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Viability and Abnormalities of the Offspring of Nucleo-cytoplasmic Hybrids between *Rana japonica* and *Rana ornativentris*

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(With 17 Text-figures and 4 Plates)

CONTENTS

INTRODUCTION

Since the nuclear transfer method was devised by BRIGGS and KING (1952) in frogs, several authors have obtained diploid nucleo-cytoplasmic hybrids between two different species or subspecies of amphibians (BRIGGS and KING, 1952; SAMBUICHI, 1957, '61; MOORE, 1958, '60; MCKINNELL, 1960; GURDON, 1961, '62; HENNEN, 1963, '64, '65, '67; KAWAMURA and NISHIOKA, 1963b, c, '72; ORTOLANI, FISCHBERG and SLATKINE, 1966; GALLIEN, L., 1970; NISHIOKA, 1972a, b, c, d). Among the various interspecific combinations, nine kinds of nucleo-cytoplasmic hybrids attained their sexual maturity (KAWAMURA and NISHIOKA, GALLIEN, NISHIOKA), although three of them were quite sterile (NISHIOKA, 1972b, c, d).

By making use of two Japanese brown frog species, *Rana japonica* and *Rana ornativentris*, KAWAMURA and NISHIOKA (1963c) obtained many diploid nucleo-cytoplasmic hybrids of reciprocal combinations. Of these nucleo-cytoplasmic hybrids, three males which were constructed of *ornativentris* cytoplasm and *japonica* nuclei produced numerous first-generation offspring by mating with normal females of the nuclear species. However, many individuals among the offspring suffered from a severe edema and died at the hatching and the late tadpole stages. The remaining tadpoles completed their metamorphosis and grew normally. Among the offspring of two nucleo-cytoplasmic hybrids there were only males or a preponderant number of males over females, while there were nearly equal number of males and females among the offspring of the other male. Twenty-two male offspring of the three male nucleo-cytoplasmic hybrids were mated with normal females of *Rana japonica* for the purpose of examining their reproductive capacity. As the result, it was found that these male offspring were generally inferior to the control male *japonica*, that is, the degree of viability in the first-generation offspring of the male nucleo-cytoplasmic hybrids was not improved in the next generation. In the same season, mating experiments for examining the reproductive capacities of female offspring of the male nucleo-cytoplasmic hybrids remained to be performed, owing to sexual immaturity of these females. On the other hand, it was clarified that all the individuals produced from two female nucleo-cytoplasmic hybrids consisting of *ornativentris* cytoplasm and *japonica* nuclei by mating with normal male *japonica* or *ornativentris* revealed severe abnormalities and died at the hatching stage.

From these results of experiments performed by KAWAMURA and NISHIOKA, it seemed very interesting for them to investigate on the problem, at what generation the poor reproductive capacities of the first-generation offspring as well as the nucleo-cytoplasmic hybrids would be improved during the sequence of generations by repeated matings with normal wild *japonica*. For the purpose of solving this problem, the present writers have hitherto obtained a great many offspring of second to ninth generations of the above male nucleo-cytoplasmic hybrids by repeated matings with the nuclear species, *Rana japonica*. In this paper, the viability, chromosomal aberrations and sexual differentiation of the individuals of four generations, from the second to the fifth, are described in detail. Preliminary notes of these subjects have occasionally made by the present writers (KAWAMURA and NISHIOKA, 1964a, b, '65, '67).

MATERIAL AND METHODS

The starting point of the present research was the first-generation offspring of three male nucleo-cytoplasmic hybrids, Nos. 61OT2, 61AT8 and 61AT12, constructed of *Rana ornativentris* cytoplasm and *Rana japonica* nuclei. The production of these offspring has been reported by KAWAMURA and NISHIOKA (1963), together with that of the nucleo-cytoplasmic hybrids. The latter were produced in the breeding season of the year 1961 by the nuclear transfer method. Frogs of the nuclear species, *Rana japonica* GUENTHER, were collected from the suburbs of Hiroshima. However, a part of the frogs used for producing descendants of nucleo-cytoplasmic hybrids in the present research were those which had been reared since the egg stage in our laboratory as controls, while the other frogs were those collected freshly from the suburbs of Hiroshima.

Ovulation of females was induced by injection of salt solution containing mashed frog pituitaries. Mating experiments were always made by the routine method of artificial insemination. Embryos and tadpoles were raised in Cl-removed tap-water. Tadpoles were mainly fed on boiled spinach, while metamorphosed frogs were usually fed on flies or bag worms, and occasionally given with pieces of a cow or chicken liver by compulsion.

The chromosomes of embryos and tadpoles were examined by the squash method after pretreatment with distilled water, in accordance with MAKINO and NISHIMURA (1952). Embryos were first immersed in colcemide solution of 20 mg./*l.* concentration for 10~15 hours, in order to obtain many mitotic figures at the metaphase. After this treatment the head and tail were removed from each embryo and mounted with distilled water on a slide glass for 10~20 minutes after eliminating yolk granules as carefully as possible. Then they were fixed and stained with aceto-orcein for 20~30 minutes, heated mildly for 20~30 seconds and squashed.

Tadpoles were not immersed in colcemide solution, because it was necessary to keep them alive for a long time in a good condition. The original and a regenerated tail-tip of each tadpole were clipped off and utilized for chromosomal observation. After heated for a moment they were squashed and stained with aceto-orcein for 30~60 minutes. In the regenerated tail-tips mitotic figures at the metaphase were always abundantly found. The number of chromosomes in each embryo or 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. These chromosome analyses were done by making use of microphotographs enlarged at about 5,000 times.

The sex of young frogs which died or were killed within one or two weeks after metamorphosis was examined by observing serial sections of their gonads. According to the inner structures of their gonads the sex was divided into the following seven categories.

1. Normal female (♀$_N$) The ovaries are quite normal; they are filled with growing auxocytes (Plate I, 1).

2. Female with under-developed ovaries (♀$_U$) The ovaries have no or only a few growing auxocytes, that is, two or three, at most, in a section of an ovary, while there are numerous oogonia and young oocytes (Plate I, 2).

3. Hermaphrodite at the beginning of sex-reversal (⚥$_1$) Multiplication of rete cells is found in the medullary parts of the gonads. In the cortical parts, there are abundant oogonia and young oocytes (Plate I, 3 and 4).

4. Hermaphrodite at the middle stage of sex-reversal (⚥$_2$) Owing to distinct multiplication of rete cells, the gonads are testicular structure in the inner part at least. However, somewhat wide areas of ovarian structure are mostly left in the outer part as remnants of the cortical layer (Plate II, 5).

5. Hermaphrodite at the last stage of sex-reversal (⚥$_3$) The gonads are testes as a whole. Nearly all the gonia are surrounded with rete cells and there

are no ovarian cavities, although there are small groups of oocytes (Plate II, 6).

6. Normal male ($♂_N$) The gonads are typical testes. However, some males of this category have a few testis-ova or oocytes in their testes (Plate II, 7).

7. Male with rudimentary testes ($♂_R$) The testes are extremely small or their germ cells are very few (Plate II, 8).

The testes of matured males in their breeding season are classified into the following five types on the basis of the amount of spermatozoa found in cross-sections of seminal tubules.

Type 1. Seminal tubules are filled with bundles of numerous normal spermatozoa. Abnormal spermatozoa and pycnotic nuclei are rarely found (Plate III, 9).

Type 2. Spermatozoa contained in each seminal tubule are far fewer than those of Type 1. However, they make small and coarse bundles. Among these bundles there are considerably numerous pycnotic nuclei and abnormal spermatozoa (Plate III, 10).

Type 3. Seminal tubules are mostly filled with abnormal spermatozoa and pycnotic nuclei. Besides, a few small bundles of normal spermatozoa are distributed here and there (Plate III, 11 and 12).

Type 4. Seminal tubules are filled with abnormal spermatozoa and pycnotic nuclei. Although there are a few normal spermatozoa distributed sparsely, bundles of the latter are nowhere to be found (Plate IV, 13 and 14).

Type 5. No normal spermatozoa are found in seminal tubules. The testes are filled with abnormal spermatozoa and pycnotic nuclei (Plate IV, 15 and 16).

In the present paper the following signs, for example, are utilized to show a specific individual or kind of individuals.

J.W64 —— *Rana japonica* collected freshly from the field in 1964

J.L64 —— *Rana japonica* raised in the laboratory and matured in the season of 1964

J(61AT8) —— First-generation offspring between a female *Rana japonica* and the male nucleo-cytoplasmic hybrid No. 61AT8

$J\{J(61AT8)\}_4$ —— Second-generation offspring between a female *Rana japonica* and a male No. 4 of the first-generation offspring, J(61AT8)

$J[J\{J(61AT8)\}_4]_2$ —— Third-generation offspring between a female *Rana japonica* and a male No. 2 of the second-generation offspring, $J\{J(61AT8)\}_4$

$J[J[J\{J(61AT8)\}_4]_2]_3$ —— Fourth-generation offspring between a female *Rana japonica* and a male No. 3 of the third-generation offspring, $J[J\{J(61AT8)\}_4]_2$

The description of developmental stages follows those of *Rana pipiens* established by Shumway(1940) and Taylor and Kollros(1946) for convenience' sake.

OBSERVATION

I. Results of mating experiments performed in 1964

1. Production of second-generation offspring

a. Parents

The two male nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8 which were produced from enucleated *ornativentris* eggs transplanted with blastula nuclei of *japonica* in the breeding season of the year 1961, were mated with female *japonica* in the next year (KAWAMURA and NISHIOKA, 1963c). By using males and females of the first-generation offspring, mating experiments were performed to produce second-generation offspring in the breeding season of the year 1964. Besides brother and sister mating, these males and females were mated with those collected from the field or raised in our laboratory since their egg stage.

i) Female parents

Twelve females in all were used for producing second-generation offspring of the two male nucleo-cytoplasmic hybrids. The body lengths and egg sizes of these female parents are presented in Table 1. Four of six female *japonica* were those which had been reared for two years from the eggs, in our laboratory, while the other two were those collected from the field. The former females were 49~50.5 mm. in body length, while the latter were 53 and 54 mm. Three females (Nos. 1~3) of first-generation offspring of each of the nucleo-cytoplasmic hybrids Nos. J(61OT2) and J(61AT8) were 47~50 mm. and 50~51 mm. in body length, respectively. For the purpose of comparing the eggs of the first-generation offspring with those of the control *japonica*, the diameters of 50 eggs taken out of each female at random were measured. As a result, the eggs of six female *japonica* were 1.45~1.60 mm. in mean diameter, while those of six female first-generation offspring were 1.41~1.64 mm.

TABLE 1

Female first-generation offspring of two nucleo-cytoplasmic hybrids and the control frogs used in the mating experiments performed in 1964

Offspring		Age	Body length	Mean diameter of 50 eggs
Kind	Individual no.	(year)	(mm.)	(mm.)
J.L64	9	2	50.5	1.60±0.03
	10	2	50.0	1.55±0.03
	11	2	49.0	1.45±0.03
	12	2	49.0	1.52±0.03
J.W64	13		53.0	1.57±0.03
	14		54.0	1.56±0.03
J(61OT2)	1	2	47.0	1.53±0.03
	2	2	47.0	1.58±0.03
	3	2	50.0	1.63±0.03
J(61AT8)	1	2	50.0	1.41±0.03
	2	2	51.0	1.64±0.03
	3	2	50.5	1.55±0.03

Example of the sign of each kind:

J———— *Rana japonica*

J(61OT2) — Offspring of the male nucleo-cytoplasmic hybrid No. 61OT2 mated with a female control *japonica*

J(61AT8) — Offspring of the male nucleo-cytoplasmic hybrid No. 61AT8 mated with a female control *japonica*

ii) Male parents

Six male *japonica* and six male first-generation offspring of the two male nucleo-cytoplasmic hybrids were used for producing second-generation offspring (Table 2). Three (Nos. 5~7) of the six *japonica* were those collected from the field. They were 45~47.5 mm. in body length. The other three (Nos. 8~10) were those which had been reared for two years since the egg stage in our laboratory. While one of them (No. 8) was small, that is, 42 mm. in body length, the remaining two frogs Nos. 9 and 10 were 47.5 and 47 mm. While the testes of the smallest male were small in size, those of the five male *japonica* were 3.2 mm. in length and 2.2 mm. in width on the average. In the inner structure of testes, all the six males were of Type 1, that is, quite normal.

