding tadpole stage. The other 143 eggs developed normally into normal tadpoles, which completed their metamorphosis. However, three frogs had abnormal hind legs. Within one month after metamorphosis 21 frogs died and 92 were preserved. The remaining 30 frogs were reared continuously. After two years, there were 22 matured frogs alive. They consisted of 9 females and 13 males.

Series VII. Offspring between two male second-generation offspring of the nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and two female *nigromaculata*

The first-generation offspring were obtained by mating the female nucleo-

cytoplasmic hybrid, (B)NN, No. 61B12T2, with a male *nigromaculata* in breeding season of the year 1962 (KAWAMURA and NISHIOKA, 1963a). The second-generation offspring were produced by mating a female first-generation offspring with a male *nigromaculata* in 1965. Two males of the second generation were mated with female *nigromaculata* in order to produce third-generation offspring.

Mating 1. Third-generation offspring from (N)NN, No. 63.3♀ × {(B)NN, No. 61B12T2}N^2, No. 1♂

While 19 of 135 normally cleaved eggs died of various kinds of abnormalities at the neurula, the tail-bud and the hatching stage, the other 116 developed normally into tadpoles which completed their metamorphosis. However, 13 frogs had abnormal hind legs. Within one month after metamorphosis 19 frogs died and 57 were preserved. The remaining 40 frogs were continuously reared. After two years, there were 32 matured frogs alive.

Mating 2. Third-generation offspring from (N)NN, No. 63.4♀ × {(B)NN, No. 61B12T2}N^2, No. 2♂

Although there were 140 normally cleaved eggs, 33 died of various kinds of abnormalities at various stages: six by the neurula, 12 at the tail-bud, 12 at the hatching and three at the feeding tadpole stage. The other 107 developed into normal tadpoles which completed their metamorphosis. Six frogs had abnormal hind legs. Within one month after metamorphosis 12 frogs died and 55 were preserved. The remaining 40 frogs were continuously reared. After two years, 34 of them were alive and sexually matured.

III. Sex and gonads

The sex and the gonads of the first-, second- and third-generation offspring of nucleo-cytoplasmic hybrids, (B)NN, were observed at the stage within two weeks after metamorphosis, together with those of the control frogs. The results are presented in Table 13.

Series I (Control A). Offspring of non-treated *nigromaculata*

In the four mating groups, a total of 198 frogs completed their metamorphosis. In each of these mating groups, the tadpoles made their landing at the age of 64.5～77.0 days on the average. The frogs were 20.5～23.0 mm. in body length immediately after metamorphosis.

Among 118 young frogs observed at the stage within two weeks after metamorphosis, there were 59 females with normal ovaries, a juvenile hermaphrodite and 58 males with normal testes. If the hermaphrodite was counted as a male, the rate of males was 50.0 percent. Of the frogs left alive, 74 attained sexual maturity. There were 38 females and 36 males among these matured frogs. When these figures were added to those of young frogs, there were 97 females and 95(49.5%) males among 192 frogs obtained by the four control matings.

Series II (Control B). Offspring of non-treated *brevipoda*

There were 100 metamorphosed frogs in the two mating groups. They were

TABLE 13

Sex of first-, second- and third-generation offspring of female

Series	Parents		Age at the time of landing (days)	No. of metamorphosed frogs	Body length immediately after metamorphosis (mm.)
	Female no.	Male no.			
I	(N)NN.63.1	(N)NN.63.1	69～77 (73.3)	40	22.0～23.0
	(N)NN.63.2	(N)NN.63.2	61～68 (64.5)	66	21.0～23.0
	(N)NN.63.3	(N)NN.63.3	71～84 (77.0)	48	20.5～23.0
	(N)NN.63.4	(N)NN.63.4	67～71 (67.4)	44	22.0～22.5
II	(B)BB.63.1	(B)BB.63.1	74～82 (78.0)	41	16.0～21.0
	(B)BB.63.2	(B)BB.63.2	79～91 (84.0)	59	17.0～20.0
III	(N)NN.63.1	(N)＋NN.63N4T7	67～75 (70.5)	140	20.0～23.0
	(N)NN.63.2	N{(N)＋NN.63N2T2}	65～73 (67.4)	140	21.5～23.0
IV	(N)NN.63.1	(B)NN.63B4T6	66～75 (70.4)	83	20.0～21. 0
		(B)NN.63B4T7	65～76 (70.2)	121	17.0～21.0
	(B)BB.63.2	(B)NN.63B4T6	67～78 (71.4)	99	17.5～20.5
		(B)NN.63B4T7	67～76 (71.3)	148	17.0～22.0
V	(B)NN.63B4T5	(N)NN.63.1	70～79 (75.0)	50	18.5～23.5
		(B)BB.63.1	72～84 (76.5)	70	17.0～20.0
	(B)NN.63B4T6	(N)NN.63.2	70～79 (75.3)	108	19.0～23.0
		(B)BB.63.2	74～81 (77.0)	117	17.0～21.5
VI	{(B)NN.61B12T2}N. 3	(N)NN.63.1	60～77 (64.3)	140	20.0～22.5
		(B)BB.63.1	77～82 (79.5)	148	18.5～20.5
	{(B)NN.61B12T2}N. 4	(N)NN.63.2	61～81 (67.0)	152	20.0～23.5
		(B)BB.63.2	70～85 (78.0)	143	17.5～22.0
VII	(N)NN.63No. 3	{(B)NN.61B12T2}N^2.1	66～74 (69.8)	116	20.5～23.0
	(N)NN.63No. 4	{(B)NN.61B12T2}N^2.2	67～74 (69.3)	107	22.0～23.0

16.0～21.0 mm. in body length immediately after metamorphosis. In each mating group, the frogs were 78.0 and 84.0 days old at the time of landing.

Among 50 frogs observed at the stage within two weeks after metamorphosis,

nucleo-cytoplasmic hybrids, (B)NN, at the juvenile or matured frog stage

Sex of frogs dead or killed within two weeks after metamorphosis								Sex of matured frogs			Sex of all frogs examined		
Total	♀N	♀U	♂1	♂2	♂R	♂N	♂(%)*	No. of frogs	♀	♂	Total	♀	♂(%)*
20	11	0	0	0	0	9	(45)	18	9	9	38	20	18 (47)
46	22	0	0	0	0	24	(52)	19	10	9	65	32	33 (51)
28	14	0	0	0	0	14	(50)	17	8	9	45	22	23 (51)
24	12	0	0	1	0	11	(50)	20	11	9	44	23	21 (48)
21	10	0	0	0	0	11	(52)	16	8	8	37	18	19 (51)
29	13	0	0	0	0	16	(55)	27	14	13	56	27	29 (52)
130	63	0	0	0	0	67	(52)	10	5	5	140	68	72 (51)
120	64	0	0	0	0	56	(47)	15	10	5	135	74	61 (45)
81	30	3	2	6	0	40	(59)				81	33	48 (59)
117	49	10	7	5	0	46	(50)				117	59	58 (50)
99	46	0	1	2	0	50	(53)				99	46	53 (53)
137	47	3	14	2	0	71	(64)				137	50	87 (64)
50	23	0	0	0	0	27	(54)				50	23	27 (54)
70	31	1	0	1	0	37	(54)				70	32	38 (54)
78	39	0	0	2	0	37	(50)	24	13	11	102	52	50 (49)
87	40	1	0	0	0	46	(53)	27	12	15	114	53	61 (54)
100	32	7	4	7	1	49	(61)	33	10	23	133	49	84 (63)
118	39	3	12	16	0	48	(64)	21	6	15	139	48	91 (65)
122	23	13	11	15	2	58	(70)	17	5	12	139	41	98 (71)
113	12	21	16	4	0	60	(71)	22	9	13	135	42	93 (69)
76	3	22	2	0	0	49	(67)	32	15	17	108	40	68 (63)
67	22	0	16	3	1	25	(67)	34	19	15	101	41	60 (59)

*··· Including hermaphrodites

there were 23 females with normal ovaries and 27(54.0%) males with normal testes. There was neither juvenile hermaphrodite nor female with under-devel-

oped ovaries. The remaining frogs were continuously reared and 43 of them attained their sexual maturity. Among these matured frogs produced by the two control matings, there were 22 females and 21(48.8%) males.

Series III (Control C). Offspring of male nucleus-exchanged *nigromaculata*

Mating 1. *Rana nigromaculata* from (N)NN, No. 63.1♀ × (N)+NN, No. 63N4T7♂

The first-generation offspring of a male nucleus-exchanged *nigromaculata* mated with a female non-treated *nigromaculata* were quite normal in development and growth. About 97% of the total number of eggs grew into metamorphosed frogs, which were 70.5 days old on the average at the time of landing and 20~23 mm. in body length immediately after metamorphosis. Among 130 young frogs there were 67(51.5%) males with normal testes and 63 females with normal ovaries. At the stage of sexual maturity, there were five females and five males among 10 frogs.

Mating 2. *Rana nigromaculata* from (N)NN, No. 63.2♀ × N{(N)+NN, No. 63N2T2}♂

The second-generation offspring of a male nucleus-exchanged *nigromaculata* mated repeatedly with female *nigromaculata* were nearly the same in development and growth as the first-generation offspring stated above. Nearly all the normally cleaved eggs became metamorphosed frogs, which were 67.4 days old on the average at the time of landing and 21.5~23 mm. in body length immediately after metamorphosis. Among 120 young frogs examined within two weeks after metamorphosis there were 56(46.7%) males with normal testes and 64 females with normal ovaries. Among 15 sexually matured frogs, there were 10 females and five males.

Series IV. The first-generation offspring between male nucleo-cytoplasmic hybrid, (B)NN, and a female *nigromaculata* or *brevipoda*

As stated above, 83 and 121 metamorphosed frogs were obtained from two male nucleo-cytoplasmic hybrids by mating with a female *nigromaculata*, while 99 and 148 metamorphosed frogs were from the same males by mating with a female *brevipoda*, respectively. The former frogs were 65~76 days old at the time of landing and 17.0~21.0 mm. in body length immediately after metamorphosis, while the latter were 67~78 days old and 17.0~22.0 mm. in body length.

Among 434 young frogs dead or preserved within two weeks after metamorphosis, there were 172 females with normal ovaries, 16 females with under-developed ovaries, 39 juvenile hermaphrodites and 207 males with normal testes. If the hermaphrodites are counted as males, the males are 56.7 percent of total frogs.

Series V. The first-generation offspring between a female nucleo-cytoplasmic hybrids, (B)NN and male *nigromaculata* or *brevipoda*

In two matings between two female nucleo-cytoplasmic hybrids and two male *nigromaculata*, 50 and 108 tadpoles completed their metamorphosis, respectively, while in the other two between these females and two male *brevipoda*, 70 and 117

metamorphosed frogs were obtained, respectively. The former frogs were 70~79 days old at the time of landing and 18.5~23.5 mm. in body length immediately after metamorphosis, while the latter were 72~84 days old and 17.0~21.5 mm.

Among 128 young frogs of the first and third matings, (B)NN × (N)NN, there were 62 females with normal ovaries, two juvenile hermaphrodites and 64 males with normal testes, while there were 71 females with normal ovaries, two females with under-developed ovaries, a juvenile hermaphrodite and 83 males with normal testes among 157 frogs of the second and fourth matings, (B)NN × (B)BB. If the juvenile hermaphrodites are counted as males, the males in the two groups of matings are 51.6 and 53.5 percent, respectively.

Among 51 frogs which were obtained by the third and fourth matings and attained their sexual maturity, there were 25 females and 26 males. When these figures were added to those of young frogs obtained by the four experimental matings, there were 160 females and 176(52.4%) males among 336 frogs.

Series VI. Second-generation offspring between female first-generation offspring of a nucleo-cytoplasmic hybrid, (B)NN, and male *nigromaculata* or *brevipoda*

In the first and third matings between two female first-generation offspring of a nucleo-cytoplasmic hybrid and two male *nigromaculata*, 292 tadpoles completed their metamorphosis. These frogs were 60~81 days old at the time of landing and 20.0~23.5 mm. in body length immediately after metamorphosis. In the other two matings between the same females and two male *brevipoda*, there were 291 metamorphosed frogs, which were 70~85 days old and 17.5~22.0 mm.

Among 222 young frogs examined in the first and third matings, there were 55 females with normal ovaries, 20 females with under-developed ovaries, 37 juvenile hermaphrodites, 3 males with rudimentary testes and 107 males with normal testes. If the juvenile hermaphrodites are counted as males, the males are 147 in number, being 66.2 percent. At the stage of sexual maturity, there were 15 females and 35 males among 50 frogs.

Among 231 young frogs of the second and fourth matings, on the other hand, there were 51 females with normal ovaries, 24 females with under-developed ovaries, 48 juvenile hermaphrodites and 108 males with normal testes. If the juvenile hermaphrodites are counted as males, as in the above matings, the males are 156(67.5%) in number. At the stage of sexual maturity, there were 15 females and 28 males among 43 frogs.

Series VII. Third-generation offspring between male second-generation offspring of a nucleo-cytoplasmic hybrid, (B)NN, and female *nigromaculata*

In the two matings, 223 tadpoles completed their metamorphosis. These frogs were 66~74 days old at the time of landing and 20.5~23.0 mm. in body length immediately after metamorphosis. Among 143 young frogs examined within two weeks after metamorphosis, there were 25 females with normal ovaries,

22 females with under-developed ovaries, 21 juvenile hermaphrodites, a male with rudimentary testes and 74 males with normal testes. If the juvenile hermaphrodites are counted as males, the latter are 96(67.1%) in number.

At the age of two years, 66 frogs attained their sexual maturity. Among these frogs, there were 34 females and 32 males. When these figures were added to those of young frogs, there were 81 females and 128(61.2%) males.

IV. Chromosomal aberrations

The chromosomes of the first-, second- and third-generation offspring of nucleo-

TABLE 14

Number of tadpoles with normal or abnormal mitoses in the offspring of female nucleo-cytoplasmic hybrids and the controls

Series	Mating group	No. of tadpoles	With normal cells only	With abnormal cells only	With normal and abnormal cells
I	(N)NN.63.1	25	25(100%)	0	0
	(N)NN.63.2	25	25(100%)	0	0
	(N)NN.63.3	25	18(72.0%)	2(8.0%)	5(20.0%)
	(N)NN.63.4	25	22(88.0%)	0	3(12.0%)
		100	90(90.0%)	2(2.0%)	8(8.0%)
II	(B)BB.63.1	20	20(100%)	0	0
	(B)BB.63.2	20	17(85.0%)	0	3(15.0%)
		40	37(92.5%)	0	3(7.5%)
III	N{(N)+NN.63N4T7}	40	36(90.0%)	0	4(10.0%)
	N^2{(N)+NN.63N2T2}	40	36(90.0%)	0	4(10.0%)
IV	{(B)NN.63B4T5}N	40	17(42.5%)	15(37.5%)	8(20.0%)
	{(B)NN.63B4T6}N	40	18(45.0%)	15(37.5%)	7(17.5%)
V	{(B)NN.61B12T2}N^2.3	40	11(27.5%)	24(60.0%)	5(12.5%)
	{(B)NN.61B12T2}N^2.4	40	16(40.0%)	21(52.5%)	3(7.5%)
VI	N[{(B)NN.61B12T2}N^2].1	40	19(47.5%)	18(45.0%)	3(7.5%)
	N[{(B)NN.61B12T2}N^2].2	45	18(40.0%)	17(37.8%)	10(22.2%)

TABLE 15

Number of mitoses with various kinds of chromosomal aberrations in

Series	Mating group	No. of mitoses	2n−1	2n normal
I	(N)NN.63.1	83	0	83(100%)
	(N)NN.63.2	75	0	75(100%)
	(N)NN.63.3	127	0	106(83.5%)
	(N)NN.63.4	74	0	70(94.6%)
		359	0	334(93.0%)
II	(B)BB.63.1	58	0	58(100%)
	(B)BB.63.2	70	0	63(90.0%)
		128	0	121(94.5%)
III	N{(N)+NN.63N4T7}	138	0	130(94.2%)
	N^2{(N)+NN.63N2T2}	136	0	128(94.1%)
IV	{(B)NN.63B4T5}N	234	15(6.4%)	136(58.1%)
	{(B)NN.63B4T6}N	224	9(4.0%)	124(55.4%)
V	{(B)NN.61B12T2}N^2.3	169	18(10.7%)	46(27.2%)
	{(B)NN.61B12T2}N^2.4	140	7(5.0%)	58(41.4%)
VI	N[{(B)NN.61B12T2}N^2].1	183	11(6.0%)	92(50.3%)
	N[{(B)NN.61B12T2}N^2].2	204	9(4.4%)	93(45.6%)

cytoplasmic hybrids, (B)NN, were observed in the tail-tips taken out of feeding tadpoles by the squash method. All these tadpoles were about 40 days old. As already stated, 40 or exceptionally 45 tadpoles with nearly normal appearance were picked out at random from each of two mating groups of each generation. Besides them, offspring of male and female non-treated *nigromaculata* or *brevipoda* and first- and second-generation offspring of a male nucleus-exchanged *nigromaculata* were examined as the controls. Twenty, 25 or 40 tadpoles were used for chromosomal observation in each mating group.