TABLE 2
Male first-generation offspring of two nucleo-cytoplasmic hybrids and the control frogs used in the mating experiments performed in 1964

Offspring		Age	Body length	Size of the testes		Inner structure	
Kinds	Individual no.	(year)	(mm.)	Left (mm.)	Right (mm.)	Type	Ploidy
J.W64	5		45	3.0×2.0	3.0×2.0	1	2n
	6		47	3.5×2.0	3.5×2.0	1	2n
	7		47.5	3.5×2.5	3.5×2.0	1	2n
J.L64	8	2	42	2.0×1.0	2.0×1.5	1	2n
	9	2	47.5	3.0×2.5	3.0×2.0	1	2n
	10	2	47	3.0×2.0	3.0×2.0	1	2n
J(61OT2)	10	2	45	3.0×2.0	3.0×2.0	1	2n
	11	2	46.5	3.0×2.0	3.0×2.0	1	2n
	12	2	47.5	3.5×2.5	3.5×2.5	2	2n
J(61AT8)	8	2	45.5	2.5×2.0	2.5×2.0	2	2n
	9	2	46	2.5×2.0	2.6×2.0	2	2n
	10	2	47	3.0×2.5	3.0×2.0	3	2n

J ——— *Rana japonica*
J(61OT2) — Offspring of the male nucleo-cytoplasmic hybrid No. 61OT2 mated with a female control *japonica*
J(61AT8) — Offspring of the male nucleo-cytoplasmic hybrid No. 61AT8 mated with a female control *japonica*

Three male first-generation offspring of each of the nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8 were 45~47.5 mm. and 45.5~47 mm. in body length, respectively. The testes of the offspring of No. 61AT8 were 2.7 mm. in length and 2.1 mm. in width on the average and of Type 2 or 3 in inner structure. Those of the offspring of No. 61OT2 were 3.2 mm. in length and 2.2 mm. in width on the average and of Type 1 or 2.

b. Mating experiments by using male and female first-generation offspring of the male nucleo-cytoplasmic hybrid No. 61OT2 (Table 3)

Set 1. A female No. 1 and a male No. 10

Mating 1. J(61OT2)♀ No. 1×J.L64♂ No. 8

Of 63 (49.6%) normally cleaved eggs two at the neurula and four at the tail-bud stage, died of various types of abnormalities. All the remaining 57 became edematous and died at the hatching stage(Fig. 1, b).

Mating 2. J(61OT2)♀ No. 1×J(61OT2)♂ No. 10

There were 74 (47.4%) normally cleaved eggs; one at the gastrula, three at

TABLE 3

Reproductive capacities of female first-generation offspring of the two male

Set	(Mating)	Parents: Female no.	Parents: Male no.	No. of eggs	No. of cleaved eggs: Normal	No. of cleaved eggs: Abnormal	No. of gastrulae: Normal	No. of gastrulae: Abnormal
1	(1)	J(61OT2).1	J.L64.8	127	63 (49.6%)	0	63 (49.6%)	0
	(2)		J(61OT2).10	156	74 (47.4%)	0	73 (46.8%)	1 (0.6%)
	(3)	J.L64.12	J.L64.8	55	53 (96.4%)	0	53 (96.4%)	0
	(4)		J(61OT2).10	116	61 (52.6%)	0	61 (52.6%)	0
2	(1)	J(61OT2).2	J.L64.9	195	143 (73.3%)	0	143 (73.3%)	0
	(2)		J(61OT2).11	232	170 (73.3%)	0	170 (73.3%)	0
	(3)	J.W64.13	J.L64.9	60	58 (96.7%)	0	58 (96.7%)	0
	(4)		J(61OT2).11	131	127 (96.9%)	0	125 (95.4%)	2 (1.5%)
3	(1)	J(61OT2).3	J.L64.10	154	147 (95.5%)	0	144 (93.5%)	0
	(2)		J(61OT2).12	117	112 (95.7%)	0	112 (95.7%)	0
	(3)	J.W64.14	J.L64.10	129	125 (96.9%)	0	125 (96.9%)	0
	(4)		J(61OT2).12	105	75 (71.4%)	0	72 (68.6%)	0
4	(1)	J(61AT8).1	J.W64.5	75	15 (20.0%)	0	15 (20.0%)	0
	(2)		J(61AT8).8	64	23 (35.9%)	0	21 (32.8%)	3 (4.7%)
	(3)	J.L64.9	J.W64.5	134	126 (94.0%)	0	126 (94.0%)	0
	(4)		J(61AT8).8	121	45 (37.2%)	0	42 (34.7%)	3 (2.5%)
5	(1)	J(61AT8).2	J.W64.6	112	67 (59.8%)	5 (4.5%)	65 (58.0%)	2 (1.8%)
			J(61AT8).9	75	56 (74.7%)	4 (5.3%)	56 (74.7%)	0
	(3)	J.L64.10	J.W64.6	84	80 (95.2%)	0	78 (92.9%)	2 (2.4%)
	(4)		J(61AT8).9	63	50 (79.4%)	0	47 (74.6%)	3 (4.8%)
6	(1)	J(61AT8).3	J.W64.7	120	98 (81.7%)	19 (15.8%)	98 (81.7%)	0
	(2)		J(61AT8).10	143	43 (30.1%)	5 (3.5%)	43 (30.1%)	0
	(3)	J.L64.11	J.W64.7	52	50 (96.2%)	0	50 (96.2%)	0
	(4)		J(61AT8).10	93	19 (20.4%)	4 (4.3%)	19 (20.4%)	0

J ———— *Rana japonica*

J(61OT2) — Offspring of the male nucleo-cytoplasmic hybrid No. 61OT2 mated with a female control *japonica*

J(61AT8) — Offspring of the male nucleo-cytoplasmic hybrid No. 61AT8 mated with a female control *japonica* the neurula and six at the tail-bud stage, died of various types of abnormalities. All the remaining 64 became edematous and died at the hatching stage.

Mating 3, Control. J.L64♀ No. 12×J.L64♂ No. 8

In this control group 53 (96.4%) eggs cleaved and developed normally into

nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8

No. of neuruale		No. of tail-bud embryos		No. of hatched tadpoles		No. of St. VI tadpoles		No. of metamorphosed frogs	
Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.
61 (48.0%)	2 (1.6%)	57 (44.9%)	4 (3.1%)	0	57 (44.9%)	0	0	0	0
70 (44.9%)	3 (1.9%)	64 (41.0%)	6 (3.8%)	0	64 (41.0%)	0	0	0	0
53 (96.4%)	0	53 (96.4%)	0	53 (96.4%)	0	52 (94.5%)	1 (1.8%)	52 (94.5%)	0
54 (46.6%)	7 (6.0%)	50 (43.1%)	4 (3.4%)	47 (40.5%)	3 (2.6%)	42 (36.2%)	5 (4.3%)	40 (34.5%)	0
116 (59.5%)	27 (13.8%)	107 (54.9%)	9 (4.6%)	92 (47.2%)	15 (7.7%)	76 (39.0%)	16 (8.2%)	67 (34.4%)	0
146 (62.9%)	24 (10.3%)	121 (52.2%)	25 (10.8%)	121 (52.2%)	0	102 (44.0%)	19 (8.2%)	94 (40.5%)	0
58 (96.7%)	0	58 (96.7%)	0	58 (96.7%)	0	57 (95.0%)	1 (1.7%)	57 (95.0%)	0
124 (94.7%)	1 (0.8%)	121 (92.4%)	3 (2.3%)	115 (87.8%)	6 (4.6%)	108 (82.4%)	7 (5.3%)	102 (77.9%)	0
141 (91.6%)	3 (1.9%)	134 (87.0%)	7 (4.5%)	70 (45.5%)	64 (41.6%)	21 (13.6%)	49 (31.8%)	0	2 (1.3%)
110 (94.0%)	2 (1.7%)	102 (87.2%)	8 (6.8%)	63 (53.8%)	39 (33.3%)	23 (19.7%)	40 (34.2%)	0	4 (3.4%)
125 (96.9%)	0	125 (96.9%)	0	124 (96.1%)	1 (0.8%)	123 (95.3%)	1 (0.8%)	123 (95.3%)	0
68 (64.8%)	4 (3.8%)	56 (53.3%)	12 (11.4%)	39 (37.1%)	17 (16.2%)	17 (16.2%)	22 (21.0%)	0	4 (3.8%)
4 (5.3%)	11 (14.7%)	0	4 (5.3%)	0	0	0	0	0	0
3 (4.7%)	18 (28.1%)	2 (3.1%)	1 (1.6%)	0	2 (3.1%)	0	0	0	0
125 (93.3%)	1 (0.7%)	125 (93.3%)	0	125 (93.3%)	0	125 (93.3%)	0	125 (93.3%)	0
37 (30.6%)	5 (4.1%)	35 (28.9%)	2 (1.7%)	30 (24.8%)	5 (4.1%)	20 (16.5%)	10 (8.3%)	15 (12.4%)	0
61 (54.5%)	4 (3.6%)	0	61 (54.5%)	0	0	0	0	0	0
56 (74.7%)	0	0	56 (74.7%)	0	0	0	0	0	0
78 (92.9%)	0	78 (92.9%)	0	78 (92.9%)	0	78 (92.9%)	0	78 (92.9%)	0
37 (58.7%)	10 (15.9%)	37 (58.7%)	0	31 (49.2%)	6 (9.5%)	4 (6.3%)	27 (42.9%)	0	4 (6.3%)
92 (76.7%)	6 (5.0%)	86 (71.7%)	6 (5.0%)	61 (50.8%)	25 (20.8%)	47 (39.2%)	14 (11.7%)	0	15 (12.5%)
32 (22.4%)	11 (7.7%)	29 (20.3%)	3 (2.1%)	22 (15.4%)	7 (4.9%)	11 (7.7%)	11 (7.7%)	0	7 (4.9%)
50 (96.2%)	0	50 (96.2%)	0	49 (94.2%)	1 (1.9%)	49 (94.2%)	0	49 (94.2%)	0
16 (17.2%)	3 (3.2%)	15 (16.1%)	1 (1.1%)	12 (12.9%)	3 (3.2%)	7 (7.5%)	5 (5.4%)	5 (5.4%)	0

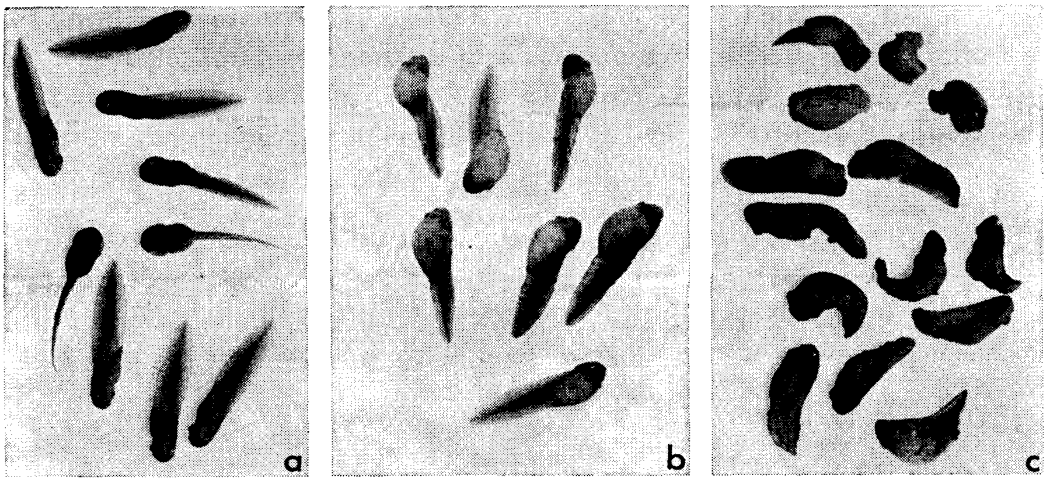

Fig. 1. Abnormalities in the second-generation offspring of the two male nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8. ×2
a, Control *Rana japonica* tadpoles at the hatching stage. b, Ascitic tadpoles among the second-generation offspring produced by mating, J(61OT2)♀ No. 1×J.L64♂ No. 8. c, Abnormal embryos among the second-generation offspring produced by mating, J.W64 ♀ No. 14×J(61OT2)♂ No. 12.

tadpoles. All the tadpoles were normally metamorphosed, except for one which died at the VI stage.