The numbers of tadpoles with normal or abnormal mitoses in each mating of each series are presented in Table 14. On the other hand, the numbers of mitoses with various kinds of chromosomal aberrations found in the tadpoles of each mating group are presented in Table 15.

Series I (Control A). Offspring of non-treated *nigromaculata*

The tadpoles used for the chromosomal analysis were 30~40 mm. in total length and quite normal in external characters. Twenty-five tadpoles were examined in each of four mating groups as the control *nigromaculata* (Table 14).

There were no chromosomal aberrations in the 50 tadpoles of two of the four mating groups. These tadpoles had normal diploid mitoses only (Fig. 4). Of the 25 tadpoles of another mating, 22 also had no other than normal diploid mitoses, while the other three had some abnormal mitoses, containing one to three aberrant chromosomes caused by deletion, translocation or the like, besides normal mitoses.

In the third mating group, 18 of the 25 tadpoles had no other than normal diploid mitoses, while two others had triploid mitoses only. Among the mitoses of another tadpole there were one or two abnormal mitoses, containing a ring derived

the offspring of female nucleo-cytoplasmic hybrids and the controls

2n abnormal	2n+1	3n±α (α=0~1)	Ring chromosome	Dicentric chromosome	Minute chromosome
0	0	0	0	0	0
0	0	0	0	0	0
14(11.0%)	0	7(5.5%)	1	0	1
4(5.4%)	0	0	0	0	0
18(5.0%)	0	7(1.9%)	1	0	1
0	0	0	0	0	0
7(10.0%)	0	0	0	0	0
7(5.5%)	0	0	0	0	0
8(5.8%)	0	0	0	0	0
8(5.9%)	0	0	0	0	0
48(20.5%)	35(15.0%)	0	11	8	27
70(31.3%)	21(9.4%)	0	5	16	44
84(49.7%)	21(12.4%)	0	6	15	16
48(34.3%)	21(15.0%)	6(4.3%)	10	6	7
41(22.4%)	39(21.3%)	0	1	18	50
69(33.8%)	33(16.2%)	0	3	12	25

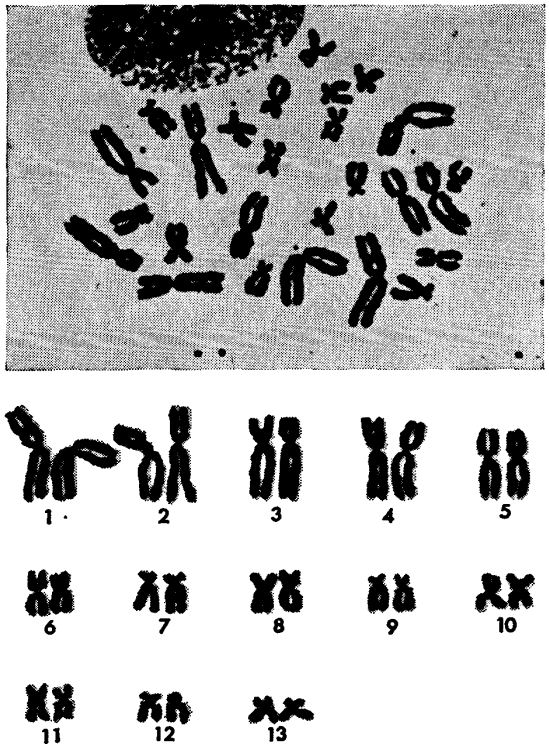

Fig. 4. Normal diploid metaphase and the karyotype of an epidermal cell of a control *Rana nigromaculata* tadpole. 2n=26. ×1500

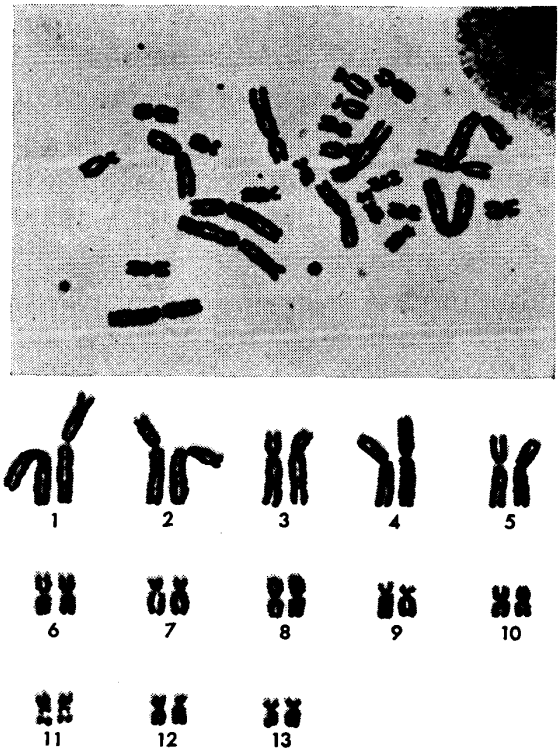

Fig. 5. Normal diploid metaphase and the karyotype of an epidermal cell of a control *Rana brevipoda* tadpole. 2n=26. ×1500

from one of the largest chromosomes (No. 1) and a minute chromosome. The other mitoses of this tadpole were all normal. In the remaining four tadpoles there were a few abnormal mitoses containing one to three aberrant chromosomes, besides normal mitoses.

Series II (Control B). Offspring of non-treated *Rana brevipoda*

The chromosomal analysis was made on 20 tadpoles of each of two mating groups (Table 14). These tadpoles were about 40 days old, 25~35 mm. in total length and externally quite normal.

The 20 tadpoles of one mating group had no other than normal diploid mitoses. Seventeen of the 20 tadpoles of another were also quite normal in having diploid mitoses only. However, in the other 3 tadpoles a few mitoses containing one to three aberrant chromosomes caused by partial deletion, translocation or the like, were intermingled with normal diploid mitoses (Fig. 5).

Series III (Control C). Offspring of male nucleus-exchanged *nigromaculata*, (N)+NN

The chromosomal analysis was made on 40 tadpoles of each mating group. These tadpoles were 40~50 mm. in total length and quite normal in appearance.

Mating 1. *Rana nigromaculata*, N{(N)+NN, No. 63N4T7}

Thirty-six of the 40 tadpoles examined had no other than normal diploid mitoses (Table 14). In the other four tadpoles there were some abnormal mitoses, besides normal diploid ones. These abnormal mitoses had one or two large, aberrant chromosomes, although their chromosomes were 26 in number.

Mating 2. *Rana nigromaculata*, $N^2\{(N)+NN, No.63N2T2\}$

While 36 of the 40 tadpoles of this second-generation offspring were quite normal diploid in karyotype, the other four were mosaics, having both normal and abnormal mitoses (Table 14). In the abnormal mitoses, there were one or two aberrant chromosomes, although the chromosomes of each mitosis were 26 in number.

Series IV. First-generation offspring of female nucleo-cytoplasmic hybrids

Mating 1. First-generation offspring, {(B)NN, No.63B4T5}N

The chromosomal analysis was made on 40 tadpoles, which were 40~50 mm. in total length and externally quite normal. Of these tadpoles, 17 had no other than normal diploid mitoses (Table 14). In 15 other tadpoles no normal diploid mitoses were found. Eight of them had one to three aberrant chromosomes in each mitosis, although there were always 26 chromosomes. In each of two of these tadpoles, all the mitoses had the same karyotype. The aberrant chromosomes were considered to be due to deletion or translocation. The other seven of the 15 tadpoles were 27 in chromosome number. In four of these seven, No. 12 chromosomes existed in triplicate (Fig. 6), in two others one of No. 12 chromosomes was a minute, owing to partial deletion of the long arm and No. 4 chromosomes existed in triplicate. In the third single tadpole, one of No. 10 chromosomes was converted into a minute, owing to partial deletion of the long arm and, moreover, No. 5 chromosome was represented in triplicate.

The remaining eight of the 40 tadpoles of the first-generation offspring had both normal diploid and various kinds of abnormal mitoses. In two tadpoles one of No. 1 chromosomes was a ring, in two others there were one or two dicentric chromosomes, and in additional two, one of No. 10 or No. 12 chromosomes was a minute, owing to partial deletion. The abnormal mitoses in one of the remaining two tadpoles were 25 in chromosome number: No. 13 existed singly. In contrast with this tadpole, the other was 27 in chromosome number, that is, No. 12 was in triplicate.

In the 40 tadpoles, a total of 234 mitoses were observed. Although 136(58.1%) of these mitoses were quite normal, the others were abnormal; in 48 diploid mitoses, one or more chromosomes were abnormal in shape. In 15 mitoses there were 25 chromosomes and in the remaining 35 there were 27 chromosomes. In the total of 234 mitoses there were 11 ring, 8 dicentric and 27 minute chromosomes.

Mating 2. First-generation offspring, {(B)NN, No.63B4T6}N

The chromosomal analysis was made on 40 tadpoles which were 40~50 mm. in total length and appeared quite normal in external characters. In 18 of these tadpoles, there were only normal diploid mitoses (Table 14), while no normal

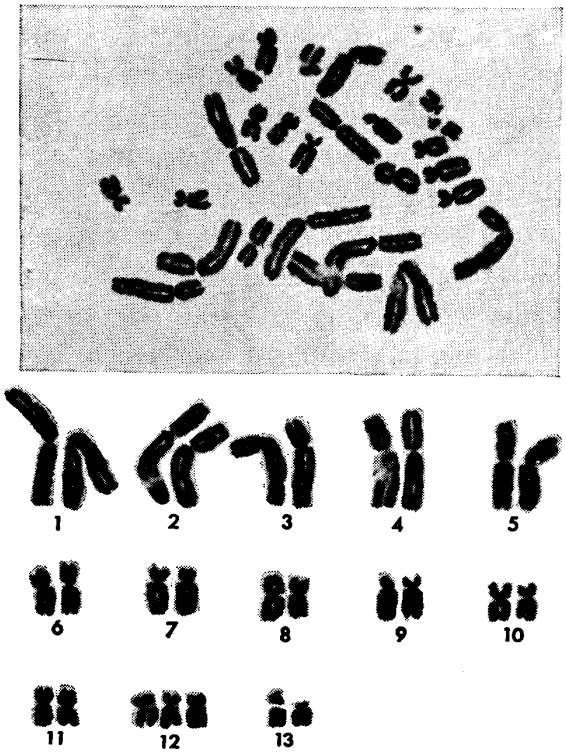

Fig. 6. Chromosomal aberration in a normally shaped tadpole, 40 days old, among the first-generation offspring, {(B)NN, No. 63B4T5} N. No. 12 chromosomes exist in triplicate.
×1500

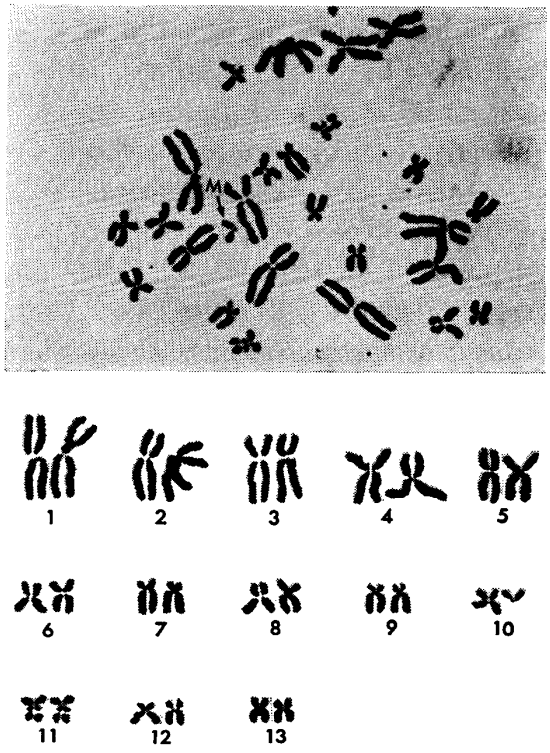

Fig. 7. Chromosomal aberration in a normally shaped tadpole, 40 days old, among the first-generation offspring, {(B)NN, No. 63B4T6} N. A minute chromosome (M) produced by deletion of the long arm of one of No. 10 chromosomes is indicated by an arrow in the metaphase plate.
×1500

diploid mitoses were found in 15 other tadpoles. Eleven of the latter had mitoses containing one to three aberrant chromosomes caused probably by partial deletion or translocation, although these tadpoles were all 26 in chromosome number (Fig. 7). In each of two of the 11 tadpoles, all the mitoses had the same karyotype. The chromosomes of the other four of the 15 tadpoles were 27 in number: the triplicate existed at No. 11 in two, at No. 12 in another and at No. 13 in the third tadpole.

The other seven of the 40 tadpoles examined had both normal diploid and abnormal mitoses. The abnormal mitoses of one tadpole were 27 in chromosome number and had a ring and a minute chromosome. Those of two other tadpoles had two dicentric chromosomes. Additional two tadpoles were 25 in chromosome number and No. 10 existed singly. In the remaining two tadpoles, the abnormal mitoses were 27 in chromosome number and No. 10 and No. 13 existed in triplicate, respectively. A total of 224 mitoses were observed in the 40 tadpoles. Among them, there were 124(55.4%) with normal diploid, 9 with 25 and 21 with 27 chromosomes. In each of the remaining 70 mitoses, more than one of the diploid chromosomes were abnormal in shape. In the total of 224 mitoses, 5 ring, 16 dicentric and 44 minute chromosomes were found.

Series V. Second-generation offspring of a female nucleo-cytoplasmic hybrid.

Mating 1. Second-generation offspring, {(B)NN, No. 61B12T2}N^2, No.3

The chromosomal analysis was made on 40 tadpoles which were 35~45 mm. in total length and externally quite normal. Eleven of them had no other than normal diploid mitoses, while 24 tadpoles had abnormal mitoses only (Tables 14 and 15). Fifteen of the latter were 26 in chromosome number and had one to three aberrant chromosomes caused probably by partial deletion or translocation (Figs. 8 and 9), although in 2 tadpoles all the mitoses of each one had the same

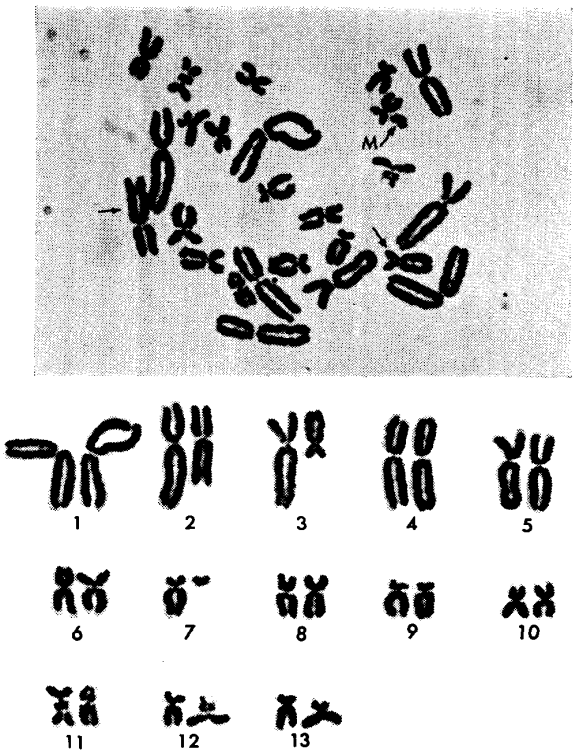

Fig. 8. Chromosomal aberration in a normally shaped tadpole, 40 days old, among the second-generation offspring, {(B)NN, No. 61B-12T2}N^2, No. 3. Deletions in Nos. 2, 3 and 7 chromosomes are indicated by arrows in the metaphase plate. M, a minute chromosome produced by deletion of the long arm of one of No. 7 chromosomes. ×1500

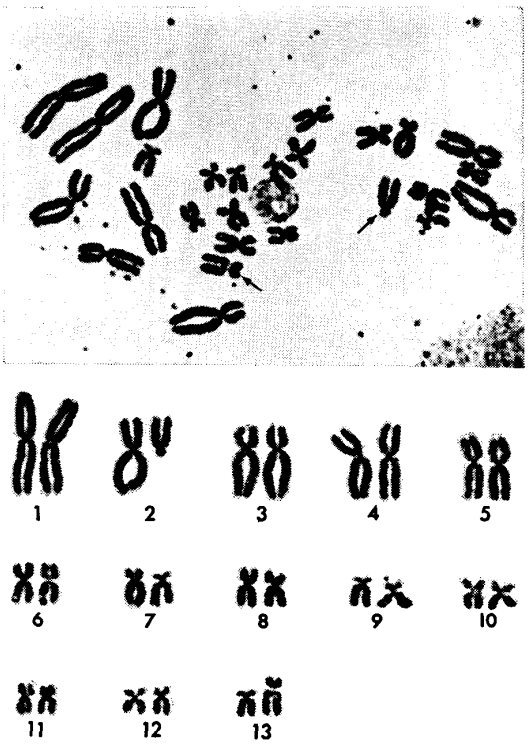

Fig. 9. Chromosomal aberration in a normally shaped tadpole, 40 days old, among the second-generation offspring, {(B)NN, No. 61B-12T2} N^2, No. 3. A deletion in one of No. 2 chromosomes and a translocation in one of No. 13 chromosomes are indicated by arrows in the metaphase plate. ×1500

karyotype. Five other tadpoles were 27 in chromosome number: the triplicate existed at No. 12 chromosome in two and at No. 13 in two tadpoles, while a ring and a fragment existed at No. 1 in the remaining tadpole (Fig. 10). The other four tadpoles were 25 in chromosome number: No. 10 in two of them and No. 13 in one existed singly, while the remaining one had a dicentric chromosome.

The other five of the 40 tadpoles examined had both normal diploid and abnormal mitoses. These abnormal mitoses were 26 in chromosome number and had a ring or a dicentric chromosome.

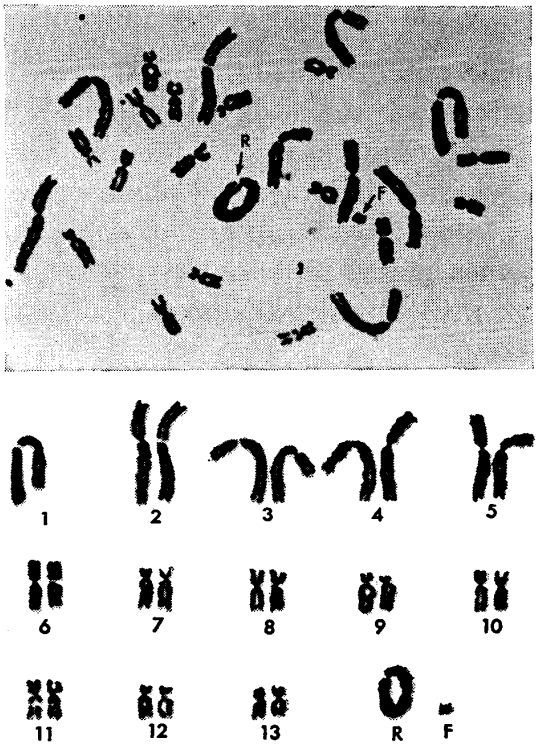

Fig. 10. Chromosomal aberration in a normally shaped tadpole, 40 days old, among the second-generation offspring, {(B)NN, No. 61B12T2}N^2, No. 3. A ring (R) and a fragment (F) existing in place of one of No. 1 chromosomes are indicated by arrows in the metaphase plate. ×1500

In the 40 tadpoles there were a total of 169 mitoses, of which 46 (27.2%) had normal diploid chromosomes. In 84(49.7%) mitoses there were more than one aberrant chromosomes, although each of the mitoses had 26 chromosomes. In 18 mitoses, there were 25 chromosomes, while there were 27 in the remaining 21 mitoses. In the total of 169 mitoses, 6 ring, 15 dicentric and 16 minute chromosomes were found.

Mating 2. Second-generation offspring, {(B)NN, No. 61B12T2}N^2, No. 4

Forty tadpoles which were 35~45 mm. in total length and externally quite normal were analysed in terms of chromosomes. While 16 tadpoles had no other mitoses than normal diploid, 21 others had abnormal mitoses only (Tables 14 and 15). Thirteen of the latter tadpoles were 26 in chromosome number and contained one to three aberrant chromosomes caused probably by partial deletion or translocation (Fig. 11), although in each of three tadpoles all the mitoses were of the same karyotype. Five other tadpoles were 27 in chromosome number: the triplicate existed at No. 8 in one, at No. 10 in one, at No. 12 in one and at No. 13 in two tadpoles. Another tadpole was 25 in chromosome number: No. 10 was single. One of the remaining two tadpoles was hypotriploid, No. 13 exisitng in a usual duplicate condition, instead of triplicate. The other tadpole was hypertriploid, No. 13 existing in quadruple (Fig. 12).

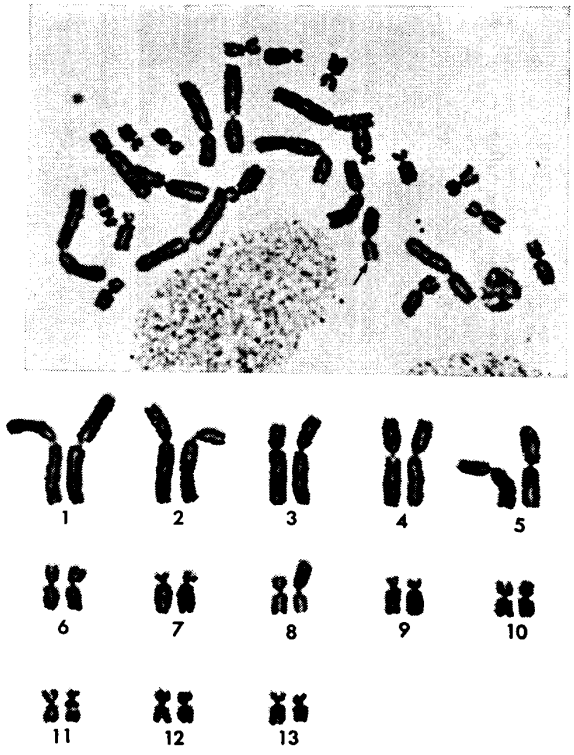

Fig. 11. Chromosomal aberration in a normally shaped tadpole, 40 days old, among the second-generation offspring, {(B)NN, No. 61B12T2} N^2, No. 4. A translocation in one of No. 8 chromosomes is indicated by an arrow in the metaphase plate. ×1500

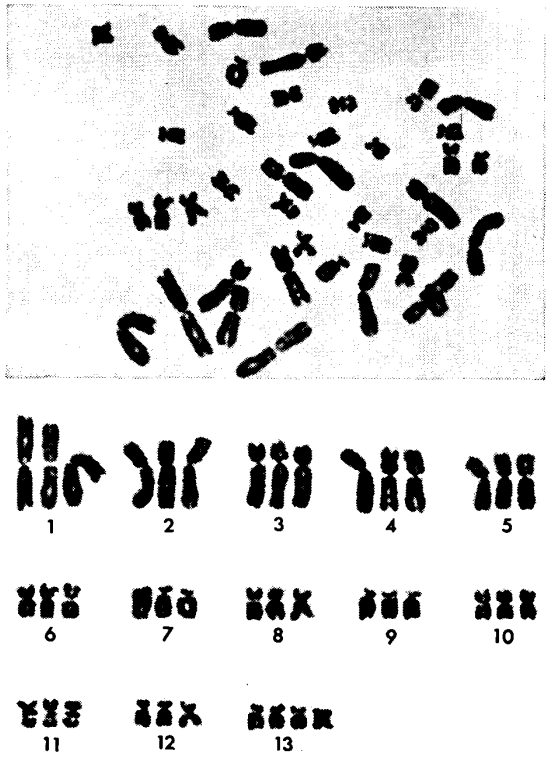

Fig. 12. Chromosomal aberration in a normally shaped tadpole, 40 days old, among the second-generation offspring, {(B)NN, No. 61B12T2} N^2, No. 4. Hypertriploid with No. 13 chromosomes existing in quadruple. ×1500

The remaining three of the forty tadpoles examined had both normal and abnormal mitoses. In two of them the abnormal mitoses contained 25 chromosomes: No. 12 existed singly. Those of the other tadpole contained an aberrant chromosome, which had been derived from one of No. 3 chromosomes by partial deletion, although there were 26 chromosomes in each mitosis.

The chromosomes of the 40 tadpoles were observed in a total of 140 mitoses. While 58(41.4%) mitoses had 26 normal chromosomes, each of 48 others contained more than one aberrant chromosomes, although there were 26 chromosomes. Besides these mitoses with 26 chromosomes, there were 7 mitoses with 25, 21 with 27 and 6 with nearly triploid chromosomes. In the total of 140 mitoses, 10 ring, 6 dicentric and 7 minute chromosomes were found.

Series VI. Third-generation offspring of a female nucleo-cytoplasmic hybrid

Mating 1. Third-generation offspring, N[{(B)NN, No. 61B12T2}N^2, No. 1]

The chromosomal analysis was made on 40 tadpoles which were 35～50 mm. in total length and quite normal in external characters. Nineteen tadpoles had no other than normal diploid mitoses, while 18 others had abnormal mitoses only (Table 14, 15). Seven of the latter were 26 in chromosome number and

contained one to three aberrant chromosomes caused probably by partial deletion or translocation, although in each of two tadpoles all the mitoses had the same karyotype. Nine tadpoles were 27 in chromosome number: No. 10 in three, No. 11 in one, No. 12 in two, No. 13 in two and No. 4 in one tadpole existed in triplicate, respectively. In the other tadpole, moreover, there was a minute chromosome, which was derived from one of No. 10 chromosomes by partial deletion. The remaining two of the 18 tadpoles were 25 in chromosome number: No. 10 in one tadpole and No. 12 in the other existed in monosomic condition, respectively.

Of the other three of the 40 tadpoles of this mating, two had both normal diploid and abnormal mitoses. In the abnormal mitoses, there were two dicentric chromosomes. The other tadpole had abnormal mitoses in which one of No. 1 chromosomes was converted into a ring, besides normal diploid mitoses.

A total of 183 mitoses were observed in the 40 tadpoles of this mating. Of these mitoses, 92(50.3%) were normal diploid, while 41 had aberrant chromosomes, although there were 26 chromosomes in each mitosis. Each of 11 mitoses had 25 chromosomes and the remaining 39 had 27. In the total mitoses, a ring, 18 dicentric and 50 minute chromosomes were found.

Mating 2. Third-generation offspring, N[{(B)NN, No. 61B12T2}N^2, No. 2]

Forty-five tadpoles, which were 35~50 mm. in total length and appeared to be quite normal, were examined. Eighteen of them had no other than normal diploid mitoses (Table 14, 15). Seventeen others had abnormal mitoses only. Although seven of these tadpoles were 26 in chromosome number, six had one to three aberrant chromosomes caused probably by partial deletion or translocation in each mitosis, and the other had abnormal mitoses, in which No. 12 existed singly and there was an additional minute chromosome. Eight other tadpoles were 27 in chromosome number: the triplicate existed at No. 12 in three and at No. 13 in three tadpoles, while the other two tadpoles had an additional minute chromosome derived from No. 13 by partial deletion. The remaining two tadpoles were 25 in chromosome number: No. 10 in one tadpole and No. 13 in the other, were singly represented, respectively.

The remaining ten of the 45 tadpoles examined had both normal and abnormal mitoses. In the abnormal mitoses of a tadpole, one of No. 2 chromosomes was converted into a ring. In those of two other tadpoles there were two dicentric chromosomes (Fig. 13). The other seven tadpoles had abnormal mitoses with one to three aberrant chromosomes caused probably by partial deletion or translocation, although these mitoses were 26 in chromosome number.

Two hundred and four mitoses in total were observed in the 45 tadpoles. Among these mitoses there were 93(45.6%) with 26 normal diploid chromosomes. In each of 69 other diploid mitoses one or more aberrant chromosomes were found. Nine of the other mitoses had 25 chromosomes and 33 had 27. In the total of 204 mitoses there were three ring, 12 dicentric and 25 minute chromosomes.

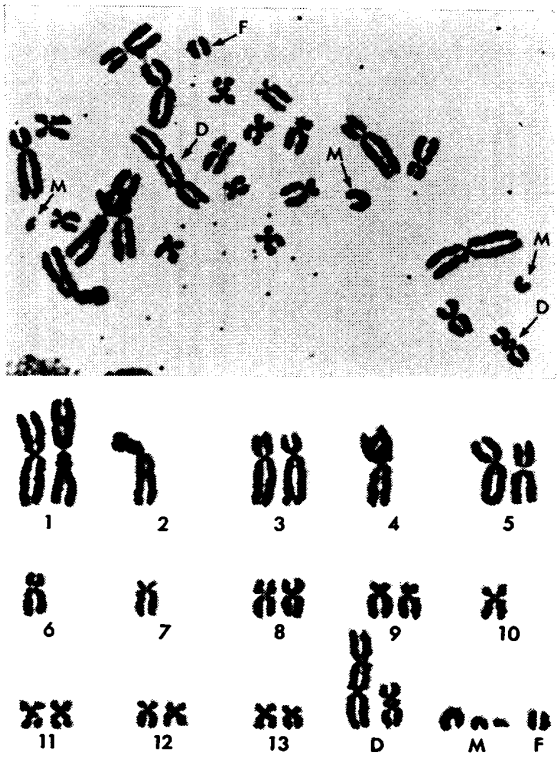

Fig. 13. Chromosomal aberration in a normally shaped tadpole, 40 days old, among the third generation offspring, N[{(B)NN, No. 61B12T2} N^2, No. 2]. Two dicentric (D), three minute (M) and a fragment (F) chromosome are indicated by arrows in the metaphase plate. ×1500

C. Experiments performed in 1968

I. Male and female parents

In the season of the year 1968, nine males and eight females were mated in order to produce the third-generation offspring of female nucleo-cytoplasmic hybrids and the controls. All these males and females were 3 years old.