Mating 4. J.L64♀ No. 12×J(61OT2)♂ No. 10

Sixty-one (52.6%) eggs cleaved normally. Seven at the neurula, four at the tail-bud, three at the hatching, five at the VI and two at the XVII stage, died of various types of abnormalities. The remaining 40 tadpoles completed their metamorphosis.

Set 2. A female No. 2 and a male No. 11

Mating 1. J(61OT2)♀ No. 2×J.L64♂ No. 9

There were 143 (73.3%) normally cleaved eggs. Twenty-seven at the neurula, nine at the tail-bud and 15 at the hatching stage died of various types of abnormalities. Sixteen others died of ill-development at the VI stage and nine died of edema by the XVII stage. The other 67 developed into normal tadpoles and completed their metamorphosis.

Mating 2. J(61OT2)♀ No. 2×J(61OT2)♂ No. 11

The normally cleaved eggs were 170 (73.3%) in number. Twenty-four and 25 embryos died of edema at the neurula and the tail-bud stage, respectively. Nineteen tadpoles died of ill-development by the VI stage. Eight others died by the XX stage and the other 94 were normally metamorphosed.

Mating 3, Control. J.W64♀ No. 13×J.L64♂ No. 9

In this control group, 58 (96.7%) eggs cleaved normally. All the eggs developed normally into tadpoles and completed their metamorphosis, except for one which died at the VI stage.

Mating 4. J.W64♀ No. 13×J(61OT2)♂ No. 11

The rate of fertilization was very high and 127(96.9%) eggs cleaved normally. However, 12 embryos died of various types of abnormalities by the hatching stage.

Seven tadpoles died of ill-development by the VI stage and six died of edema by the XVIII stage. All the remaining 102 tadpoles were normally metamorphosed.

Set 3. A female No. 3 and a male No. 12

Mating 1. J(61OT2)♀ No. 3×J.L64♂ No. 10

The rate of fertilization was very high and 147(95.5%) eggs cleaved normally. Six by the neurula, seven at the tail-bud, 64 at the hatching and 49 by the VI stage, died of edema. Nineteen of the other tadpoles died at the metamorphosing stage: they had ill-developed forelegs and incompletely degenerated tails. Although the remaining two tadpoles completed their metamorphosis, their forelegs were abnormal.

Mating 2. J(61OT2)♀ No. 3×J(61OT2)♂ No. 12

The rate of fertilization was very high and 112(95.7%) eggs cleaved normally. Two at the neurula, eight at the tail-bud, and 39 at the hatching stage, died of edema. The other 63 developed into tadpoles. However, 40 of them became suddenly thin since the V or VI stage and died soon afterward. Four died of edema at the XVIII stage and 15 died at the XXI or XXII stage, without protruding their forelegs. The remaining four completed their metamorphosis, although they had no forelegs.

Mating 3, Control. J.W64♀ No. 14×J.L64♂ No. 10

There were 125(96.9%) normally cleaved eggs. Two of them died at the hatching or feeding stage and the others grew into tadpoles which were normally metamorphosed.

Mating 4. J.W64♀ No. 14×J(61OT2)♂ No. 12

Although there were 75(71.4%) normally cleaved eggs, seven by the neurula, 12 at the tail-bud, 17 at the hatching and 22 by the VI tadpole stage, died of various types of abnormalities (Fig. 2, c). Three of the others died of edema at the XVII or XVIII stage. All the remaining 14 had abnormal forelegs; ten died at the XXI or XXII stage and the other four completed their metamorphosis.

c. Mating experiments by using male and female first-generation offspring of the male nucleo-cytoplasmic hybrid No. 61AT8 (Table 3)

Set 4. A female No. 1 and a male No. 8

Mating 1. J(61AT8)♀ No. 1×J.W64♂ No. 5

Normally cleaved eggs were 15(20.0%) in number. All of them died of various types of abnormalities at the neurula and tail-bud stages.

Mating 2. J(61AT8)♀ No. 1×J(61AT8)♂ No. 8

There were 23(35.9%) normally cleaved eggs. However, all of them died of various types of abnormalities by the hatching stage, that is, three at the gastrula, 18 at the neurula, one at the tail-bud and two at the hatching stage.

Mating 3, Control. J.L64♀ No. 9×J.W64♂ No. 5

In this control group the rate of fertilization was very high: 126(94.0%) eggs cleaved normally. All of them developed normally into tadpoles which completed their metamorphosis, except for one embryo. All these metamorphosed frogs were quite normal in external characters.

Mating 4. J.L64♀ No. 9×J(61AT8)♂ No. 8

Although there were 45(37.2%) normally cleaved eggs, 15 of them died of various types of abnormalities at various stages: three at the gastrula, five at the neurula, two at the tail-bud and five at the hatching stage. The other 30 developed normally into feeding tadpoles. However, 10 tadpoles died of ill-development at the IV stage and five others died of edema at the XVII stage. Fifteen tadpoles eventually completed their metamorphosis.

Set 5. A female No. 2 and a male No. 9

Mating 1. J(61AT8)♀ No. 2×J.W64♂ No. 6

There were 67(59.8%) normally cleaved eggs. Two of them died at the gastrula stage, while four others died at the neurula. All the remaining 61 embryos died simultaneously of edema at the tail-bud stage (Fig. 2).

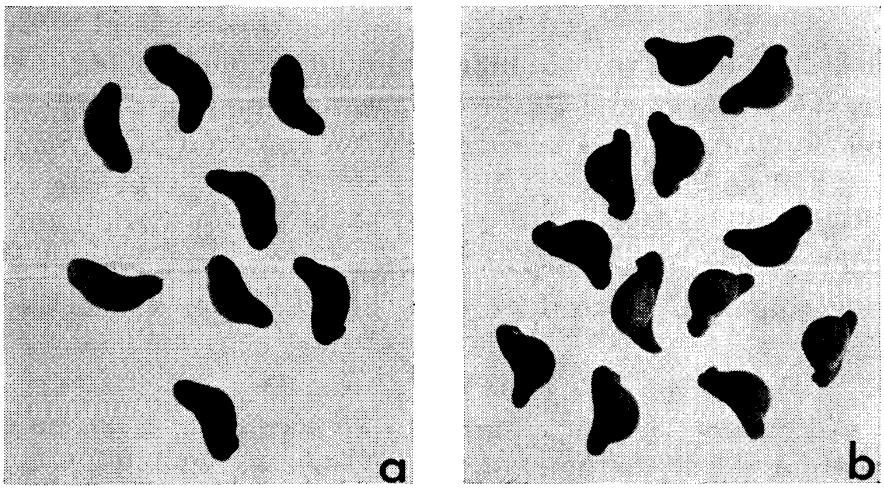

Fig. 2. Edematous embryos among the second-generation offspring of a nucleo-cytoplasmic hybrid. ×2.5
a, Control *Rana japonica* embryos at the tail-bud stage. b, Edematous second-generation offspring produced by mating, J(61AT8) ♀ No. 2×J.W64♂ No. 6.

Mating 2. J(61AT8)♀ No. 2×J(61AT8)♂ No. 9

Fifty-six (74.7%) cleaved and developed normally until their tail-bud stage. However, all of them became edematous simultaneously and died soon later.

Mating 3, Control. J.L64♀ No. 10×J.W64♂ No. 6

The rate of fertilization was very high: there were 80(95.2%) normally cleaved eggs. All but two of them developed normally into tadpoles and completed their metamorphosis.

Mating 4. J.L64♀ No. 10×J(61AT8)♂ No. 9

The rate of fertilization was comparatively high. Of 50(79.4%) normally cleaved eggs three at the gastrula, 10 at the neurula and six at the hatching stage, died of various types of abnormalities. Twenty-seven of the remaining 31 tadpoles were ill-developed at the IV stage and died later. The other four developed normally and completed their metamorphosis. However, the forelegs were so poor in development that they could not support the bodies, while the hind legs were quite normal.

Set 6. A female No. 3 and a male No. 10

Mating 1. J(61AT8)♀ No. 3×J.W64♂ No. 7

The rate of fertilization was very high and 98(81.7%) eggs cleaved normally. However, six at the neurula, six at the tail-bud, 25 at the hatching, 14 at the IV stage and 17 at the XII～XVII stages, died of edema. Although the other 30 grew normally during the period of their tadpole stages, 15 of them died at the matemorphosing stage; their forelegs did not protrude, while the degeneration of their tails was in progress. In the remaining 15 tadpoles the forelegs hardly protruded or they were so slender and abnormal that they could not support the bodies, although the tails degenerated perfectly.

Mating 2. J(61AT8)♀ No. 3×J(61AT8)♂ No. 10

Of 43(30.1%) normally cleaved eggs 11 at the neurula, three at the tail-bud, seven at the hatching and 11 at the tadpole stage, died of edema. Although the other 11 tadpoles grew normally, four died at the metamorphosing stage and seven died within one week after the completion of metamorphosis. All these frogs had abnormal forelegs.

Mating 3, Control. J.L64♀ No. 11×J.W64♂ No. 7

The rate of fertilization was very high. All the 50 normally cleaved eggs developed normally into normal tadpoles, except that one died at the hatching stage. All the tadpoles were normally metamorphosed.

Mating 4. J.L64♀ No. 11×J(61AT8)♂ No. 10

There were 19(20.4%) normally cleaved eggs; seven died of various types of abnormalities by the hatching stage, five were ill-developed and died at the VI stage and two died of edema at the XVII stage. The remaining five completed normally their metamorphosis.

2. Production of third-generation offspring

a. Parents

For the purpose of obtaining third-generation offspring of the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12 which had developed from enucleated eggs of *Rana ornativentris* transplanted with blastula nuclei of *Rana japonica* in the breeding season of the year 1961, the following procedures were taken. First-generation offspring of these male nucleo-cytoplasmic hybrids were obtained by mating with female *japonica* in the breeding season of the year 1962. Next, second-generation offspring of the male nucleo-cytoplasmic hybrids were obtained by mating of males of the first-generation offspring with female *japonica* in the breeding season of 1963. The results of experiments concerning the production of these nucleo-cytoplasmic hybrids and their first- and second-generation offspring were already reported by us in a previous paper (Kawamura and Nishioka, 1963c). Six female and twelve male second-generation offspring obtained from the matings, J♀×J(61OT2)♂, Nos. 1, 4, 8 and 9, two female and six male second-generation offspring from the matings, J♀×J(61AT8)♂, Nos. 4 and 6, and four female and four male second-generation offspring from the matings, J♀ ×J(61AT12)♂, Nos. 3 and 6, were mated with male and female *japonica* raised in our laboratory or collected from the field.

i) Female parents

In order to produce third-generation offspring, twenty females in all were utilized (Table 4). Twelve of them were 1-year-old second-generation offspring of the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12, while five others were female *japonica*, reared for a year since the egg stage in our laboratory and the other three were those collected from the field. The twelve second-generation offspring were 33.5~49 mm. in body length and 1.40~1.66 mm. in the mean diameter of 50 eggs, while the eight *japonica* were 35.5~47.5 mm. and 1.46~1.62 mm., respectively. Accordingly, there was no distinct difference in egg size between those of the second-generation offspring and normal *japonica*.

TABLE 4

Female second-generation offspring of three nucleo-cytoplasmic hybrids and the control frogs used in the mating experiments performed in 1964

Second-generation offspring		Age	Body length	Mean diameter of 50 eggs
Kind	Individual no.	(year)	(mm.)	(mm.)
J.L64	1	1	45.0	1.62±0.03
	2	1	42.0	1.57±0.03
	3	1	35.5	1.46±0.03
J.W64	4		46.0	1.60±0.04
	5		47.5	1.60±0.04
	6		42.5	1.52±0.03
J.L64	7	1	40.5	1.47±0.03
	8	1	41.0	1.50±0.03
J{J(61OT2)}$_9$	1	1	33.5	1.45±0.03
J{J(61OT2)}$_8$	1	1	40.0	1.50±0.03
	2	1	49.0	1.66±0.03
	3	1	41.0	1.54±0.03
	4	1	41.0	1.50±0.03
	5	1	42.0	1.49±0.03
J{J(61AT8)}$_4$	1	1	40.5	1.62±0.04
	2	1	41.0	1.60±0.04
J{J(61AT12)}$_3$	1	1	45.0	1.60±0.03
	2	1	43.5	1.55±0.03
J{J(61AT12)}$_6$	1	1	39.0	1.40±0.03
	2	1	40.5	1.46±0.03

Example of the sign of each kind:

J———— *Rana japonica*

J(61AT8)———— First-generation offspring of the male nucleo-cytoplasmic hybrid No. 61AT8 mated with a female *japonica*

J{J(61AT8)}$_4$———— Second-generation offspring of a male No. 4 of the first-generation offspring No. J(61AT8) mated with a female *japonica*

ii) Male parents

As normal *japonica*, four males were utilized for mating experiments (Table 5). Two of them were those reared for a year since the egg stage in our laboratory and 42.5 and 45 mm. in body length. Their testes were 1.8 mm. in length and 1.1 mm. in width on the average and of Type 1 in inner structure. The other two males

TABLE 5

Male second-generation offspring of three nucleo-cytoplasmic hybrids and the control frogs used in the mating experiments performed in 1964

Second-generation offspring		Age	Body length	Size of the testes		Inner structure	
Kind	Individual no.	(year)	(mm.)	Left (mm.)	Right (mm.)	Type	Ploidy
J.L64	2	1	45.0	1.5×1.0	1.5×1.0	1	2n
	4	1	42.5	2.0×1.5	2.0×1.0	1	2n
J.W64	1		48.0	2.5×1.5	2.5×1.5	1	2n
	3		47.0	2.5×1.5	2.5×1.5	1	2n
J{J(61OT2)}1	1	1	43.0	2.0×1.5	2.0×1.5	2	2n
	2	1	43.0	1.5×1.0	2.0×1.0	4	2n
	3	1	42.0	1.5×1.0	1.5×1.0	2	2n
	4	1	41.0	2.0×1.5	2.0×1.0	4	2n
	5	1	41.0	2.0×1.5	2.0×1.5	1	2n
	6	1	40.5	1.5×1.0	1.5×1.0	4	2n
J{J(61OT2)}4	1	1	40.0	2.0×1.0	2.0×1.0	4	2n
	2	1	35.5	2.0×1.0	2.0×1.0	3	2n
	3	1	41.0	1.5×1.0	2.0×1.0	5	2n
	4	1	40.5	1.5×1.0	1.0×1.0	5	2n
J{J(61OT2)}9	1	1	42.0	2.0×1.0	2.0×1.0	1	2n
	2	1	42.0	2.0×1.5	2.0×1.5	2	2n
J{J(61AT8)}4	1	1	43.0	2.0×1.5	2.0×1.5	4	2n
	2	1	41.5	2.5×1.5	2.5×1.5	1	2n
J{J(61AT8)}6	1	1	40.5	2.0×1.5	2.0×1.0	4	2n
	2	1	42.0	2.0×1.0	2.5×1.0	3	2n
	3	1	43.0	1.0×1.0	1.0×0.5	5	2n
	4	1	42.5	2.5×1.5	2.0×1.5	1	2n
J{J(61AT12)}3	1	1	40.0	2.0×1.5	2.0×1.5	1	2n
	2	1	40.5	2.5×1.5	2.5×2.0	2	2n
J{J(61AT12)}6	1	1	41.0	2.0×1.5	2.0×1.5	1	2n
	2	1	42.5	2.0×1.5	2.0×1.5	1	2n

were collected from the field. They were 47 and 48 mm. in body length. Their testes were 2.5 mm. in length and 1.5 mm. in width and of Type 1 in inner structure.

All the 22 second-generation males, used for producing third-generation offspring of nucleo-cytoplasmic hybrids were one year old. Twelve, six and four of them were offspring originated from the three nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12, respectively. Six (Nos. 1∼6) of the first twelve were those produced by mating between a female *japonica* and a first-generation male No. 1 of the nucleo-cytoplasmic hybrid No. 61OT2, while the other six were by mating with two first-generation males Nos. 4 and 9. Of the second six males, two were those obtained by mating between a female *japonica* and a first-generation male No. 4 of the nucleo-cytoplasmic hybrid No. 61AT8 and four were obtained by mating with another first-generation male No. 6. The third four males came from the nucleo-cytoplasmic hybrid No. 61AT12. Two of them were produced by mating between a female *japonica* and a first-generation male No. 3, while the other two were by mating with another first-generation male No. 6.

TABLE 6

Reproductive capacities of second-generation offspring

Set	(Mating)	Parents: Female no.	Parents: Male no.	No. of eggs	No. of cleaved eggs: Normal	No. of cleaved eggs: Abnormal	No. of gastrulae: Normal	No. of gastrulae: Abnormal
1	(1)	J{J(61OT2)}$_{9}$.1	J.W64.3	31	26 (83.9%)	0	26 (83.9%)	0
	(2)		J{J(61OT2)}$_{9}$.1	57	30 (52.6%)	0	30 (52.6%)	0
	(3)	J.L64.7～8	J{J(61OT2)}$_{9}$.1	75	45 (60.0%)	0	45 (60.0%)	0
	(4)		J{J(61OT2)}$_{9}$.2	55	10 (18.2%)	2 (3.6%)	10 (18.2%)	0
2	(1)	J{J(61OT2)}$_{8}$.1	J.W64.3	57	10 (17.5%)	14 (24.6%)	9 (15.8%)	1 (1.8%)
	(2)		J{J(61OT2)}$_{1}$.1	136	5 (3.7%)	21 (15.4%)	5 (3.7%)	0
	(3)	J{J(61OT2)}$_{8}$.2	J.W64.3	140	20 (14.3%)	4 (2.9%)	20 (14.3%)	0
	(4)		J{J(61OT2)}$_{1}$.2	168	1 (0.6%)	12 (7.1%)	1 (0.6%)	0
	(5)	J{J(61OT2)}$_{8}$.3	J.W64.3	51	50 (98.0%)	0	50 (98.0%)	0
	(6)		J{J(61OT2)}$_{1}$.3	142	4 (2.8%)	27 (19.0%)	4 (2.8%)	0
	(7)	J{J(61OT2)}$_{8}$.4	J.W64.3	213	22 (10.3%)	59 (27.7%)	22 (10.3%)	0
	(8)		J{J(61OT2)}$_{1}$.4	143	3 (2.1%)	36 (25.2%)	3 (2.1%)	0
	(9)	J{J(61OT2)}$_{8}$.5	J.W64.3	110	75 (68.2%)	0	75 (68.2%)	0
	(10)		J{J(61OT2)}$_{1}$.5	151	146 (96.7%)	0	146 (96.7%)	0
	(11)	J.L64.7～8	J.W64.3	40	38 (95.0%)	0	38 (95.0%)	0
	(12)		J{J(61OT2)}$_{1}$.1	121	30 (24.8%)	5 (4.1%)	30 (24.8%)	0
	(13)		J{J(61OT2)}$_{1}$.2	154	3 (1.9%)	0	3 (1.9%)	0
	(14)		J{J(61OT2)}$_{1}$.3	75	21 (28.0%)	0	21 (28.0%)	0
	(15)		J{J(61OT2)}$_{1}$.4	70	3 (4.3%)	0	3 (4.3%)	0
	(16)		J{J(61OT2)}$_{1}$.5	64	26 (40.6%)	0	26 (40.6%)	0
	(17)		J{J(61OT2)}$_{1}$.6	51	1 (2.0%)	0	1 (2.0%)	0
3	(1)	J.L64.7～8	J{J(61OT2)}$_{4}$.1	86	11 (12.8%)	4 (4.7%)	9 (10.5%)	2 (2.3%)
	(2)		J{J(61OT2)}$_{4}$.2	42	3 (7.1%)	0	3 (7.1%)	0
	(3)		J{J(61OT2)}$_{4}$.3	50	1 (2.0%)	0	1 (2.0%)	0
	(4)		J{J(61OT2)}$_{4}$.4	62	0	0	0	0

of the male nucleo-cytoplasmic hybrid No. 61OT2

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of St.VI tadpoles		No. of metamorphosed frogs	
Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.
26 (83.9%)	0	26 (83.9%)	0	22 (71.0%)	4 (12.9%)	22 (71.0%)	0	17 (54.8%)	0
30 (52.6%)	0	30 (52.6%)	0	28 (49.1%)	2 (3.5%)	26 (45.6%)	2 (3.5%)	21 (36.8%)	0
44 (58.7%)	1 (1.3%)	44 (58.7%)	0	41 (54.7%)	3 (4.0%)	41 (54.7%)	0	37 (49.3%)	0
10 (18.2%)	0	6 (10.9%)	4 (7.3%)	6 (10.9%)	0	6 (10.9%)	0	1 (1.8%)	5 (9.1%)
9 (15.8%)	0	9 (15.8%)	0	9 (15.8%)	0	9 (15.8%)	0	0	9 (15.8%)
5 (3.7%)	0	4 (2.9%)	1 (0.7%)	4 (2.9%)	0	4 (2.9%)	0	0	4 (2.9%)
20 (14.3%)	0	20 (14.3%)	0	20 (14.3%)	0	15 (10.7%)	5 (3.6%)	0	8 (5.7%)
1 (0.6%)	0	1 (0.6%)	0	1 (0.6%)	0	1 (0.6%)	0	0	1 (0.6%)
50 (98.0%)	0	36 (70.6%)	14 (27.5%)	30 (58.8%)	6 (11.8%)	28 (54.9%)	2 (3.9%)	0	24 (47.1%)
4 (2.8%)	0	4 (2.8%)	0	3 (2.1%)	1 (0.7%)	3 (2.1%)	0	0	3 (2.1%)
22 (10.3%)	0	22 (10.3%)	0	22 (10.3%)	0	20 (9.4%)	2 (0.9%)	0	8 (3.8%)
3 (2.1%)	0	0	3 (2.1%)	0	0	0	0	0	0
75 (68.2%)	0	75 (68.2%)	0	70 (63.6%)	5 (4.5%)	70 (63.6%)	0	65 (59.1%)	0
143 (94.7%)	3 (2.0%)	136 (90.1%)	7 (4.6%)	119 (78.8%)	17 (11.3%)	119 (78.8%)	0	71 (47.0%)	0
38 (95.0%)	0	37 (92.5%)	1 (2.5%)	37 (92.5%)	0	37 (92.5%)	0	37 (92.5%)	0
30 (24.8%)	0	30 (24.8%)	0	28 (23.1%)	2 (1.7%)	28 (23.1%)	0	28 (23.1%)	0
3 (1.9%)	0	3 (1.9%)	0	2 (1.3%)	1 (0.6%)	2 (1.3%)	0	0	1 (0.6%)
21 (28.0%)	0	1 (1.3%)	20 (26.7%)	1 (1.3%)	0	1 (1.3%)	0	0	1 (1.3%)
3 (4.3%)	0	3 (4.3%)	0	3 (4.3%)	0	3 (4.3%)	0	3 (4.3%)	0
25 (39.1%)	1 (1.6%)	24 (37.5%)	1 (1.6%)	24 (37.5%)	0	24 (37.5%)	0	24 (37.5%)	0
1 (2.0%)	0	1 (2.0%)	0	1 (2.0%)	0	1 (2.0%)	0	1 (2.0%)	0
1 (1.2%)	8 (9.3%)	0	1 (1.2%)	0	0	0	0	0	0
3 (7.1%)	0	2 (4.8%)	1 (2.4%)	2 (4.8%)	0	2 (4.8%)	0	2 (4.8%)	0
0	1 (2.0%)	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0

All the first-generation males were 40∼43 mm. in body length, except one male. The testes of these 22 females were 1.9 mm. in length and 1.3 mm. in width on the average. In the inner structure of testes, there were seven males of Type 1, four of Type 2, two of Type 3, six of Type 4 and three of Type 5. The testes of these males of Types 1, 2, 3, 4 and 5, were 2.1 mm. and 1.4 mm., 2.0 mm. and 1.4 mm., 2.1 mm. and 1.0 mm., 1.9 mm. and 1.2 mm., and 1.3 mm. and 0.9 mm. in length and width, on the average, respectively. Accordingly, there were no distinct differences in size among the testes of various types, except those of Type 5 in two of the three males.

b. Mating experiments by using male and female second-generation offspring of the male nucleo-cytoplasmic hybrid No. 61OT2 (Table 6)

Set 1. A female No. 1 and two males Nos. 1 and 2 produced from a male first-generation offspring No. 9

Mating 1. J{J(61OT2)}$_9$♀ No. 1×J.W64♂ No. 3

There were 26(83.9%) normally cleaved eggs. At the hatching stage four embryos died of abnormal structures. Seventeen embryos developed normally and completed their metamorphosis. The remaining five were not metamorphosed even at the age of 150 days. As they were less than 30 mm. in total length and very thin, they were killed and preserved.