1. Testes of male parents

Among the nine males, there were three *nigromaculata* and six second-generation offspring of two female nucleo-cytoplasmic hybrids, (B)NN, Nos. 61B12T2 and 61B13T3. The first-generation offspring of these nucleo-cytoplasmic hybrids had been produced by mating with male *nigromaculata* in 1962 (KAWAMURA and NISHIOKA, 1963a). The second-generation offspring had been obtained in 1965 by mating female first-generation offspring with male *nigromaculata*.

The male *nigromaculata* were those which had been raised as the control of the experiments performed in 1965. They were 52.5～57.0 mm. in body length. The testes of these males were 3.0～4.0 mm. in length and 2.0～2.5 mm. in width, and of Type 1 in inner structure, that is, the seminal tubules were filled with

TABLE 16
Testes of male parents used for mating experiments performed in 1968

Individual no.	Age (year)	Body length (mm.)	Size of the testes		Inner structure
			Left (mm.)	Right (mm.)	
(N)NN.65.1	3	54.0	3.0×2.0	3.0×2.0	Type 1
(N)NN.65.2	3	52.5	4.0×2.5	3.5×2.5	Type 1
(N)NN.65.3	3	57.0	4.0×2.5	4.0×2.5	Type 1
{(B)NN.61B12T2}N².3	3	52.0	3.5×2.0	3.5×2.5	Type 1
{(B)NN.61B12T2}N².4	3	59.0	4.5×3.0	4.5×3.0	Type 1
{(B)NN.61B12T2}N².5	3	62.0	4.5×3.5	4.5×3.5	Type 1
{(B)NN.61B13T3}N².1	3	57.5	3.5×3.0	3.5×2.5	Type 1
{(B)NN.61B13T3}N².2	3	52.0	3.0×2.0	3.0×2.0	Type 1
{(B)NN.61B13T3}N².3	3	51.5	2.5×2.0	3.0×2.5	Type 1

bundles of normal spermatozoa (Table 16).

The six male second-generation offspring were 51.5~62.0 mm. in body length. Their testes were 2.5~4.5 mm. in length and 2.0~3.5 mm. in width, and of Type 1 in inner structure, that is, quite normal and very similar in size and structure to those of the control *nigromaculata*.

2. Eggs of female parents

The eight females used for the mating experiments in 1968 consisted of three *nigromaculata* and five sisters of the male second-generation offspring.

The female *nigromaculata* were 55.0~57.0 mm., 55.8 mm. on the average, in body length. They had 1325~1720 eggs, 1525 on the average, which were 1.7~2.0 mm. in diameter (Table 17).

TABLE 17
Eggs of female parents used for mating experiments performed in 1968

Individual no.	Age (year)	Body length (mm.)	No. of eggs	Mean diameter of 50 eggs (mm.)
(N)NN.65.1	3	55.0	1720	1.7±0.02
(N)NN.65.2	3	57.0	1530	1.9±0.02
(N)NN.65.3	3	55.5	1325	2.0±0.02
{(B)NN.61B12T2}N².1	3	55.0	1266	2.0±0.02
{(B)NN.61B12T2}N².2	3	57.5	1145	2.0±0.03
{(B)NN.61B12T2}N².3	3	60.0	1322	2.0±0.04
{(B)NN.61B13T3}N².1	3	57.0	1466	1.8±0.02
{(B)NN.61B13T3}N².2	3	62.0	1625	1.8±0.02

{(B)NN.61B12T2}N² —— Offspring of the female {(B)NN.61B12T2}N mated with a male *nigromaculata*, (N)NN.

{(B)NN.61B13T3}N² —— Offspring of the female {(B)NN.61B13T3}N mated with a male *nigromaculata*, (N)NN.

The females of the second generations were 55.0~62.0 mm., 58.3 mm. on the average, in body length. Their eggs were 1145~1625, 1365 on the average, in number and 1.8~2.0 mm. in diameter. Accordingly, there were no great differences in these respects between the females of the second generations and the con-

trol *nigromaculata*. However, two of these females, {(B)NN, No. 61B12T2}N^2, Nos. 2 and 3, at least seemed to have comparatively few eggs for their body length, as shown in Table 17. Moreover, there was considerable unequality in size among the eggs of each of the two females.

II. Mating experiments

The third-generation offspring were produced by mating five female second-generation offspring of two female nucleo-cytoplasmic hybrids with a male *nigromaculata*, as well as by mating a female *nigromaculata* with six male second-generation offspring (Table 18).

Series I (Control). Offspring of female and male *nigromaculata*, (N)NN

Three matings were made between three females and three males. Out of total 647 eggs obtained from the three females, 626(96.8%) cleaved normally. While a few of the normally cleaved eggs became abnormal and died during the embryonal stage, the others developed normally. Immediately after the hatching stage, there were 609(94.1%) normal tadpoles. Afterwards, five tadpoles became abnormal and died. A total of 604(93.4%) tadpoles grew normally and completed their metamorphosis.

Series II. Offspring between three female second-generation offspring of a female nucleo-cytoplasmic hybrid, (B)NN, No. 61B12T2 and a male *nigromaculata*

Mating 1. Third-generation offspring from {(B)NN, No. 61B12T2}N^2, No. 1♀ × (N)NN, No. 65.1♂

Out of 203 eggs, 57(28.1%) cleaved normally. Ten of these normally cleaved eggs became abnormal and died during the embryonal stage; the others hatched normally and became normal tadpoles. However, 11 tadpoles died of various abnormalities later. The remaining 36(17.7%) completed their metamorphosis.

Mating 2. Third-generation offspring from {(B)NN, No. 61B12T2}N^2, No. 2♀ × (N)NN, No. 65.1♂

Normal cleavages occurred in 154(79.4%) of 194 eggs. A small number of embryos became abnormal and 131(67.5%) hatched normally. While seven tadpoles gradually died, the other 124(63.9%) became normal, metamorphosed frogs.

Mating 3. Third-generation offspring from {(B)NN, No. 61B12T2}N^2, No. 3♀ × (N)NN, No. 65.1♂

The rate of normal cleavages was high, being 92.2%. While seven of 213 embryos became abnormal at the hatching stage, the other 206(89.2%) hatched normally. Normal, metamorphosed frogs were 190(82.3%) in number.

Series III. Offspring between two female second-generation offspring of a female nucleo-cytoplasmic hybrid, (B)NN, No. 61B13T3, and a male *nigromaculata*

TABLE 18
Viability of third-generation offspring of female

Series	parents		No. of eggs	No. of cleaved eggs		No. of neurulae	
	Female no.	Male no.		Normal	Abnormal	Normal	Abnormal
I	(N)NN.65.1	(N)NN.65.1	219	213 (97.3%)	0	212 (96.8%)	1 (0.5%)
	(N)NN.65.2	(N)NN.65.2	203	199 (98.0%)	0	195 (96.1%)	3 (1.5%)
	(N)NN.65.3	(N)NN.65.3	225	214 (95.1%)	0	213 (94.7%)	0
II	{(B)NN.61B12T2}N².1	(N)NN.65.1	203	57 (28.1%)	0	53 (26.1%)	2 (1.0%)
	{(B)NN.61B12T2}N².2		194	154 (79.4%)	0	147 (75.8%)	4 (2.1%)
	{(B)NN.61B12T2}N².3		231	213 (92.2%)	0	213 (92.2%)	0
III	{(B)NN.61B13T3}N².1	(N)NN.65.1	266	219 (82.3%)	3 (1.1%)	215 (80.8%)	4 (1.5%)
	{(B)NN.61B13T3}N².2		270	258 (95.6%)	1 (0.4%)	255 (94.4%)	1 (0.4%)
IV	(N)NN.65.1	{(B)NN.61B12T2}N².3	257	127 (49.4%)	3 (1.2%)	122 (47.5%)	4 (1.6%)
		{(B)NN.61B12T2}N².4	238	212 (89.1%)	0	205 (86.1%)	3 (1.3%)
		{(B)NN.61B12T2}N².5	229	76 (33.2%)	0	76 (33.2%)	0
V	(N)NN.65.1	{(B)NN.61B13T3}N².1	206	43 (20.9%)	0	42 (20.4%)	1 (0.5%)
		{(B)NN.61B13T3}N².2	194	160 (82.5%)	0	152 (78.4%)	4 (2.1%)
		{(B)NN.61B13T3}N².3	245	202 (82.4%)	0	196 (80.0%)	2 (0.8%)

Mating 1. Third-generation offspring from {(B)NN, No. 61B13T3}N², No. 1♀ × (N)NN, No. 65.1♂

In this mating, 219(82.3%) of 266 eggs cleaved normally. While four, eleven and two of them became abnormal and died at the neurula, tail-bud and hatching stage, respectively, the other 202(75.9%) hatched normally. During the tadpole stage, 40 died; the remaining 162(60.9%) completed their metamorphosis.

Mating 2. Third-generation offspring from {(B)NN, No. 61B13T3}N², No. 2♀ × (N)NN, No. 65.1♂

The rate of normal cleavages was very high: 258(95.6%) of 270 eggs cleaved normally. While a small number of embryos died at various stages, 249(92.2%) hatched and grew into normal tadpoles. Normally metamorphosed frogs were 222(82.2%) in number.

Series IV. Offspring between a female *nigromaculata* and three male second-generation offspring of a female nucleo-cytoplasmic

nucleo-cytoplasmic hybrids, (B)NN, and the controls

No. of tail-bud embryos		No. of hatched tadpoles		No. of tadpoles at X stage		No. of metamorphosed frogs	
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
208 (95.0%)	4 (1.8%)	205 (93.6%)	3 (1.4%)	205 (93.6%)	0	204 (93.2%)	0
195 (96.1%)	0	194 (95.6%)	1 (0.5%)	194 (95.6%)	0	194 (95.6%)	0
212 (94.2%)	1 (0.4%)	210 (93.3%)	2 (0.9%)	208 (92.4%)	0	206 (91.6%)	0
51 (25.1%)	2 (1.0%)	47 (23.2%)	4 (2.0%)	37 (18.2%)	0	36 (17.7%)	0
142 (73.2%)	5 (2.6%)	131 (67.5%)	11 (5.7%)	125 (64.4%)	0	124 (63.9%)	0
213 (92.2%)	0	206 (89.2%)	7 (3.0%)	201 (87.0%)	0	190 (82.3%)	0
204 (76.7%)	11 (4.1%)	202 (75.9%)	2 (0.8%)	202 (75.9%)	0	162 (60.9%)	0
252 (93.3%)	3 (1.1%)	249 (92.2%)	3 (1.1%)	240 (88.9%)	0	222 (82.2%)	
110 (42.8%)	12 (4.7%)	106 (41.2%)	4 (1.6%)	93 (36.2%)	0	80 (31.1%)	0
195 (81.9%)	10 (4.2%)	188 (79.0%)	7 (2.9%)	172 (72.3%)	0	157 (66.0%)	0
76 (33.2%)	0	64 (27.9%)	12 (5.2%)	60 (26.2%)	0	52 (22.7%)	0
40 (19.4%)	2 (1.0%)	31 (15.0%)	9 (4.4%)	25 (12.1%)	0	22 (10.7%)	0
141 (72.7%)	11 (5.7%)	138 (71.1%)	3 (1.5%)	123 (63.4%)	0	106 (54.6%)	0
179 (73.1%)	17 (6.9%)	158 (64.5%)	21 (8.6%)	150 (61.2%)	0	145 (59.2%)	0

hybrid, (B)NN, No. 61B12T2

Mating 1. Third-generation offspring from (N)NN, No. 65.1♀ × {(B)NN, No. 61B12T2}N^2, No. 3♂

In this mating, 127(49.4%) of 257 eggs cleaved normally. While a small number of embryos died of various abnormalities at various stages, 106(41.2%) hatched normally. Eighty (31.1%) tadpoles completed their metamorphosis.

Mating 2. Third-generation offspring from (N)NN, No. 65.1♀ × {(B)NN, No. 61B12T2}N^2, No. 4♂

The rate of cleavages was high, being 89.1%. Twenty-four embryos became abnormal and 188(79.0%) hatched normally. Thirty-one tadpoles died and the other 157(66.0%) passed over the stage of metamorphosis and became normal frogs.

Mating 3. Third-generation offspring from (N)NN, No. 65.1♀ × {(B)NN, No. 61B12T2}N^2, No. 5♂

The rate of cleavages was low, being 33.2%. Out of 76 normally cleaved eggs, 64(27.9%) hatched normally. The other 12 became abnormal at this stage and soon died. While 12 other tadpoles died, the remaining 52(22.7%) completed their metamorphosis.

Series V. Offspring between a female *nigromaculata* and three male second-generation offspring of a female nucleo-cytoplasmic hybrid, (B)NN, No. 61B13T3

Mating 1. Third-generation offspring from (N)NN, No. 65.1♀ × {(B)NN, No. 61B13T3}N^2, No. 1♂

In this mating, 43(20.9%) of 206 eggs cleaved normally. Of these cleaved eggs, one, two and nine became abnormal and died at the neurula, tail-bud and hatching stage, respectively. The remaining 31(15.0%) embryos hatched and became normal tadpoles. While nine of these tadpoles died, the other 22(10.7%) completed their metamorphosis.

Mating 2. Third-generation offspring from (N)NN, No. 65.1♀ × {(B)NN, No. 61B13T3}N^2, No. 2♂

Normal cleavages occurred in 160(82.5%) of 194 eggs. At the neurula stage, there were 152 normal embryos. Four, eleven and three of these embryos became abnormal and died at the neurula, tail-bud and hatching stage, respectively. The remaining 138(71.1%) hatched and became normal tadpoles. While 32 tadpoles died by the stage of metamorphosis, the other 106(54.6%) normally passed over this stage.

Mating 3. Third-generation offspring from (N)NN, No. 65.1♀ × {(B)NN, No. 61B13T3}N^2, No. 3♂

The rate of normal cleavages was 82.4%. At the neurula stage, there were 196 normal embryos. While 17 and 21 embryos became abnormal and died at the tail-bud and hatching stage, respectively, 158(64.5%) hatched and became normal tadpoles. However, 13 of the latter died in this stage; the other 145 (59.2%) completed their metamorphosis.

CONCLUSION AND DISCUSSION

1. Reproductive capacity of nucleo-cytoplasmic hybrids

In Japanese pond frog species, KAWAMURA and NISHIOKA (1963a) have reported that two female nucleo-cytoplasmic hybrids, (B)NN, composed of *Rana brevipoda* cytoplasm and *Rana nigromaculata* nuclei and a female nucleo-cytoplasmic hybrid of the reciprocal combination, (N)BB, were fertile, although inferior to the control *nigromaculata* or *brevipoda* in reproductive capacity. By mating with a male *nigromaculata* or *brevipoda* about half (43~58%) of eggs of the two (B)NN females and most (90~97%) of those of the (N)BB cleaved normally. While the cleaved eggs of the (N)BB female mostly developed into metamorphosed frogs, those of one of the (B)NN females mostly died at the hatching stage and only a very small number of them developed into metamorphosed frogs. From the normally cleaved

eggs of the other (B)NN female, however, a considerable number of metamorphosed frogs were obtained. In the present research, it was found that two female nucleo-cytoplasmic hybrids, (B)NN, were very similar to the above (N)BB females in reproductive capacity, when mated with male *nigromaculata* or *brevipoda*, as presented in Table 12 (Series V).

Besides the nucleo-cytoplasmic hybrids between the two pond frog species, KAWAMURA and NISHIOKA (1963b) have reported on those of two Japanese brown frog species. Two female and three male nucleo-cytoplasmic hybrids constructed of *Rana ornativentris* cytoplasm and *Rana japonica* nuclei were sexually matured. The males were found to be fertile to some extent: a small number of *japonica* eggs which had been fertilized with sperms of these males developed into matured frogs. Most males of the latter were also fertile to some extent. The two female nucleo-cytoplasmic hybrids produced no normal tadpoles by mating with male *japonica* or *ornativentris*, differing from the male nucleo-cytoplasmic hybrids, although most of the eggs attained the hatching stage after normal fertilization. Recently, GALLIEN, C.-L. (1970) obtained a male and two females of sexually matured nucleo-cytoplasmic hybrids between *Pleurodeles waltlii* and *P. poireti*. They were fertile and produced progeny by mating with a male or a female of the nuclear species.

In the present research, the reproductive capacities of male nucleo-cytoplasmic hybrids produced from the two Japanese pond frog species, *Rana nigromaculata* and *R. brevipoda*, were further examined. Figures 14~16 show schematically the results of some mating experiments performed in 1965 and 1966, in order

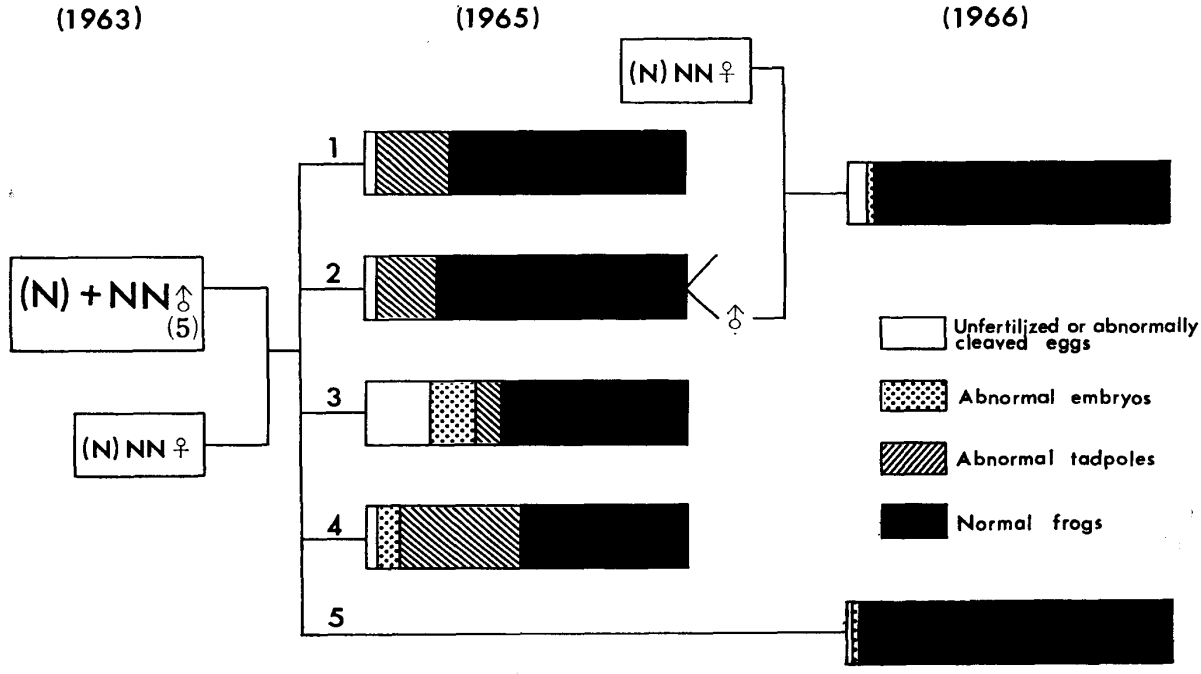

Fig. 14. Diagrammatic representation of the viabilities of the first- and second-generation offspring of five male nucleus-exchanged *Rana nigromaculata*, (N)+NN.
1, No. 63N1T1. 2, No. 63N2T2. 3, No. 63N2T3. 4, No. 63N3T4. 5, No. 63N4T7. (N)NN, *Rana nigromaculata* collected from the field.

to make easy to follow. Fig. 14 corresponds to Series II in Table 6 and Series III in Table 12; Fig. 15 to Series VII and VIII in Table 6 and Series IV in Table 12; Fig. 16 to Series III and IX in Table 6. Each of the rectangular frames standing in line in each figure shows the total number of eggs of each mating. A white, a dotted, a shaded and a black area of each frame show percentages of unfertilized or abnormally cleaved eggs, abnormal embryos, abnormal tadpoles, and normal metamorphosed frogs, respectively. Cross-striped areas found in some rectangular flames show percentages of metamorphosed frogs with abnormal forelegs. As noticed by these figures, male nucleo-cytoplasmic hybrids between the two species were remarkably inferior to male nucleus-exchanged *nigromaculata* in reproductive capacity. Although the latter were mostly inferior to the control *nigromaculata,* about 97% of *nigromaculata* eggs became normal frogs by insemination with sperm of one of them. By two other males, more than 73~78% of *nigromaculata* eggs developed into normal frogs, while 53~58% did so by the remaining two males (Fig. 14).

Differing from these nucleus-exchanged frogs as well as the control *nigromaculata* or *brevipoda,* male nucleo-cytoplasmic hybrids were generally poor in reproductive capacity (Figs. 15 and 16). Four of ten male nucleo-cytoplasmic hybrids, (B)NN, constructed of *brevipoda* cytoplasm and *nigromaculata* nuclei produced no or only a few metamorphosed frogs by mating with female *nigromaculata,* as presented in Tables 6 (Series IV and VII) and 12 (Series IV). Another male produced a small number of frogs with ill-developed hind legs. By mating with five of the other six males, 32~54% of *nigromaculata* eggs developed into metamorphosed frogs, while 74% of *nigromaculata* eggs became frogs by mating with the remaining one frog. Concerning the reproductive capacities of the male (B)NN nucleo-cytoplasmic hybrids, very similar results to the above-stated ones were obtained by mating nine of them with female *brevipoda,* as presented in Tables 6 (Series V and VIII) and 12 (Series IV). Male nucleo-cytoplasmic hybrids, (N)BB, constructed of *nigromaculata* cytoplasm and *brevipoda* nuclei, were far poorer in reproductive capacity than those of the reciprocal combination (Fig. 16). By mating with *brevipoda* females, four of nine (N)BB nucleo-cytoplasmic hybrids, produced no metamorphosed frogs, while each of the other five produced a very small number of frogs, which is equal to 9~16% of total eggs, as presented in Table 6 (Series III and IX).

There were rough correlations between the inner structures of the testes of male nucleo-cytoplasmic hybrids and the percentages of normally cleaved eggs produced by using their sperm. By ten male (B)NN nucleo-cytoplasmic hybrids whose testes were all of Type 1, 70~98% of *nigromaculata* or *brevipoda* eggs cleaved normally, while 87~100% of eggs of the same females did so by mating with male *nigromaculata* or *brevipoda* in the control series (Tables 6 and 12). Among nine (N)BB nucleo-cytoplasmic hybrids, there were three of Type 1, three of Type 2 and three of Type 3. With sperms of two of the three Type 1 males, 80% of *brevipoda* eggs cleaved normally, while 54% did so with sperm of the other Type 1 male. The three males of Type 2 produced normal cleavages in 54~64% of *brevipoda*

eggs, and the three of Type 3 produced normal cleavages in 21～23%. The first-generation offspring of the 19 male (B)NN and (N)BB nucleo-cytoplasmic hybrids were generally very low in viability, regardless of the types of their testes. No normal frogs were obtained from two (B)NN and two (N)BB males with Type 1 testes. Two of the three (N)BB males with Type 2 testes also produced no frogs. In contrast with these males, the three (N)BB males with Type 3 testes produced metamorphosed frogs which corresponded in number to only 9～16% of the *brevipoda* eggs used for insemination. Although a male, (B)NN, No. 63B1T1, produced the most numerous metamorphosed frogs among the 19 nucleo-cytoplasmic hybrids by mating with *nigromaculata* or *brevipoda* females, they were far fewer

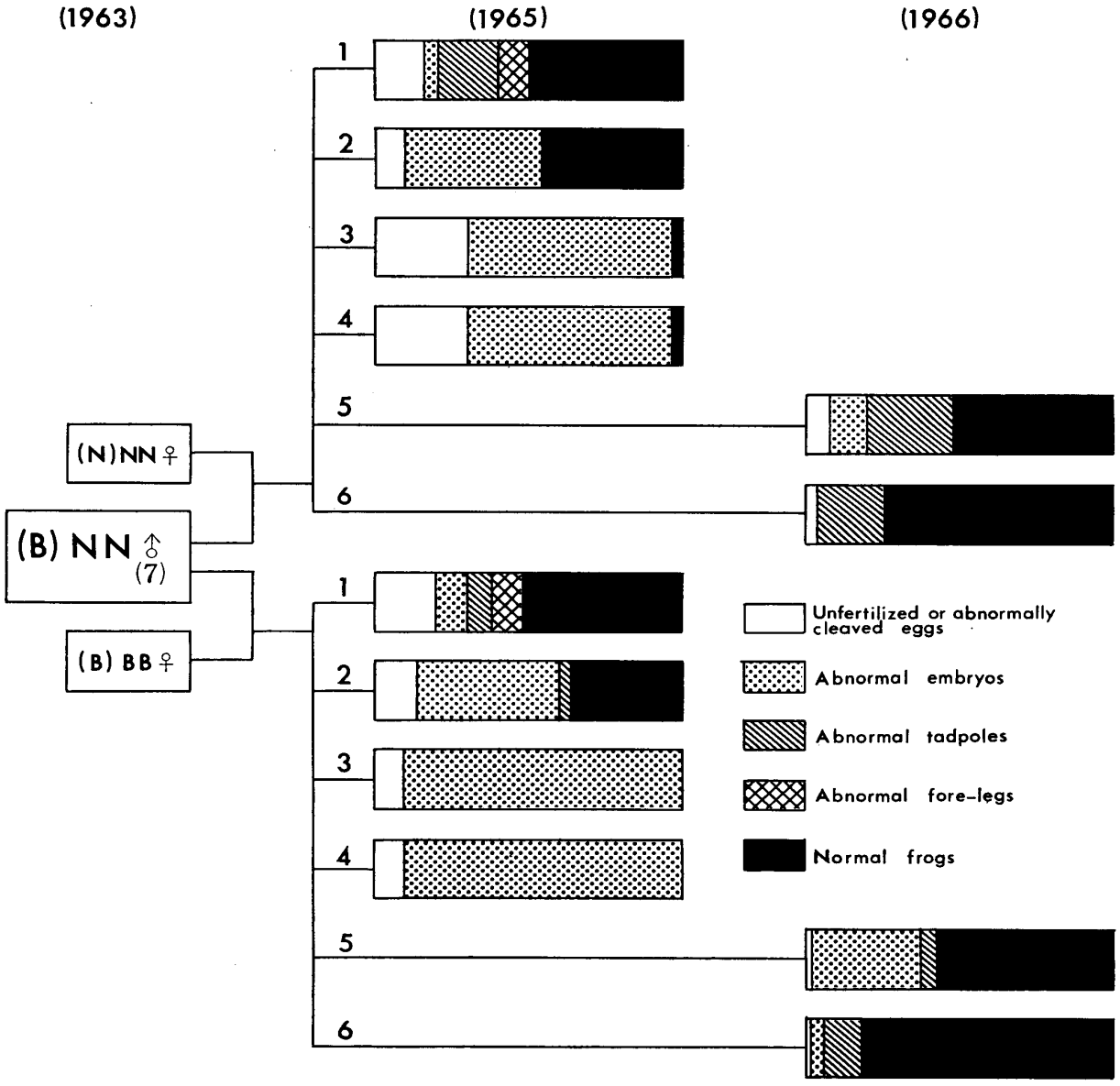

Fig. 15. Diagrammatic representation of the viabilities of the first-generation offspring of six male nucleo-cytoplasmic hybrids, (B)NN, consisting of *Rana brevipoda* cytoplasm and *Rana nigromaculata* nuclei. 1, No. 63B2T1. 2, No. 63B2T2. 3, No. 63B2T3. 4, No. 63B2T4. 5, No. 63B4T6. 6, No. 63B4T7. (N)NN, *Rana nigromaculata* collected from the field. (B)BB, *Rana brevipoda* collected from the field.

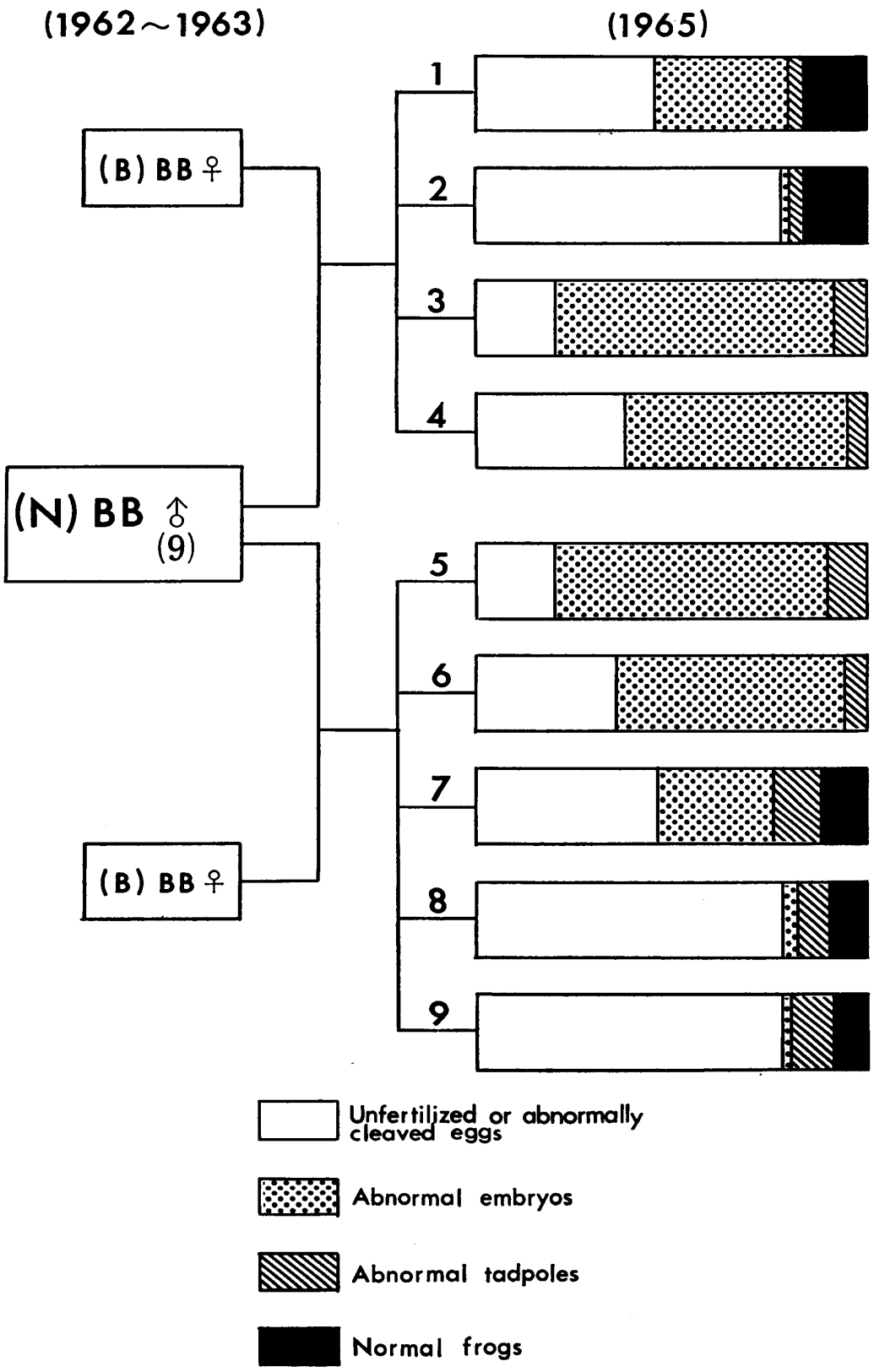

Fig. 16. Diagrammatic representation of the viabilities of the first-generation offspring of nine male nucleo-cytoplasmic hybrids, (N)BB, consisting of *Rana nigromaculata* cytoplasm and *Rana brevipoda* nuclei. 1, No. 62N1T1. 2, No. 62N2T1. 3, No. 62N3T1. 4, No. 62N4T1. 5, No. 63N1T1. 6, No. 63N1T2. 7, No. 63N3T3. 8, No. 63N3T4. 9, No. 63N4T5. (B)BB, *Rana brevipoda* collected from the field.

than those in the control series. Moreover, there were some frogs with ill-developed forelegs. Another male, (B)NN, No. 63B2T1, also produced a considerable number of abnormal frogs with ill-developed forelegs by mating with *nigromaculata* or *brevipoda* females, besides numerous normal ones.