Mating 2. J{J(61OT2)}$_9$♀ No. 1×J{J(61OT2)}$_9$♂ No. 1

Of 30(52.6%) normally cleaved eggs two died of edema at each of the hatching, the VI and the XIII stage. Twenty-one tadpoles completed their metamorphosis and the other three were not metamorphosed even at the age of 150 days. As they became very thin, they were killed and preserved soon afterward.

Mating 3. J.L64♀ Nos. 7 and 8×J{J(61OT2)}$_9$♂ No. 1

There were 45(60.0%) normally cleaved eggs. One and three died of abnormal structures at the neurula and the hatching stage, respectively. The other 41 developed normally into tadpoles. While 37 of these tadpoles completed their metamorphosis 82 to 91 days after the fertilization, the other four were not metamorphosed even 150 days after the fertilization; they were very thin.

Mating 4. J.L64♀ Nos. 7 and 8×J{J(61OT2)}$_9$♂ No. 2

There were ten (18.2%) normally cleaved eggs. Four of them died of abnormal structures at the tail-bud stage. Although the other six completed their metamorphosis, only one frog was quite normal. The other five had ill-developed forelegs.

Set 2. Five females Nos. 1∼5 and six males Nos. 1∼6 produced from a male first-generation offspring No. 1

Mating 1. J{J(61OT2)}$_8$♀ No. 1×J.W64♂ No. 3

Ten (17.5%) eggs cleaved normally. While one egg died at the gastrula stage, the other nine developed normally into tadpoles and completed their metamorphosis. However, the forelegs of these frogs were ill-developed and hardly protruded from the body walls.

Mating 2. J{J(61OT2)}$_8$♀ No. 1×J{J(61OT2)}$_1$♂ No. 1

Only five (3.7%) eggs cleaved normally. One of them died of edema at the tail-bud stage; the other four developed normally into tadpoles and completed their metamorphosis. But these frogs had abnormally slender forelegs which were too feeble to support their bodies.

Mating 3. J{J(61OT2)}$_{8}$♀ No. 2×J.W64♂ No. 3

Twenty (14.3%) eggs cleaved normally. Five of them died of edema at the VI tadpole stage. Eight others developed normally into tadpoles and completed their metamorphosis 62 to 66 days after the fertilization. But their forelegs were ill-developed and hardly protruded from the body walls. The remaining seven tadpoles were not metamorphosed even 150 days after the fertilization.

Mating 4. J{J(61OT2)}$_{8}$♀ No. 2×J{J(61OT2)}$_{1}$♂ No. 2

Only one (0.6%) egg cleaved normally. Although this egg developed normally into a tadpole and completed metamorphosis, there were no forelegs.

Mating 5. J{J(61OT2)}$_{8}$♀ No. 3×J.W64♂ No. 3

The rate of fertilization was very high and there were 50(98.0%) normally cleaved eggs. Fourteen and six of the latter died of edema at the tail-bud and the hatching stage, respectively. Although the other 30 eggs became feeding tadpoles, nearly all of them deposited aragonite crystals in the dorsal skin as a few white flecks by the VI∼XVII stages. Six tadpoles died by the metamorphosing stage. The other 24 tadpoles completed their metamorphosis, although their forelegs were slender and could not support their bodies. On the backs of these frogs there were a few white flecks of aragonite crystals deposited in the skin as found at their tadpole stage (Fig. 3). They died or were killed within one to two weeks after the metamorphosis.

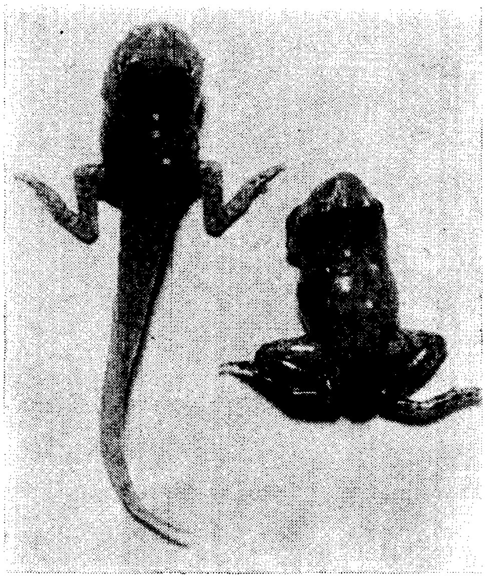

Fig. 3. Aragonite crystals deposited on the backs of frogs with ill-developed forelegs. These frogs were found among the second-generation offspring produced by mating, J{J(61OT2)}$_{8}$♀ No. 3×J.W64♂ No. 3. ×1.5

Mating 6. J{J(61OT2)}$_{8}$♀ No. 3×J{J(61OT2)}$_{1}$♂ No. 3

Only four (2.8%) eggs cleaved normally, while 27(19.0%) did abnormally.

One of the normally cleaved eggs died of edema at the hatching stage, while the other three developed normally into tadpoles. However, aragonite crystals were deposited on the backs of these tadpoles at the XV stage. Moreover, they had no forelegs, although they completed their metamorphosis.

Mating 7. J{J(61OT2)}$_8$♀ No. 4×J.W64♂ No. 3

There were 22(10.3%) normally cleaved eggs. Two of the latter died of edema at the VI tadpole stage and eight others developed into metamorphosed frogs

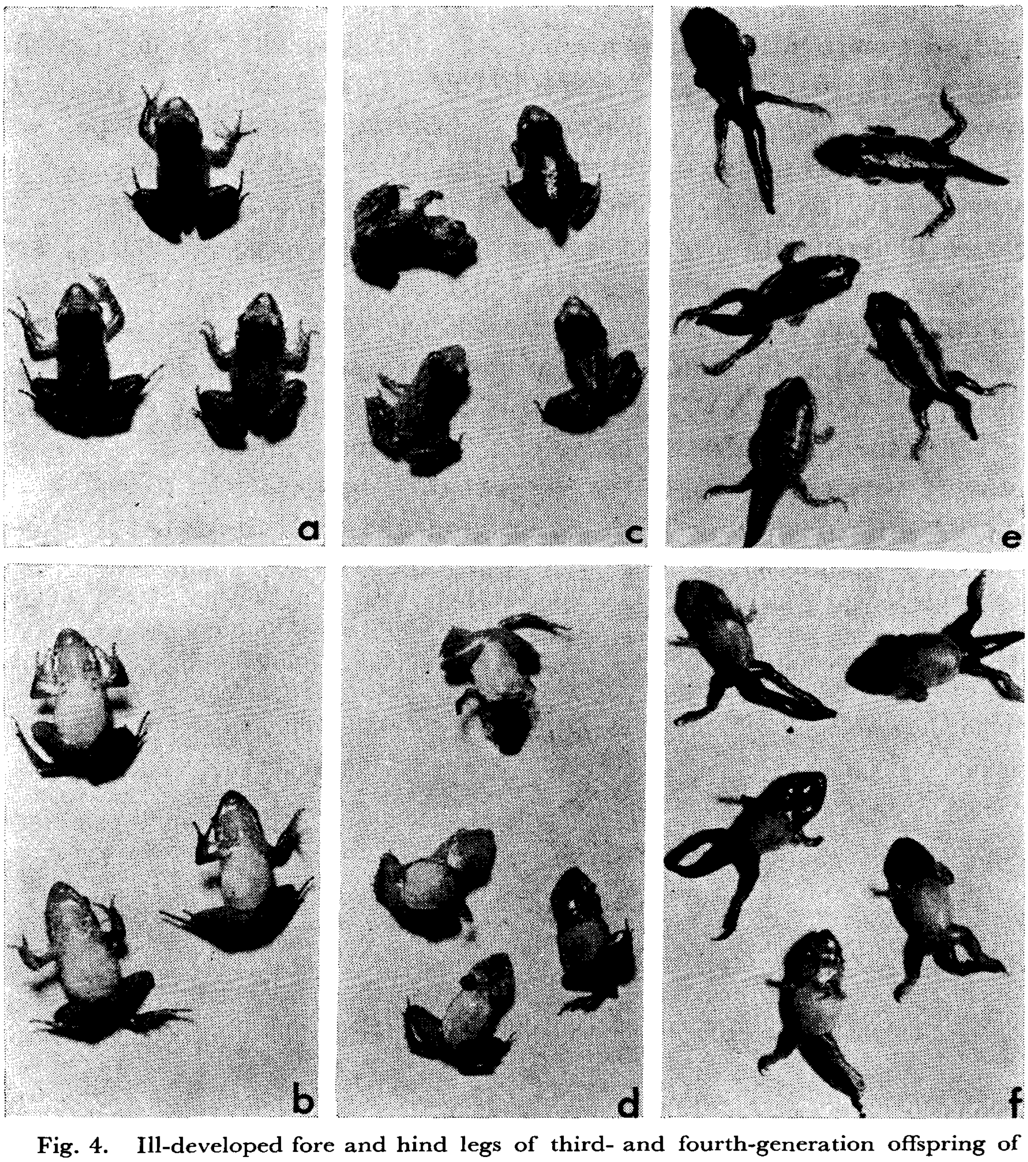

Fig. 4. Ill-developed fore and hind legs of third- and fourth-generation offspring of a nucleo-cytoplasmic hybrid. ×1

a and b, Dorsal and ventral views of normal frogs produced by mating, J. L64♀ Nos. 7~8 ×J.W64♂ No. 3.

c and d, Dorsal and ventral views of abnormal frogs with ill-developed forelegs among the third-generation offspring produced by mating, J{J(61OT2)}$_8$♀ No. 4×J.W64♂ No. 3.

e and f, Dorsal and ventral views of abnormal, metamorphosing frogs with ill-developed hind legs among the fourth-generation offspring produced by mating, J.W65♀ Nos. 1~3× [J{J(61OT2)}$_4$]$_2$J♂ No. 4.

with abnormal forelegs which were too small and slender to support their bodies (Fig. 4, c and d). The remaining 12 developed into thin tadpoles which were not metamorphosed even at the age of 150 days.

Mating 8. J{J(61OT2)}$_8$♀ No. 4×J{J(61OT2)}$_1$♂ No. 4

Only three (2.1%) eggs cleaved normally. All these eggs died of edema at the tail-bud stage.

Mating 9. J{J(61OT2)}$_8$♀ No. 5×J.W64♂ No. 3

There were 75(68.2%) normally cleaved eggs. Five of them died of edema at the hatching stage and 65 others developed normally into metamorphosed frogs. The remaining five developed into thin tadpoles which were about 28.5 mm. in total length at the age of 150 days and had no possibility to be metamorphosed.

Mating 10. J{J(61OT2)}$_8$♀ No. 5×J{J(61OT2)}$_1$♂ No. 5

Normally cleaved eggs were 146(96.7%) in number. Three, seven and 17 of them died of various types of abnormalities at the neurula, the tail-bud and the hatching stage, respectively. The other developed normally into tadpoles. While 20 of the latter died of abnormalities at the X～XVII stages, 71 others completed their metamorphosis. The remaining 28 tadpoles were ill-developed, being about 29.0 mm. in total length at the age of 150 days. They had no possibility to be metamorphosed.

Mating 11, Control. J.L64♀ Nos. 7 and 8×J.W64♂ No. 3

Thirty-eight (95.0%) of 40 eggs cleaved normally. All the cleaved eggs developed normally into metamorphosed frogs (Fig. 4, a and b), except one which died of abnormal structure at the tail-bud stage.

Mating 12. J.L64♀ Nos. 7 and 8×J{J(61OT2)}$_1$♂ No. 1

There were 30(24.8%) normally cleaved eggs. While two of them became abnormal at the hatching stage, the other 28 developed normally into metamorphosed frogs.

Mating 13. J.L64♀ Nos. 7 and 8 ×J{J(61OT2)}$_1$♂ No. 2

The rate of fertilization was very low; only three (1.9%) eggs cleaved normally. One of them died of abnormal structures at the hatching stage and another died of edema at the XII stage. The remaining tadpole completed its metamorphosis, although there were no forelegs.