From the matters stated above, it is certain that the eggs and spermatozoa of nucleo-cytoplasmic hybrids between two different frog species are mostly incompetent in the function giving rise to normal development, even if they are normal in appearance. The poverty of normal spermatozoa in some male nucleo-cytoplasmic hybrids is probably due to stumbling of meioses in many spermatocytes. Such abnormal meioses seem attributable to distinct or indistinct chromosomal aberrations which have occurred in the spermatocytes, as a result of disharmony between the chromosomes and the foreign cytoplasm.

The fact that distinct abnormalities in the number and gross structure of chromosomes occur in nucleo-cytoplasmic hybrids composed of *Rana pipiens* nuclei and *Rana sylvatica* cytoplasm has been ascertained by HENNEN (1961, '63). However, the relation between *Rana pipiens* and *Rana sylvatica* is far more remote than that between *Rana nigromaculata* and *Rana brevipoda,* as the reciprocal diploid hybrids of the former two species all die at the early gastrula stage. In contrast with these two species, the relation between *Rana pipiens* and *Rana palustris* is fairly similar to that of *Rana nigromaculata* and *Rana brevipoda*, because *Rana pipiens* produce normal viable diploid hybrids by ordinary crosses with *Rana palustris.* HENNEN (1962, '65, '67, '72) observed that reciprocal nucleo-cytoplasmic hybrids between *Rana pipiens* and *Rana palustris* as well as embryos produced by transferring their nuclei into enucleated eggs of the nuclear species after division for one to three blastula generations in foreign cytoplasm possessed normal chromosome complements, although the nucleo-cytoplasmic hybrids were very abnormal in the post-blastula development.

In KAWAMURA and NISHIOKA's paper (1963a) a great majority of hatched tadpoles produced by the enucleation and the nuclear transplantation method between *Rana nigromaculata* and *Rana brevipoda* were not examined in terms of their karyotypes, as they were abnormal and inviable. Accordingly, it was unknown whether they were normal diploids or not. The remaining viable tadpoles were examined and ascertained to be diploids, that is, nucleo-cytoplasmic hybrids, except a few which were triploids obtained undesignedly, owing to failure of enucleation. Four male nucleo-cytoplasmic hybrids and 20 males and females of first-generation offspring produced from two female nucleo-cytoplasmic hybrids, were utilized as material in the present researches. Although it was unknown whether the chromosome complements of these nucleo-cytoplasmic hybrids were perfectly normal or not in the minute structures of each chromosome, there was no doubt about that their somatic cells repeated very actively their mitotic divisions, as they were remarkably better in growth than the controls were. It is very probable that the first distinct abnormalities of the nucleo-cytoplasmic hybrids appeared in chromosomes during the meioses of spermatocytes or oocytes in their gonads. At the same time it is believed that the chromosomes of the

nucleo-cytoplasmic hybrids were in such a condition as they were apt to give rise to aberrations and that various aberrations actually occurred in the chromosome complements of germ cells during meiotic divisions.

2. Reproductive capacity of the offspring of nucleo-cytoplasmic hybrids

A male first-generation offspring of a male nucleus-exchanged *nigromaculata* mated with a female *nigromaculata* was completely normal in reproductive capacity, just as a male 3-year-old nucleus-exchanged *nigromaculata* was, as shown in Fig. 14.

More than 93% of the *nigromaculata* eggs used for both matings became normal frogs. In the controls of the experiments producing second-generation offspring of a female nucleo-cytoplasmic hybrid, all the male and female *nigromaculata* or *brevipoda* were quite normal in reproductive capacity. More than 84 and 80% of *nigromaculata* eggs developed into normal frogs in 1965 and 1966, respectively, by insemination with sperms of male *nigromaculata,* while more than 84 and 82% of *brevipoda* eggs did in 1965 and 1966, respectively, with sperms of male *brevipoda.* Differing from the control *nigromaculata* and *brevipoda,* male and female first-generation offspring of female nucleo-cytoplasmic hybrids were mostly very poor in reproductive capacity, as presented in Tables 6 and 12. The results of mating experiments performed during the years 1962~1968 between the (B)NN nucleo-cytoplasmic hybrids or their offspring and male or female *nigromaculata* are shown schematically in Fig. 17.

Four of 12 male first-generation offspring of two (B)NN female nucleo-cytoplasmic hybrids mated with male *nigromaculata* did produce no metamorphosed frogs, in spite of high rates of normally cleaved eggs, by mating with female *nigromaculata.* Four other males were distinctly low in fertilizing capacity and consequently produced only a few normal frogs. In contrast with these males, the other four produced considerably numerous frogs: 57~75 and 40% of *nigromaculata* eggs became normal frogs after insemination with sperms of three and one of them, respectively. Six of these 12 male first-generation offspring gave similar results by mating with female *brevipoda* to those obtained by mating with female *nigromaculata.* No or a few normal frogs were produced after insemination with sperms of four males, while 25 or 44% of eggs developed into frogs by mating with the other two males (Table 7).

In contrast with the males, five females of the above-stated first-generation offspring were fairly good in reproductive capacity, when mated with male *nigromaculata* or *brevipoda* in 1965 and 1966. In three of these five females, 57~70% of eggs became normal, metamorphosed frogs, although the rate of normal frogs in the matings with their brothers were 3~22 percent (Table 7). These low rates were mainly due to the poverty of the males in reproductive capacity. One year later, the other two females were mated with male *nigromaculata* or *brevipoda,* and it was found that they were very good in reproductive capacity, that is, 87~91% of eggs became normal frogs (Table 12).

Two female and three male first-generation offspring of a female (B)NN nucleo-cytoplasmic hybrid mated with a male *brevipoda* were similar in reproduc-

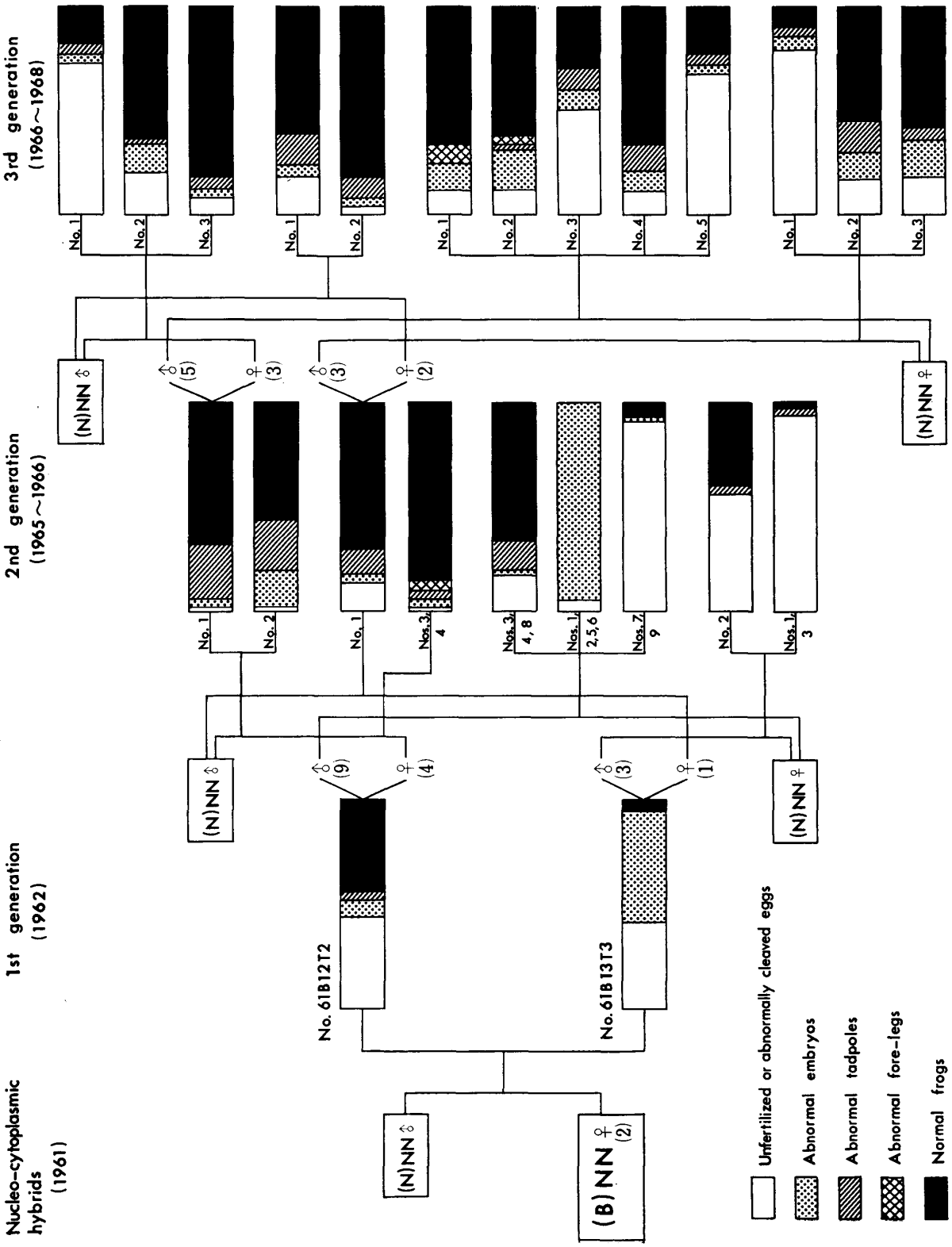

Fig. 17. Diagrammatic representation of the viabilities of the first- to third-generation offspring of female nucleo-cytoplasmic hybrids, (B)NN, consisting of *Rana brevipoda* cytoplasm and *Rana nigromaculata* nuclei. (N)NN, *Rana nigromaculata* collected from the field.

tive capacity to the male first-generation offspring obtained by mating with male *nigromaculata*. By mating with a male *nigromaculata* or *brevipoda*, 26~48% of eggs of the two females became normal frogs, while 10~40% of eggs of the same two females and 0~42% of eggs of female *nigromaculata* and *brevipoda* developed into normal frogs by insemination with sperms of the three male first-generation offspring.

The reproductive capacities of second-generation offspring of a female (B)NN nucleo-cytoplasmic hybrid mated repeatedly with male *nigromaculata* are shown in Fig. 17. Two males used for experiments in 1966 were somewhat inferior in reproductive capacity to the four control males: by mating with the former, 63 and 67% of *nigromaculata* eggs became normal frogs, while 80~89% did so by mating with the latter. In the control series in 1968, 92~96% of total eggs of three male *nigromaculata* developed into normal frogs. Five females and six males of the second generation offspring were all inferior in reproductive capacity to the control frogs. By insemination with sperms of male *nigromaculata*, 18~82% of eggs of the five females became normal frogs, while 11~66% of *nigromaculata* eggs did so by the six males. In third-generation offspring, there were more numerous individuals which became abnormal at the embryonal stages than those in the control series. If the males were compared with the females in reproductive capacity, it was obvious that the former were considerably inferior to the latter, just as the male first-generation offspring were (Fig. 17). On the other hand, the female and male second-generation offspring were very similar in reproductive capacity to the female and male first-generation offspring. The male second-generation offspring did not seem to have been improved in this capacity, as compared with those of first-generation offspring as well as the nucleo-cytoplasmic hybrids.

It was remarkable that the testes of 12 male first-generation offspring obtained by mating between female (B)NN nucleo-cytoplasmic hybrids and a male *nigromaculata* were not always normal in inner structure. There were four males of each Type 1, Type 2 and Type 3 among them. As a matter of course, there were one of Type 2 and two of Type 3 among three male first-generation offspring obtained by mating between a female (B)NN nucleo-cytoplasmic hybrid and a male *brevipoda*. Between these types of testes and the percentages of cleaved eggs, there was an intimate relation, just as found in male nucleo-cytoplasmic hybrids: six males with Type 3 testes produced no or a few cleaved eggs by mating with female *nigromaculata* or *brevipoda*, while by insemination with sperms of four males with Type 1 testes, 87~96% of *nigromaculata* eggs cleaved normally (Tables 4 and 7). However, these males with Type 1 testes were not always good in the production of frogs: two of them produced no frogs by mating with female *nigromaculata*, while by the other two, 67 and 75% of *nigromaculata* eggs developed into normal frogs. By the six males with Type 3 testes, no or only a few frogs were produced.

In six male second-generation offspring of female (B)NN nucleo-cytoplasmic hybrids mated repeatedly with male *nigromaculata*, it was found that their testes

were all of Type 1. However, all these males were not quite normal in reproductive capacity, since only 11～66% of *nigromaculata* eggs became frogs by insemination with their sperms.

The seven female first-generation offspring obtained by mating between a female (B)NN nucleo-cytoplasmic hybrid and a male *nigromaculata* or *brevipoda* were quite similar in egg size to those of the control *nigromaculata,* although there were somewhat larger deviations from mean sizes (Tables 5 and 11). In a female, 55 mm. in body length, produced by mating with a male *nigromaculata* at least, the number of eggs were surely smaller than that in the control *nigromaculata,* just as in the two females produced by mating with a male *brevipoda.* Among the seven female second-generation offspring, there were also three which had fewer eggs for the body lengths than those of the controls. Moreover, there were larger deviations from mean sizes in the eggs of two females. Such larger deviations in egg size as well as smaller numbers of eggs seem to indicate the existence of some abnormal processes in the oogenesis of these females. It is probable that this in turn caused the females to have lower reproductive capacities than the control females.