Mating 14. J.L64♀ Nos. 7 and 8×J{J(61OT2)}$_1$♂ No. 3

There were 21(28.0%) normally cleaved eggs. However, 20 of them died of edema at the tail-bud stage. The remaining one developed into a metamorphosed frog, although it had no forelegs.

Mating 15. J.L64♀ Nos. 7 and 8×J{J(61OT2)}$_1$♂ No. 4

Only three (4.3%) eggs cleaved normally. These developed normally into metamorphosed frogs.

Mating 16. J.L64♀ Nos. 7 and 8×J{J(61OT2)}$_1$♂ No. 5

Normally cleaved eggs were 26(40.6%) in number. Two eggs died of abnormal structures at the neurula and tail-bud stages. The other 24 developed normally into metamorphosed frogs.

Mating 17. J.L64♀ Nos. 7 and 8×J{J(61OT2)}$_1$♂ No. 6
Only one (2.0%) egg cleaved normally. This egg developed normally into a metamorphosed frog.

Set 3. Four males Nos. 1～4 produced from a male first-generation offspring No. 4

Mating 1. J.L64♀ Nos. 7 and 8×J{J(61OT2)}$_4$♂ No. 1
There were 11 (12.8%) normally and four abnormally cleaved eggs. Two, eight and one died of various types of abnormalities at the gastrula, the neurula and the tail-bud stage, respectively. No embryos survived further.

Mating 2. J.L64♀ Nos. 7 and 8×J{J(61OT2)}$_4$♂ No. 2
Only three (7.1%) eggs cleaved normally. One of them died of abnormal structures at the tail-bud stage and the other two developed normally into metamorphosed frogs.

Mating 3. J.L64♀ Nos. 7 and 8×J{J(61OT2)}$_4$♂ No. 3
Only one egg cleaved normally. But this egg died of abnormal structures at the neurula stage.

Mating 4. J.L64♀ Nos. 7 and 8×J{J(61OT2)}$_4$♂ No. 4
No eggs were fertilized.

c. Mating experiments by using male and female second-generation offspring of the male nucleo-cytoplasmic hybrid No. 61AT8 (Table 7)

TABLE 7

Reproductive capacities of second-generation offspring of the

Set	(Mating)	Parents		No. of	No. of cleaved eggs		No. of gastrulae	
		Female no.	Male no.	eggs	Normal	Abnormal	Normal	Abnormal
1	(1)	J{J(61AT8)}$_4$.1	J.W64.1	84	37 (44.0%)	2 (2.4%)	37 (44.0%)	0
	(2)		J{J(61AT8)}$_4$.1	97	45 (46.4%)	0	42 (43.3%)	3 (3.1%)
	(3)	J{J(61AT8)}$_4$.2	J.W64.1	95	76 (80.0%)	6 (6.3%)	76 (80.0%)	0
	(4)		J{J(61AT8)}$_4$.2	113	84 (74.3%)	0	84 (74.3%)	0
	(5)	J.L64.1～3	J.W64.1	78	68 (87.2%)	0	68 (87.2%)	0
	(6)		J{J(61AT8)}$_4$.1	64	8 (12.5%)	0	8 (12.5%)	0
	(7)		J{J(61AT8)}$_4$.2	77	66 (85.7%)	0	66 (85.7%)	0
2	(1)	J.W64.4～6	J.L64.2	71	65 (91.5%)	0	65 (91.5%)	0
	(2)		J{J(61AT8)}$_6$.1	133	29 (21.8%)	0	29 (21.8%)	0
	(3)		J{J(61AT8)}$_6$.2	129	27 (20.9%)	0	27 (20.9%)	0
	(4)		J{J(61AT8)}$_6$.3	53	0	0	0	0
	(5)		J{J(61AT8)}$_6$.4	62	51 (82.3%)	0	51 (82.3%)	0

Set 1. Two females Nos. 1 and 2 and two males Nos. 1 and 2 produced from a male first-generation offspring No. 4

Mating 1. $J\{J(61AT8)\}_4$♀ No. 1 × J.W64♂ No. 1

There were 37(44.0%) normally cleaved eggs. All these eggs died simultaneously of edema at the hatching stage after they developed normally passing over the tail-bud stage.

Mating 2. $J\{J(61AT8)\}_4$♀ No. 1 × $J\{J(61AT8)\}_4$♂ No. 1

Three and one of 45(46.4%) normally cleaved eggs died of abnormalities at the gastrula and the neurula stage. All the remaining 41 embryos died simultaneously at the hatching stage.

Mating 3. $J\{J(61AT8)\}_4$♀ No. 2 × J.W64♂ No. 1

Of 76(80.0%) normally cleaved eggs four at the neurula, five at the tail-bud, three at the hatching and two at the VI stage, died of various types of abnormalities. Six tadpoles died of edema at the XVII stage and five others died at the XX and XXI stages. The other 51 tadpoles completed normally their metamorphosis.

Mating 4. $J\{J(61AT8)\}_4$♀ No. 2 × $J\{J(61AT8)\}_4$♂ No. 2

Of 84(74.3%) normally cleaved eggs eight at the neurula, ten at the tail-bud, two at the hatching and four by the VI stage, died of various types of abnormalities. Seven tadpoles died of edema by the XVII stage and three others died by the

male nucleo-cytoplasmic hybrid No. 61AT8

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of St. VI tadpoles		No. of metamorphosed frogs	
Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.
37 (44.0%)	0	37 (44.0%)	0	0	37 (44.0%)	0	0	0	0
41 (42.3%)	1 (1.0%)	41 (42.3%)	0	0	41 (42.3%)	0	0	0	0
72 (75.8%)	4 (4.2%)	67 (70.5%)	5 (5.3%)	64 (67.4%)	3 (3.2%)	62 (65.3%)	2 (2.1%)	51 (53.7%)	0
76 (67.3%)	8 (7.1%)	66 (58.4%)	10 (8.8%)	64 (56.6%)	2 (1.8%)	60 (53.1%)	4 (3.5%)	46 (40.7%)	0
68 (87.2%)	0	68 (87.2%)	0	65 (83.3%)	3 (3.8%)	65 (83.3%)	0	65 (83.3%)	0
3 (4.7%)	5 (7.8%)	1 (1.6%)	2 (3.1%)	1 (1.6%)	0	1 (1.6%)	0	1 (1.6%)	0
66 (85.7%)	0	46 (59.7%)	20 (26.0%)	36 (46.8%)	10 (13.0%)	36 (46.8%)	0	34 (44.2%)	0
65 (91.5%)	0	65 (91.5%)	0	65 (91.5%)	0	63 (88.7%)	2 (2.8%)	63 (88.7%)	0
22 (16.5%)	7 (5.3%)	0	22 (16.5%)	0	0	0	0	0	0
26 (20.2%)	1 (0.8%)	26 (20.2%)	0	26 (20.2%)	0	15 (11.6%)	11 (8.5%)	5 (3.9%)	0
0	0	0	0	0	0	0	0	0	0
51 (82.3%)	0	51 (82.3%)	0	42 (67.7%)	9 (14.5%)	37 (59.7%)	5 (8.1%)	24 (38.7%)	0

XXII stage. The normally metamorphosed frogs were 46 in number. The remaining four tadpoles were not metamorphosed even 150 days after the fertilization and eventually died becoming thin.

Mating 5, Control. J.L64♀ Nos. 1～3×J.W64♂ No. 1

In this control group there were 68(87.2%) normally cleaved eggs. Except for three eggs which died of abnormal structures at the hatching stage, all the cleaved eggs developed into normally metamorphosed frogs.

Mating 6. J.L64♀ Nos. 1～3×J{J(61AT8)}$_4$♂ No. 1

The rate of fertilization was very low; there were only eight (12.5%) normally cleaved eggs. Five and two of them died of abnormal structures at the neurula and the tail-bud stage, respectively. The remaining one developed normally and became a metamorphosed frog.

Mating 7. J.L64♀ Nos. 1～3×J{J(61AT8)}$_4$♂ No. 2

There were 66(85.7%) normally cleaved eggs. However, twenty and ten of them died of edema at the tail-bud and the hatching stage, respectively. Two tadpoles died at the metamorphosing stage and the other 34 completed their metamorphosis.

Set 2. Four males Nos. 1～4 produced from a male first-generation offspring No. 6

Mating 1, Control. J.W64♀ Nos. 4～6×J.L64♂ No. 2

There were 65(91.5%) normally cleaved eggs. While two of them died of ill-development by the VI stage, the other 63 developed normally into metamorphosed frogs.

Mating 2. J.W64♀ Nos. 4～6×J{J(61AT8)}$_6$♂ No. 1

Although there were 29(21.8%) normally cleaved eggs, seven and 22 died of edema at the neurula and the tail-bud stage, respectively; no eggs developed further.

Mating 3. J.W64♀ Nos. 4～6×J{J(61AT8)}$_6$♂ No. 2

Twenty-seven (20.9%) eggs cleaved normally. One of them died of abnormal structures and the others developed into tadpoles. However, eleven, five and five died of edema at the VI, XVII and XX stage, respectively. Only five tadpoles completed their metamorphosis.

Mating 4. J.W64♀ Nos. 4～6×J{J(61AT8)}$_6$♂ No. 3

No eggs were fertilized.

Mating 5. J.W64♀ Nos. 4～6×J{J(61AT8)}$_6$♂ No. 4

There were 51(82.3%) normally cleaved eggs. Nine and five of them died of edema or some other types of abnormalities at the hatching and the VI tadpole stage, respectively. Three other tadpoles died at the metamorphosing stage. While 24 of the others completed their metamorphosis, the remaining ten died becoming thin at the age of about 150 days, without being metamorphosed (Fig. 5, a).

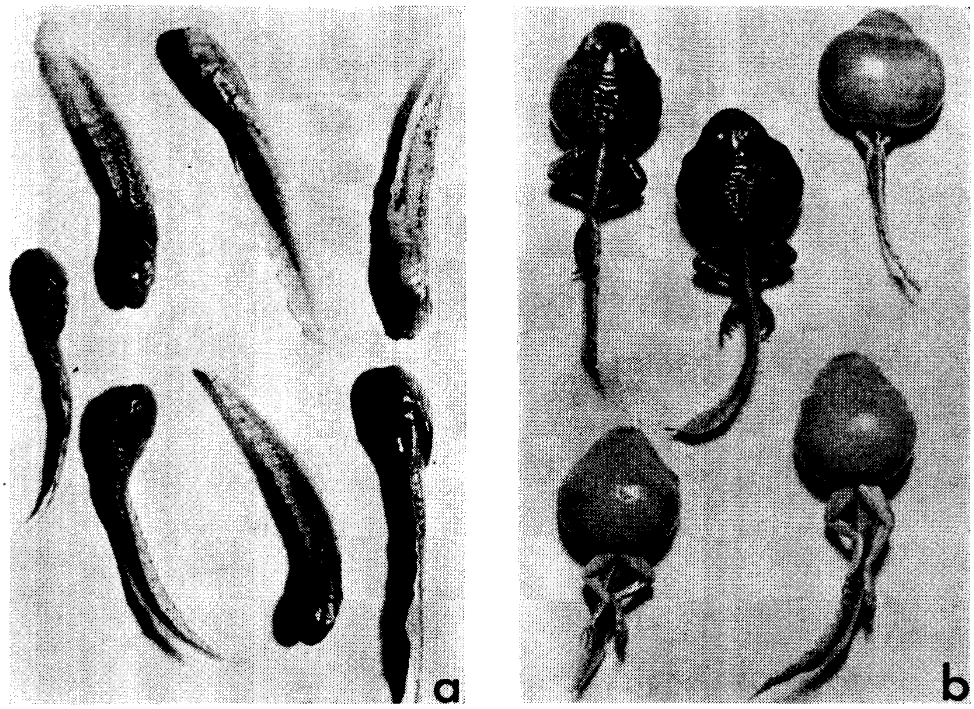

Fig. 5. Ill-developed and ascitic tadpoles among the third-generation offspring of nucleo-cytoplasmic hybrids. ×2/3

a, Ill-developed tadpoles among the third-generation offspring produced by mating, J.W64♀ Nos. 4∼6×J{J(61AT8)}$_6$♂ No. 4.

b, Ascitic tadpoles among the third-generation offspring produced by mating, J{J(61AT12)}$_6$♀ No. 1×J{J(61AT12)}$_6$♂ No. 1.

d. Mating experiments by using male and female second-generation offspring of the male nucleo-cytoplasmic hybrid No. 61AT12 (Table 8)

Set 1. Two females Nos. 1 and 2 and two males Nos. 1 and 2 produced from a male first-generation offspring No. 3

Mating 1. J{J(61AT12)}$_3$♀ No. 1×J.L64♂ No. 4

There were 38(62.3%) normally cleaved eggs. Three at the tail-bud and three others at the hatching stage died of various types of abnormalities. By the VI stage 16 others died of ill-development. At the metamorphosing stage four tadpoles died. Of the remaining 12 tadpoles seven completed their metamorphosis 78 to 86 days after the fertilization, although their forelegs were not protruded. These abnormal frogs died or were killed before long. The other five tadpoles were ill-developed and about 26∼27 mm. in total length 150 days after the fertilization. They were very defective in the development of teeth and too thin to be metamorphosed.