The first-generation offspring of male nucleo-cytoplasmic hybrids were similar to those of female ones in that there were some males with abnormal testes. In the control series all the 15 *nigromaculata* and one *brevipoda* males had testes of Type 1 (Table 8). In this respect, the first-generation offspring of a male nucleus-exchanged *nigromaculata* were quite the same as the control *nigromaculata.* Differing from these males of the control series and the offspring of the male nucleus-exchanged *nigromaculata,* six and four of 23 male first-generation offspring between two male (N)BB nucleo-cytoplasmic hybrids and two female *brevipoda* were of Type 2 and Type 3 in the inner structures of their testes, respectively. Among young frogs of these first-generation offspring which were dead or killed within one month after metamorphosis, there were nearly equal numbers of males and females and no juvenile hermaphrodites. Accordingly, the males with testes of Type 2 or 3 did not seem to be sex-reversed ones. On the other hand, one of five male first-generation offspring between two male (B)NN nucleo-cytoplasmic hybrids and three female *nigromaculata* was of Type 3, while the other four were of Type 1. Among the first-generation offspring of the same two male (B)NN nucleo-cytoplasmic hybrids mated with two female *brevipoda,* there were four with Type 2, 39 with Type 3 and no males with Type 1 testes, similar to ordinary hybrids between *nigromaculata* and *brevipoda.*

In both the sex ratio and the inner structures of testes, the second-generation offspring of two female (B)NN nucleo-cytoplasmic hybrids were somewhat different from the controls (Table 9). In each of the six control matings of *nigromaculata* or *brevipoda,* 47～52% of the total number of frogs examined were males. When examined within one month after metamorphosis, 45～53% of frogs were males and there were no juvenile hermaphrodites, except for two which were found in the control *nigromaculata.* The testes of 57 *nigromaculata* and 92 *brevipoda* males which were sexually matured were all of Type 1, that is, quite normal in inner

structure. In the second-generation offspring of the nucleo-cytoplasmic hybrids, males were generally more or less fewer than females. The most scanty number of males was found in second-generation offspring of a male No. 8 of the first-generation offspring, {(B)NN, No. 61B12T2}N, mated with female *nigromaculata, brevipoda* or sisters of the same first-generation offspring. Among 155 young frogs of four matings examined within one month after metamorphosis, there were only four (2.6%) males and no juvenile hermaphrodites. After more than one year there were 5(10%) males among 49 sexually matured frogs obtained by the same matings. Moreover, two of them which had been produced by brother and sister mating were examined in terms of the type of their gonads and it became clear that both were of Type 2. Such a situation seems to show that the male parent of these second-generation offspring was a genetic female, XX, and produced XX offspring by mating with female parents, although a few of the offspring became phenotypic males.

Among the frogs of second-generation offspring of a male No. 2 of the first-generation offspring between another female (B)NN nucleo-cytoplasmic hybrid No. 61B13T3 and a male *nigromaculata,* mated with female *nigromaculata, brevipoda* or a sister of the same first-generation offspring, males were far fewer than females, too. In these three kinds of matings of this male, 28, 33 and 17% of frogs were males, respectively, although the frogs were not large in number. Five of 13 matured males produced by mating with female *nigromaculata* had testes of Type 2 or 3, while the others had those of Type 1. The testes of a matured male produced by brother and sister mating were of Type 2. Those of three males produced by mating with female *brevipoda* were of Type 2 or 3, as a matter of course. In the second-generation offspring of two males Nos. 3 and 4 of the first-generation offspring, {(B)NN, No. 61B12T2}N, mated with female *nigromaculata,* 43 and 42% of frogs were males, respectively. The testes of two matured males were of Type 1. In the second-generation offspring of a male No. 7, there were four males and four females. While the testes of two of these males were of Type 1, those of the other two were of Type 2 or 3. In two groups of second-generation offspring containing 24 and 21 frogs, which were produced by brother and sister mating of the first-generation offspring, {(B)NN, No. 61B12T2}N or {(B)NN, No. 61B13T3}N, there were 46 and 29% males, respectively. The testes of four of eight matured males in these groups were of Type 2 or 3, while those of the other four were of Type 1.

On the other hand, in the second-generation offspring of a female No. 1 of the first-generation offspring, {(B)NN, No. 61B12T2}N, mated with a male *nigromaculata* and *brevipoda* in 1965 (Table 9), 46 and 15% of frogs were males, respectively, while in those of female No. 2 of the same first-generation offspring mated with a male *nigromaculata* in 1965, 58% were males. The testes of six of 19 sexually matured males produced from the two females Nos. 1 and 2 by mating with male *nigromaculata* were of Type 2 or 3, while those of the other 13 were of Type 1. In the second-generation of a female No. 3 of the same first-generation offspring mated with a male *nigromaculata* and *brevipoda* in 1966, 63 and 65% of

frogs were males, respectively (Table 13). While those of another female No. 4 of the same first-generation offspring mated with a male *nigromaculata* and *brevipoda* in the same year, 71 and 69% were males, respectively. In the second-generation offspring of a female first-generation offspring derived from the other female nucleo-cytoplasmic hybrid, (B)NN, No. 61B13T3, mated with a male *nigromaculata,* 62% of frogs were males. The testes of two matured males of the latter were of Type 1. These results seem to show that both males and females of first- and second-generation offspring of the female nucleo-cytoplasmic hybrids were generally abnormal in sex-determination or sex-differentiation, as compared with their controls.

Such abnormalities were also found in the second-generation offspring produced from the first-generation offspring between a female (B)NN nucleo-cytoplasmic hybrid and a male *brevipoda.* In the second-generation offspring of a male No. 2 mated with a *nigromaculata* and a *brevipoda* female, 45 and 14% of frogs were males, while in the second-generation offspring of two females Nos. 1 and 2 mated with a *nigromaculata* male, 63 and 75% of frogs were males, and in those mated with a *brevipoda* male, 75 and 54% were males, respectively. In the second-generation offspring produced from these frogs by brother and sister mating, 67% were males. Although it is a matter of course, nearly half of sexually matured males produced from them by mating with a *nigromaculata* or *brevipoda* or by brother and sister mating had testes of Type 2 or 3.

It is noteworthy that various kinds of chromosomal aberrations were abundantly found in normally shaped tadpoles of first-, second- and third-generation offspring of nucleo-cytoplasmic hybrids (Tables 14 and 15). In the control *nigromaculata* or *brevipoda,* more than 72% of tadpoles of each mating group had no chromosomal aberrations. Only two out of both 100 *nigromaculata* and 40 *brevipoda* tadpoles were constructed of cells with aberrant chromosome complements. No such individuals were found in 40 tadpoles of each of first and second generations produced from male nucleus-exchanged *nigromaculata* mated with females of the same species. Ninety percent of these tadpoles consisted of normal diploid cells only. Differing from these, tadpoles constructed of normal diploid cells were more or less fewer than those with abnormal cells in each of first-, second- and third-generation offspring of female (B)NN nucleo-cytoplasmic hybrids. Moreover, the rate of tadpoles with abnormal cells did not decrease with the progress of generation, in spite of mating with the nuclear species. Concerning their cellular constitution, 38~60% of 40 or 45 tadpoles of each mating group in the three generations were constructed of cells with aberrant chromosome complements, while 8~22% were of a mixture of normal diploid and abnormal cells. Among the abnormal cells found in the offspring of nucleo-cytoplasmic hybrids there were various kinds of chromosome complements, such as diploid including one to three abnormal chromosomes, hyper-diploid, hypo-diploid and nearly triploid. Nearly all the hyper- and hypo-diploid and nearly triploid tadpoles as well as a part of the abnormally diploid ones consisted respectively of cells with one kind of chromosome complement, while the remaining tadpoles had two

or three kinds.

It is very probable that the chromosomal abnormality of each tadpole with one kind of chromosome complement was established at the time of fertilization by an abnormal gamete of a nucleo-cytoplasmic hybrid or its offspring used as a male or female parent. Such an abnormal gamete is probably produced by an abnormal meiosis at the gametogenesis or an abnormal mitosis of the ancestral germ cell. In contrast with these chromosomal abnormalities, those of tadpoles constructed of a mixture of cells with different chromosome complements seem to have occurred after the first cleavage by abnormal mitoses. It does not seem meaningless to repeat in this place that the male parents of the third-generation offspring had testes which were quite normal in inner structure (Table 10).

What is the origin of the poor reproductive capacity of the offspring of nucleo-cytoplasmic hybrids mated with their nuclear species? This origin seems to result in the low fertilization capacity of their gametes, weak viability of the next generation embryos, and frequent production of juvenile hermaphrodites and matured males with scanty sperm in the testes. Although this goes back to deterioration of chromosomes exposed to foreign cytoplasm in germ cells of nucleo-cytoplasmic hybrids, extensive chromosomal aberrations which occurred in these germ cells during meiotic divisions seem scarcely inherited to the second- and third-generation offspring. Nearly all these chromosomal aberrations might be lost during gametogenesis or by producing inviable individuals in the next generation. In brown frogs, KAWAMURA and NISHIOKA (1972) have reported that inviable embryos found in several generations derived from nucleo-cytoplasmic hybrids generally had very aberrant chromosome complements. In the present researches, it was also ascertained that there were numerous individuals with slight degrees of chromosomal abnormalities among tadpoles with nearly normal appearances (Tables 14 and 15). The present authoress recently confirmed that more than half of trisomics in *Rana nigromaculata* completed their metamorphosis, although they were all under-developed and small in size (unpublished). Accordingly, it seems possible that some of trisomics, monosomics or other tadpoles with slightly abnormal diploid chromosomes in the first- and second-generation offspring of nucleo-cytoplasmic hybrids developed passing over metamorphosis. It seems also undeniable that a part of them attained sexual maturity and produced gametes. However, the fact that various kinds of severe abnormalities were produced by gametes of male and female offspring of nucleo-cytoplasmic hybrids do not seem to be explained on the basis of inheritance of such chromosomal abnormalities as found in the tadpoles with nearly normal appearances. As a matter of course, various abnormalities can not be explained by dominant or recessive gene mutations caused by foreign cytoplasm in germ cells of nucleo-cytoplasmic hybrids, since they appeared repeatedly in the first-, second- and third-generation offspring, in spite of mating with normal males or females of the nuclear species. Extensive chromosomal aberrations must have occurred *de novo* during the meiotic divisions of some germ cells of males and females of the first- and second-generation offspring of nucleo-cytoplasmic

hybrids in the same way as presumed to occur in those of male and female nucleo-cytoplasmic hybrids themselves. This presumption naturally necessitates another presumption that the chromosomes of germ cells of nucleo-cytoplasmic hybrids have numerous latent damages produced by the foreign cytoplasm and that these latent damages apt to manifest their effects and produce various chromosomal aberrations as well as new latent damages during meioses of germ cells in both nucleo-cytoplasmic hybrids and their offspring. Such latent damages would be seen on lampbrush chromosomes of auxocytes of females of the strains derived from nucleo-cytoplasmic hybrids. It might be possible, on the other hand, that these latent damages are exaggeratively manifested in cultured cells by a change of physico-chemical conditions.

SUMMARY

1. First-, second- and third-generation offspring were produced from nucleo-cytoplasmic hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with the nuclear or cytoplasmic species or by brother and sister mating.

2. The inner structures of the testes of male parents which were used for producing the next generation offspring as well as the number and size of eggs of female parents were examined. When the inner structures of testes were divided into the following three types — Type 1, being nearly normal, Type 2, containing a small number of small bundles of normally shaped spermatozoa and Type 3, containing only a few normally shaped spermatozoa — there were three with Type 1, three with Type 2 and three with Type 3 testes among nine 2- or 3-year-old male (N)BB nucleo-cytoplasmic hybrids. The testes of eight 2-year-old male (B)NN nucleo-cytoplasmic hybrids were all of Type 1. Of twelve 3-year-old male first-generation offspring of two female (B)NN nucleo-cytoplasmic hybrids mated with males of the nuclear species, *nigromaculata,* four had Type 3, four others had Type 2 and the third four had Type 1 testes. The testes of three males of first-generation offspring of one of the two female (B)NN nucleo-cytoplasmic hybrids mated with a male of the cytoplasmic species, *brevipoda,* were of Type 2 or 3. The testes of eight male second-generation offspring of the same nucleo-cytoplasmic hybrids, mated repeatedly with the nuclear species, *nigromaculata,* were all of Type 1.

Five 3-year-old and two 4-year-old female first-generation offspring of two female (B)NN nucleo-cytoplasmic hybrids mated with a male *nigromaculata* or *brevipoda* had eggs which were ununiform in size. Moreover, in five of them at least, the eggs were remarkably fewer than those of the control *nigromaculata.* The same tendency was found in three of five 3-year-old female second-generation offspring produced from the female (B)NN nucleo-cytoplasmic hybrids by repeated matings with males of the nuclear species, *nigromaculata.* The eggs of two 3-year-old female (B)NN nucleo-cytoplasmic hybrids were also ununiform in size; besides they were somewhat smaller than those of the control *nigromaculata.*

3. Four 2-year-old male nucleus-exchanged *nigromaculata* produced fairly

numerous first-generation offspring by mating with female *nigromaculata,* that is, 52.7~78.4% of the eggs became normal metamorphosed frogs. Another 3-year-old male nucleus-exchanged *nigromaculata* was extremely good in reproductive capacity; by mating with a female *nigromaculata* 96.6% of eggs became normal metamorphosed frogs. A one-year-old male first-generation offspring of a male nucleus-exchanged *nigromaculata* was also very good and produced normal metamorphosed frogs from 93% of *nigromaculata* eggs. By mating of control male and female *nigromaculata* or *brevipoda,* more than 79%, mostly more than 90% of eggs became normal metamorphosed frogs.

4. Five of nine male (N)BB nucleo-cytoplasmic hybrids produced normal, metamorphosed frogs from 9~16% of eggs by mating with five females of the nuclear species, *brevipoda.* The other four males produced no frogs, although more than 50% of eggs became normal neurulae. Six of ten male (B)NN nucleo-cytoplasmic hybrids produced normal metamorphosed frogs from 32~74% of eggs, by mating with six females of the nuclear species, *nigromaculata.* The other four males produced no or only a few metamorphosed frogs by mating with the same females, although 25~69% of eggs became normal tail-bud embryos. Nine of these male nucleo-cytoplasmic hybrids were mated with five females of the cytoplasmic species, *brevipoda.* Six of them produced normal metamorphosed frogs from 36~82% of eggs, while all the eggs of the remaining three could not develop passing over the hatching stage. By mating of two female (B)NN nucleo-cytoplasmic hybrids with male *nigromaculata,* 53 and 74% of eggs became normal metamorphosed frogs, respectively, while 58 and 83% of eggs attained the same stage by mating of the above two females with male *brevipoda.*

5. While four of twelve male first-generation offspring obtained from two female (B)NN nucleo-cytoplasmic hybrids mated with males of the nuclear species, *nigromaculata,* produced normal metamorphosed frogs from 40~75% of eggs by mating with three female *nigromaculata,* four others produced no frogs by mating with the same females, although 83~95% of eggs became normal tail-bud embryos. By the other four males, only 1~12% of eggs cleaved normally and 1~8% of eggs became normal metamorphosed frogs.

Three of five female first-generation offspring obtained by the above matings produced normal metamorphosed frogs from 57~70% eggs by mating with two males of the nuclear species, *nigromaculata.* However, only 4~22% of eggs of these three females cleaved normally and 3~22% became metamorphosed frogs by mating with six brothers. On the other hand, 87~91% became normal metamorphosed frogs by mating the other two female first-generation offspring with a male *nigromaculata* or *brevipoda.*

6. Two of three male first-generation offspring between a female (B)NN nucleo-cytoplasmic hybrid and a male of the cytoplasmic species, *brevipoda,* could scarcely produce frogs by mating with three female *nigromaculata* or three female *brevipoda.* The other male produced normal metamorphosed frogs from 21% of *nigromaculata* and 42% of *brevipoda* eggs. These three males produced normal metamorphosed frogs from 10~40% of eggs by mating with their two sisters.

7. Female and male second-generation offspring obtained from two female (B)NN nucleo-cytoplasmic hybrids by repeated matings with male *nigromaculata* were mated with male or female *nigromaculata*, again; 18～82% of the eggs of five female second-generation offspring became normal metamorphosed frogs, while their eight brothers produced normal metamorphosed frogs from 11～67% of *nigromaculata* eggs. By seven control matings between the male and female *nigromaculata* used in these mating experiments, more than 88% of eggs, except for 80% in one mating, became normal metamorphosed frogs.