Mating 2. J{J(61AT12)}$_3$♀ No. 1×J{J(61AT12)}$_3$♂ No. 1

Of 11(34.4%) normally cleaved eggs one at the tail-bud and two at the hatching stage died of abnormal structures. Three others died of ill-development at the VI stage. These tadpoles were very defective in the development of teeth. The remaining five tadpoles began to deposit a few white flecks constructed of aragonite crystals in the dorsal skin of the body at the X stage and died by the XVIII stage.

TABLE 8
Reproductive capacities of second-generation offspring

Set	(Mating)	Parents: Female no.	Parents: Male no.	No. of eggs	No. of cleaved eggs: Normal	No. of cleaved eggs: Abnormal	No. of gastrulae: Normal	No. of gastrulae: Abnormal
1	(1)	J{J(61AT12)}$_{3}$.1	J.L64.4	61	38 (62.3%)	7 (11.5%)	38 (62.3%)	0
	(2)		J{J(61AT12)}$_{3}$.1	32	11 (34.4%)	0	11 (34.4%)	0
	(3)	J{J(61AT12)}$_{3}$.2	J.L64.4	74	41 (55.4%)	5 (6.8%)	41 (55.4%)	0
	(4)		J{J(61AT12)}$_{3}$.2	49	11 (22.4%)	6 (12.2%)	11 (22.4%)	0
	(5)	J.L64.1∼3	J.L64.4	52	50 (96.2%)	0	50 (96.2%)	0
	(6)		J{J(61AT12)}$_{3}$.1	71	52 (73.2%)	0	52 (73.2%)	0
	(7)		J{J(61AT12)}$_{3}$.2	75	11 (14.7%)	0	6 (8.0%)	5 (6.7%)
2	(1)	J{J(61AT12)}$_{6}$.1	J.L64.4	85	63 (74.1%)	0	63 (74.1%)	0
	(2)		J{J(61AT12)}$_{6}$.1	94	65 (69.1%)	0	65 (69.1%)	0
	(3)	J{J(61AT12)}$_{6}$.2	J.L64.4	88	53 (60.2%)	2 (2.3%)	53 (60.2%)	0
	(4)		J{J(61AT12)}$_{6}$.2	81	56 (69.1%)	0	54 (66.7%)	2 (2.5%)
	(5)	J.L64.1∼3	J{J(61AT12)}$_{6}$.1	103	71 (68.9%)	6 (5.8%)	65 (63.1%)	3 (2.9%)
	(6)		J{J(61AT12)}$_{6}$.2	116	69 (59.5%)	4 (3.4%)	69 (59.5%)	0

Mating 3. J{J(61AT12)}$_3$♀ No. 2×J.L64♂ No. 4

There were 41(55.4%) normally cleaved eggs. Eleven of them died of edema by the hatching stage and the other 30 developed into tadpoles. Nine of the latter died of edema at the VI∼X stages. The remaining 21 tadpoles began to deposit aragonite crystals on their backs at the XVII stage. Nine of these tadpoles died of edema before the metamorphosing stage. The other 12 completed their metamorphosis, although their forelegs did not appear. As there was no promise that these abnormal frogs would live longer, they were all killed and preserved.

Mating 4. J{J(61AT12)}$_3$♀ No. 2×J{J(61AT12)}$_3$♂ No. 2

Eleven (22.4%) eggs cleaved normally. These eggs developed normally into tadpoles. However, the latter began to deposit aragonite crystals in the dorsal skin of the body at the XVII stage. Although they showed good growth and completed their metamorphosis, their forelegs did not appear. All these abnormal frogs were killed and preserved a short time later, as they seemed to perish sooner or later.

Mating 5, Control. J.L64♀ Nos. 1∼3×J.L64♂ No. 4

of the male nucleo-cytoplasmic hybrid No. 61AT12

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of St. VI tadpoles		No. of metamorphosed frogs	
Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.
38 (62.3%)	0	35 (57.4%)	3 (4.9%)	32 (52.5%)	3 (4.9%)	16 (26.2%)	16 (26.2%)	0	7 (11.5%)
11 (34.4%)	0	10 (31.3%)	1 (3.1%)	8 (25.0%)	2 (6.3%)	5 (15.6%)	3 (9.4%)	0	0
40 (54.1%)	1 (1.4%)	37 (50.0%)	3 (4.1%)	30 (40.5%)	7 (9.5%)	21 (28.4%)	9 (12.2%)	0	12 (16.2%)
11 (22.4%)	0	11 (22.4%)	0	11 (22.4%)	0	11 (22.4%)	0	0	11 (22.4%)
50 (96.2%)	0	50 (96.2%)	0	50 (96.2%)	0	50 (96.2%)	0	50 (96.2%)	0
52 (73.2%)	0	41 (57.7%)	11 (15.5%)	34 (47.9%)	7 (9.9%)	29 (40.8%)	5 (7.0%)	29 (40.8%)	0
6 (8.0%)	0	3 (4.0%)	3 (4.0%)	3 (4.0%)	0	3 (4.0%)	0	3 (4.0%)	0
63 (74.1%)	0	60 (70.6%)	3 (3.5%)	55 (64.7%)	5 (5.9%)	52 (61.2%)	3 (3.5%)	51 (60.0%)	0
63 (67.0%)	2 (2.1%)	60 (63.8%)	3 (3.2%)	60 (63.8%)	0	49 (52.1%)	11 (11.7%)	14 (14.9%)	0
53 (60.2%)	0	50 (56.8%)	3 (3.4%)	47 (53.4%)	3 (3.4%)	46 (52.3%)	1 (1.1%)	45 (51.1%)	0
53 (65.4%)	1 (1.2%)	46 (56.8%)	7 (8.6%)	33 (40.7%)	13 (16.0%)	30 (37.0%)	3 (3.7%)	27 (33.3%)	0
65 (63.1%)	0	62 (60.2%)	3 (2.9%)	55 (53.4%)	7 (6.8%)	52 (50.5%)	3 (2.9%)	44 (42.7%)	0
69 (59.5%)	0	68 (58.6%)	1 (0.9%)	63 (54.3%)	5 (4.3%)	63 (54.3%)	0	44 (37.9%)	0

There were 50(96.2%) normally cleaved eggs. All these eggs developed normally into metamorphosed frogs.

Mating 6. J.L64♀ Nos. 1～3×J{J(61AT12)}$_3$♂ No. 1

The normally cleaved eggs were 52(73.2%) in number. By the VI stage 23 individuals died of various types of abnormalities. The other 29 completed their metamorphosis.

Mating 7. J.L64♀ Nos. 1～3×J{J(61AT12)}$_3$♂ No. 2

Eleven (14.7%) eggs cleaved normally. Five and three of them died of abnormal structures at the gastrula and the tail-bud stage, respectively. The remaining three completed their metamorphosis.

Set 2. Two females Nos. 1 and 2 and two males Nos. 1 and 2 produced from a male first-generation offspring No. 6

Mating 1. J{J(61AT12)}$_6$♀ No. 1×J.L64♂ No. 4

There were 63(74.1%) normally cleaved eggs. Eleven of them died of various types of abnormalities by the VI stage. Another tadpole died at the metamorphosing stage and the other 51 completed their metamorphosis.

Mating 2. J{J(61AT12)}$_6$♀ No. 1×J{J(61AT12)}$_6$♂ No. 1

There were 65(69.1%) normally cleaved eggs. Two and three of them died of abnormal structures at the neurula and the tail-bud stage, respectively. The other 60 eggs developed into tadpoles. Eleven tadpoles died of edema at the VI stage and 35 others died of edema by the XX stage (Fig. 5, b). The remaining 14 tadpoles completed their metamorphosis.

Mating 3. J{J(61AT12)}$_6$♀ No. 2×J.L64♂ No. 4

Of 53(60.2%) normally cleaved eggs seven died of various types of abnormalities by the VI stage. Another tadpole died at the XVII stage. The remaining 45 tadpoles completed their metamorphosis.

Mating 4. J{J(61AT12)}$_6$♀ No. 2×J{J(61AT12)}$_6$♂ No. 2

Of 56(69.1%) normally cleaved eggs two at the gastrula, one at the neurula, seven at the tail-bud and 13 at the hatching stage, died of various types of abnormalities. The other 33 eggs developed into tadpoles. Three of the latter died of edema at the VI stage and three others died in the XX～XXIII stages. The remaining 27 tadpoles completed normally their metamorphosis.

Mating 5. J.L64♀ Nos. 1～3×J{J(61AT12)}$_6$♂ No. 1

Of 71(68.9%) normally cleaved eggs 16 died of various types of abnormalities by the hatching stage. While three of the remaining tadpoles died of ill-development owing to ill-formation of teeth, 44 others completed their metamorphosis 82～87 days after the fertilization and the other eight were thin tadpoles, being 25.5～26.5 mm. in total length, at the age of 150 days. These tadpoles appeared to have no possibility to be metamorphosed.

Mating 6. J.L64♀ Nos. 1～3×J{J(61AT12)}$_6$♂ No. 2

There were 69(59.5%) normally cleaved eggs. By the hatching stage six eggs died of abnormal structures. The other 63 eggs developed into tadpoles; 44 completed their metamorphosis 80～85 days after the fertilization and 19 were still tadpoles, 26.0～28.5 mm. in total length, at the age of 150 days. The latter were very thin and had no promise to be metamorphosed.

3. Chromosomal aberrations

a. Chromosomes of second-generation offspring

Mitotic figures were observed in edematous embryos and externally normal tadpoles of all but one second generation of the two male nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8 as well as in the controls to examine their chromosomal aberrations (Figs. 6 and 7). The results of observations are presented in Tables 9 and 10.

i) Edematous embryos

A part of the edematous embryos were second-generation offspring produced from a female No. 1 of first-generation offspring of No. 61OT2 by mating with a male *japonica* No. 8 or by brother and sister mating with a male No. 10. These embryos were at the stages 20～22. The other embryos were those produced from a female No. 2 of first-generation offspring of No. 61AT8 by mating with a male *japonica* No. 6 or by brother and sister mating with a male No. 9. They were at the stages 17～18.

TABLE 9

Mitoses with chromosomal aberrations in second-generation offspring of the two male nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8

Parents		Analysed embryos or tadpoles		No. of analysed mitoses					No. of mitoses	
				Total	$2n-\alpha$	2n		$2n+\alpha$	With dicentric chromosome	With ring chromosome
Female no.	Male no.	Stage	No.			Abnorm.	Norm.			
J(61OT2).1	J.L64.8	20~22 e	23	82	67	10	1	4	32	2
	J(61OT2).10	20~22 e	29	123	98	3	20	2	35	4
J.L64.12	J.L64.8	III~V n	40	120	1	2	117	0	0	0
	J(61OT2).10	III~V n	30	119	14	6	98	1	0	0
J(61OT2).2	J.W64.9	III~V n	30	129	18	3	106	2	0	0
	J(61OT2).11	III~V n	40	177	26	14	132	5	0	0
J.W64.13	J.W64.9	III~V n	20	67	2	1	64	0	0	0
	J(61OT2).11	III~V n	30	106	20	2	81	3	0	0
J(61OT2).3	J.W64.10	IV~VI n	21	111	13	65	31	2	0	0
	J(61OT2).12	IV~VI n	23	123	16	71	34	2	0	0
J.W64.14	J.W64.10	IV~VI n	30	88	0	1	87	0	0	0
	J(61OT2).12	IV~VI n	17	105	20	44	39	2	0	0
J.L64.9	J.W64.5	IV~VI n	40	140	1	1	138	0	0	0
	J(61AT8).8	IV~VI n	20	78	8	4	63	3	0	0
J(61AT8).2	J.W64.6	17~18 e	32	132	93	12	21	6	41	1
	J(61AT8).9	17~18 e	25	111	75	6	22	8	26	2
J.L64.10	J.W64.6	IV~VI n	20	45	0	0	45	0	0	0
	J(61AT8).9	IV~VI n	4	18	1	11	6	0	0	3
J(61AT8).3	J.L64.7	III~IV n	28	148	23	94	27	4	3	0
	J(61AT8).10	III~IV n	11	60	5	43	11	1	0	0
J.L64.11	J.L64.7	III~IV n	20	63	1	0	62	0	0	0
	J(61AT8).10	III~IV n	7	25	4	4	17	0	0	0

e, Edema n, Normal $\alpha=1$ or 2 Stage — SHUMWAY 1940, Anat. Rec. 78: 139
TAYLOR and KOLLROS 1946, Anat. Rec. 94: 7.