8. The sex ratios of first-, second- and third-generation offspring of male nucleo-cytoplasmic hybrids and the control frogs were examined. The main results were as follows.

In the control matings, 47～51% of 38～161 *nigromaculata* and 47～52% of 37～180 *brevipoda* were males. In four of five matings between male nucleus-exchanged *nigromaculata* and normal female *nigromaculata*, 39～51% of 100～140 frogs were males, while 24% of 75 frogs were males in the other mating. In two matings between two male (N)BB nucleo-cytoplasmic hybrids and four female *brevipoda*, 45% of 51 and 51% of 83 frogs were males, respectively. In two matings between two male (B)NN nucleo-cytoplasmic hybrids and five female *nigromaculata*, 45% of 105 and 36% of 67 frogs were males, while in two matings between these males and four female *brevipoda*, 51% of 158 and 59% of 138 frogs were males, respectively.

In the second-generation offspring of nucleo-cytoplasmic hybrids, there were much or less large deviations from the 1 : 1 sex ratio. The most remarkable deviations were found in the next generation of a male first-generation offspring between a female (B)NN nucleo-cytoplasmic hybrid and a male *nigromaculata*. Only 2～9% of 35～99 frogs produced from this male by mating with three female *nigromaculata* or three female *brevipoda*, or by brother and sister mating were males, while in two matings between two other males of this first generation and the three female *nigromaculata*, 43% of 118 and 42% of 120 frogs were males, respectively. In five matings of five female first-generation offspring between two female (B)NN nucleo-cytoplasmic hybrids and a male *nigromaculata*, with four male *nigromaculata*, 46～71% of 101～214 frogs produced were males. In the matings of three male *brevipoda* and three female first-generation offspring between two female (B)NN nucleo-cytoplasmic hybrid and a male *nigromaculata*, 15% of 175, 65% of 139 or 69% of 135 frogs obtained were males.

In the next generation of a female first-generation offspring between a female (B)NN nucleo-cytoplasmic hybrid and a male *brevipoda*, 63～75% of 24～112 frogs produced by mating with a male *nigromaculata*, a male *brevipoda* or by brother and sister mating were males. In the next generations obtained from a sister of this female by mating with a male *nigromaculata* or a male *brevipoda*, there were males corresponding to 75% of 83 or 54% of 61 frogs.

Concerning the sex ratio of third-generation offspring of a female (B)NN nucleo-cytoplasmic hybrid mated repeatedly with *nigromaculata*, it was known that there were 128 males and 81 females among 209 frogs obtained by two matings

between two male second-generation offspring and two female *nigromaculata.*

9. While all the testes of matured males of *nigromaculata* and *brevipoda* produced by the control matings and offspring between male nucleus-exchanged and female untreated *nigromaculata* were quite normal in inner structure, those of first- and second-generation offspring of nucleo-cytoplasmic hybrids mated with the nuclear species were not always normal. There were a goodly number of males with testes of Type 2 or 3 in the first-generation offspring of a male (N)BB nucleo-cytoplasmic hybrid mated with female *brevipoda* and also in the second-generation offspring produced from female (B)NN nucleo-cytoplasmic hybrids by repeated matings with *nigromaculata.* Males with Type 2 or 3 testes were abundantly found among frogs produced from first-generation offspring between the female (B)NN nucleo-cytoplasmic hybrids and male *nigromaculata* by brother and sister mating.

Matured male first-generation offspring between male (B)NN nucleo-cytoplasmic hybrids and female *brevipoda* had testes of Type 2 or 3. Those of second-generation offspring produced from first-generation offspring between female (B)NN nucleo-cytoplasmic hybrids and male *nigromaculata* by mating with *brevipoda* had testes of Type 2 or 3, too. In each kind of second-generation offspring produced from first-generation offspring between a female (B)NN nucleo-cytoplasmic hybrid and male *brevipoda,* by mating with *nigromaculata* or *brevipoda,* or by brother and sister mating, there were matured males of Type 1, 2 or 3 in a considerable number.

10. In each series of the control *nigromaculata, brevipoda* and first- and second-generation offspring produced from a male nucleus-exchanged *nigromaculata* by mating with female *nigromaculata,* about 90% of tadpoles with normal appearances consisted of normal diploid cells, while the others consisted of a mixture of normal diploid and abnormal cells or rarely of abnormal cells only.

In the first-, second- and third-generation offspring of female (B)NN nucleo-cytoplasmic hybrids mated with the nuclear species, *nigromaculata,* a majority of tadpoles with normal appearances were constructed of abnormal diploid cells or a mixture of normal diploid and abnormal cells, while the remaining tadpoles were solely of normal diploid cells. Among these abnormal cells, there were abnormal diploid containing one to three aberrant chromosomes, hypo- or hyper-diploid, or nearly triploid cells. In these abnormal chromosomal complements, various kinds of aberrant chromosomes, such as minute, dicentric or ring, were frequently found. Such kinds of aberrant chromosomes were not found in the above control tadpoles.

11. The existence of latent damages in the chromosomes of nucleo-cytoplasmic hybrids and their offspring was presumed in order to explain the low reproductive capacities of the first- and second-generation offspring produced by mating with the nuclear species.

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EXPLANATION OF PLATES

PLATE I

Cross-sections of seminal tubules of the testes of matured male nucleo-cytoplasmic hybrids consisting of *Rana nigromaculata* cytoplasm and *Rana brevipoda* nuclei, male nucleus-exchanged *nigromaculata* and the control male *nigromaculata*. All these males were two years old. On the basis of the amount of spermatozoa in cross-sections of seminal tubules as found in Plates I and II, the testes of matured males were divided into three types.

×300

1. Type 1 testis of a control male *nigromaculata*, (N)NN, No.63.2.
2. Type 1 testis of a male nucleus-exchanged *nigromaculata*, (N)+NN, No.63N2T2.
3. Type 2 testis of a male nucleo-cytoplasmic hybrid, (N)BB, No.63N3T3.
4. Type 3 testis of a male nucleo-cytoplasmic hybrid, (N)BB, No.63N3T4.

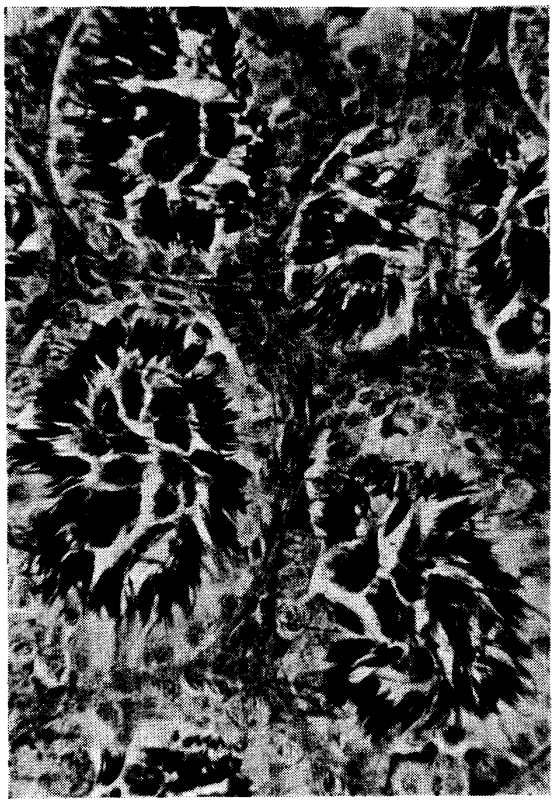

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PLATE II

Cross-sections of seminal tubules of the testes of matured male first-generation offspring of nucleo-cytoplasmic hybrids consisting of *Rana brevipoda* cytoplasm and *Rana nigromaculata* nuclei. All these males were three years old. ×300

5. Type 1 testis of a male first-generation offspring, {(B)NN, No. 61B12T2}N, No. 1.
6. Type 2 testis of a male first-generation offspring, {(B)NN, No. 61B12T2}N, No. 2.
7. Type 3 testis of a male first-generation offspring, {(B)NN, No. 61B13T3}N, No. 1.
8. Type 2 testis of a male first-generation offspring, {(B)NN, No. 61B12T2}B, No. 2.

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PLATE III

Cross-sections of the gonads of young frogs killed one month after metamorphosis. According to the inner structures of gonads as shown in the eight figures of Plates III and IV, the sex of young frogs was divided into six categories. ×300

9. Normal ovary of a female ♀$_N$ among the control *nigromaculata* produced by mating, (N)NN, No.62.4♀ × (N)NN, No.62.3♂.
10. Under-developed ovary of a female ♀$_U$ among the second-generation offspring produced by mating, {(B)NN, No.61B12T2}N, No.2 ♀ × (N)NN, No.62.3 ♂.
11. Gonad of a hermaphrodite ⚥$_1$ at the first phase of sex-reversal among the second-generation offspring produced by mating, {(B)NN, No.61B12T2}N, No.1 ♀ × (B)BB, No.62.2 ♂.
12. Gonad of a hermaphrodite ⚥$_1$ at the first phase of sex-reversal among the second-generation offspring produced by mating, {(B)NN, No.61B12T2}N, No.1 ♀ × (N)NN, No.62.3 ♂.

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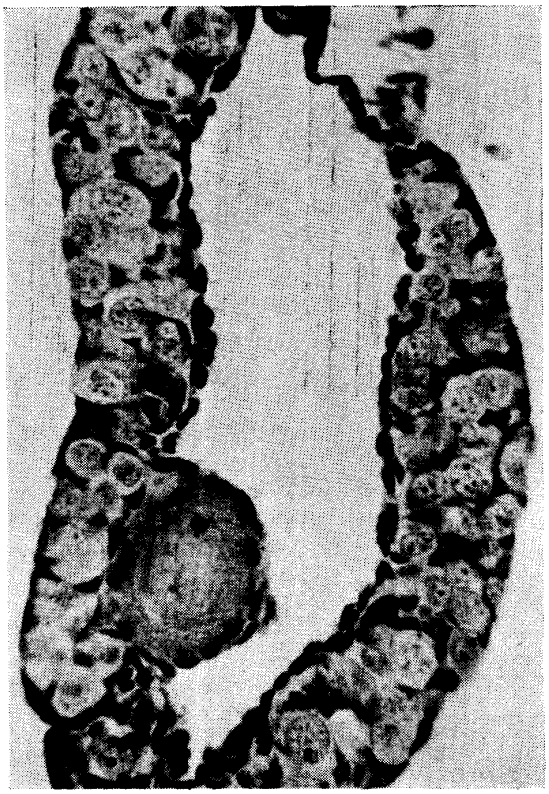

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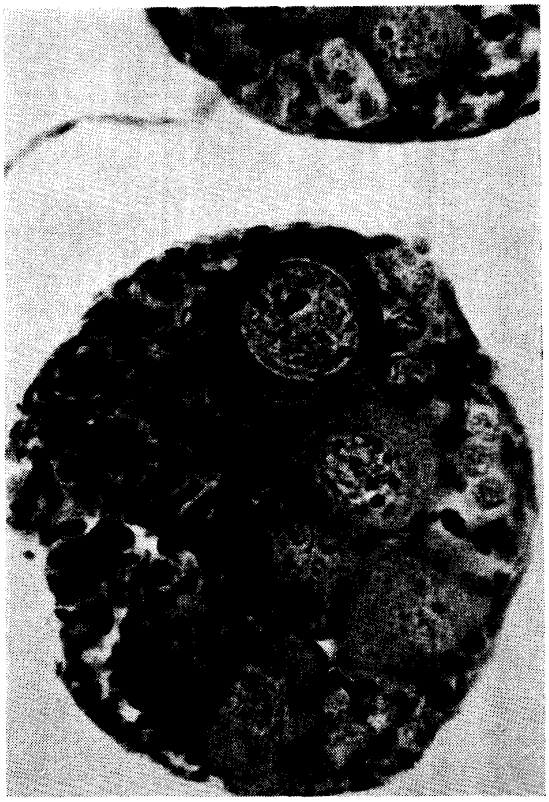

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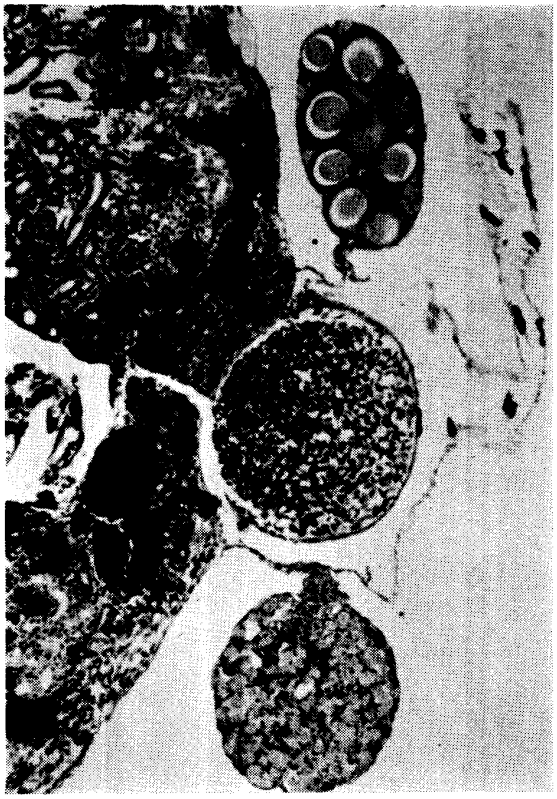
PLATE IV

Cross-sections of the gonads of young frogs killed one month after metamorphosis.

13. Gonads of a hermaphrodite ♀$_1$ at the first phase of sex-reversal among the second-generation offspring produced by mating, {(B)NN, No. 61B13T3}N, No. 1 ♀ × (N)NN, No. 62.4 ♂. ×100
14. Gonad of a hermaphrodite ♀$_2$ at the second phase of sex-reversal among the second-generation offspring produced by mating, {(B)NN, No. 61B12T2}N, No. 1 ♀ × (N)NN, No. 62.3 ♂. ×300
15. Normal testis of a male ♂$_N$ among the control *nigromaculata* produced by mating, (N)NN, No. 62.4 ♀ × (N)NN, No. 62.3 ♂. ×300
16. Rudimentary testis of a male ♂$_R$ among the second-generation offspring produced by mating, {(B)NN, No. 61B12T2}N, No. 1 ♀ × (N)NN, No. 62.3 ♂. ×300



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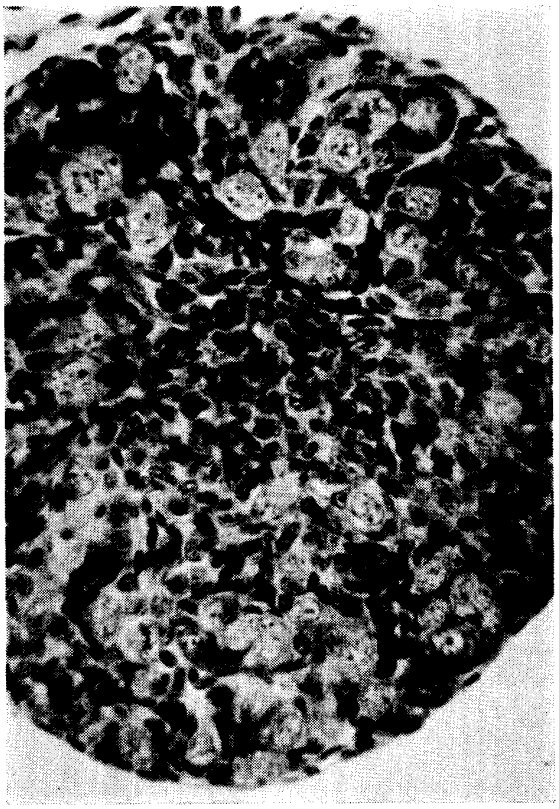

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