TABLE 10

Numbers of embryos and tadpoles with chromosomal aberrations in second-generation offspring of the two male nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8

Parents		Analysed embryos or tadpoles		With normal cells only	With abnormal cells only				With normal and abnormal cells
					Pure		Mosaic		
Female no.	Male no.	Stage	No.		2n	2n±α	2n	2n, 2n±α	
J(61OT2).1	J.L64.8	20∼22 e	23	0	1	6	2	13	1
	J(61OT2).10	20∼22 e	29	0	0	6	0	13	10
J.L64.12	J.L64.8	III∼V n	40	37	0	0	0	0	3
	J(61OT2).10	III∼V n	30	18	1	0	0	1	10
J(61OT2).2	J.W64.9	III∼V n	30	17	0	0	0	2	11
	J(61OT2).11	III∼V n	40	24	1	0	1	1	13
J.W64.13	J.W64.9	III∼V n	20	18	0	0	0	0	2
	J(61OT2).11	III∼V n	30	20	0	0	0	3	7
J(61OT2).3	J.W64.10	IV∼VI n	21	0	4	0	7	0	10
	J(61OT2).12	IV∼VI n	23	0	4	0	6	0	13
J.W64.14	J.W64.10	IV∼VI n	30	29	0	0	0	0	1
	J(61OT2).12	IV∼VI n	17	0	2	0	4	1	10
J.L64.9	J.W64.5	IV∼VI n	40	38	0	0	0	0	2
	J(61AT8).8	IV∼VI n	20	11	0	0	2	2	5
J(61AT8).2	J.W64.6	17∼18 e	32	0	0	0	0	16	16
	J(61AT8).9	17∼18 e	25	0	0	0	0	13	12
J.L64.10	J.W64.6	IV∼VI n	20	20	0	0	0	0	0
	J(61AT8).9	IV∼VI n	4	0	0	0	0	0	4
J(61AT8).3	J.L64.7	III∼IV n	28	0	1	1	15	5	6
	J(61AT8).10	III∼IV n	11	0	1	0	5	0	5
J.L64.11	J.L64.7	III∼IV n	20	19	0	0	0	0	1
	J(61AT8).10	III∼IV n	7	3	0	0	1	0	3

Chromosomes were examined in 448 mitoses of 109 edematous embryos belonging to the four matings stated above. Normal diploid chromosomes were found in 64(14.3%) mitoses only, while the other mitoses were hypo- or hyperdiploid or abnormal diploid. The hypodiploid mitoses were most abundant among abnormal mitoses, that is, there were 333(74.3%). In this type of mitoses there were usually 25, rarely 24 chromosomes, instead of 26 and various kinds of chromosomal aberrations, such as elimination, translocation and formation of dicentric chromosomes were found. The next type was an abnormal diploid. In the mitoses of this type, there were fragmentation, translocation and formation of ring chromosomes. The third type of abnormal mitoses was a hyperdiploid; the chromosomes were 27 or 28 in number. Fragmentation, translocation and existence of minute, ring or additional normal chromosomes were observed.

There were no edematous embryos which consisted of normal diploid cells only (Table 10). They were always constructed of a mixture of normal and abnormal cells or of only abnormal cells. Among the 109 edematous embryos, there were 39 consisting of a mixture of normal and abnormal cells, 55, of a mixture of abnormal diploid and hypo- or hyperdiploid cells, 12, of hypo- or hyperdiploid cells, 2, of more than two kinds of abnormal diploid cells and one, of a kind of abnormal diploid cells.

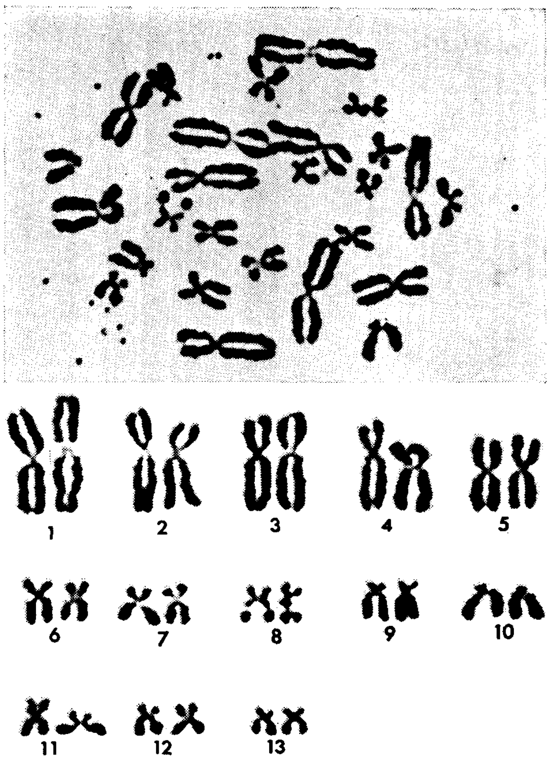

Fig. 6. Normal diploid metaphase plate and its karyotype of an epidermal cell of a control *Rana japonica* tadpole. ×1500

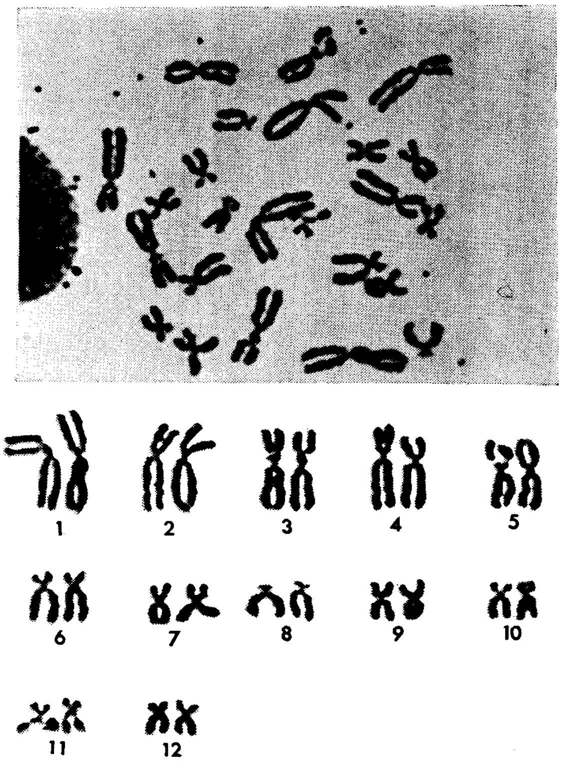

Fig. 7. Normal diploid metaphase plate and its karyotype of an epidermal cell of a control *Rana ornativentris* tadpole. ×1500

ii) Tadpoles with a normal appearance

The externally normal tadpoles used for chromosomal observations were those produced by 12 experimental matings, that is, from female or male first-generation offspring of No. 61OT2 or 61AT8 by mating with male or female *japonica* or by brother and sister mating, besides the controls obtained by six matings. They were hind limb-bud tadpoles at the stages III~VI which were picked at random out of the tadpoles of each mating. All of them were quite normal in external characters and behaviors at least. In the six control matings, 523 mitoses of 170 tadpoles were examined and it was found that 513(98.1%) of them were normal diploid. Among the remaining ten mitoses, there were five hypodiploid and five abnormal diploid. Differing from these control tadpoles, there were numerous abnormal mitoses in the second-generation offspring of the nucleo-cytoplasmic hybrids (Table 9). Among 451 mitoses of 108 tadpoles produced from six first-generation males by mating with female *japonica*, there were 67 (14.9%) hypodiploid, 71(15.7%) abnormal diploid and 9(2.0%) hyperdiploid, besides 304(67.4%) normal diploid. In 78 second-generation offspring produced from three first-generation females by mating with male *japonica*, there were 54(13.9%) hypodiploid, 162(41.8%) abnormal diploid and 8(2.1%) hyperdiploid, besides 164(42.3%) normal diploid among 388 mitoses. In 74 second-generation offspring produced from the same three females by brother and sister mating with three first-generation males, there were 47(13.1%) hypodiploid, 128(35.6%)

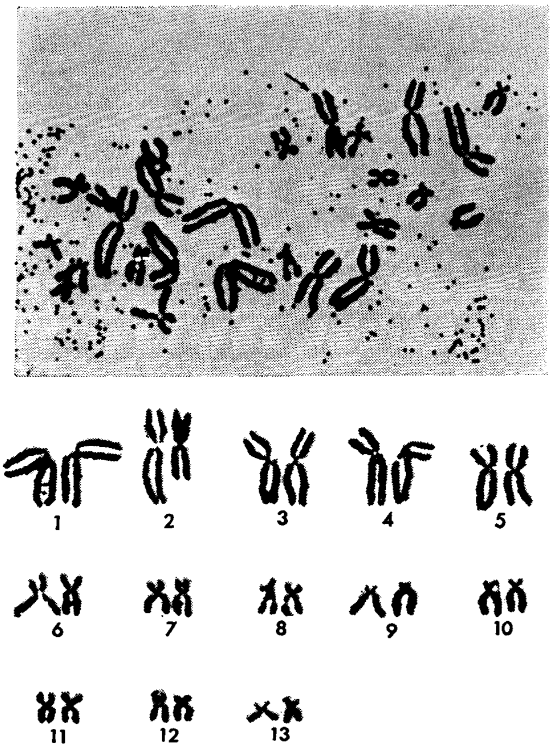

Fig. 8. Chromosomal aberration in a normally shaped tadpole at the stage III among the second-generation offspring produced by mating, J(61AT8) ♀ No. 3 × J.W64 ♂ No. 7. A deletion in one of No. 2 chromosomes is indicated in the metaphase plate by an arrow. × 1500

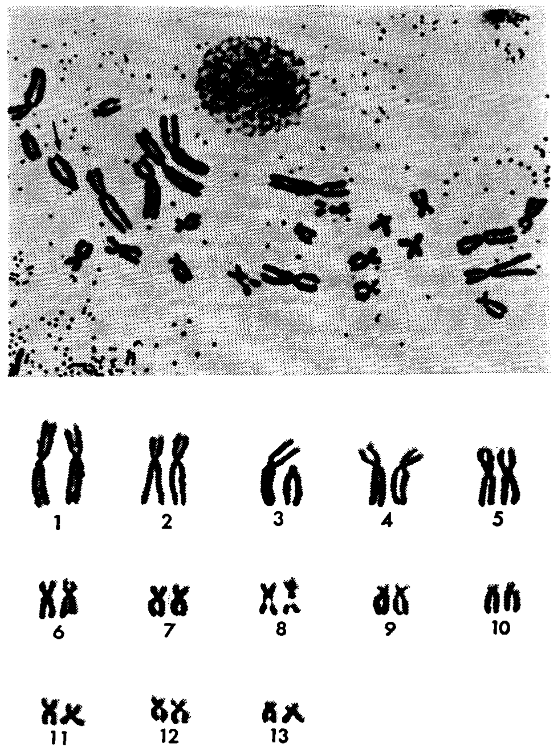

Fig. 9. Chromosomal aberration in a normally shaped tadpole at the stage IV among the second-generation offspring produced by mating, J(61AT8) ♀ No. 3 × J.W64 ♂ No. 7. A deletion in one of No. 3 chromosomes is indicated in the metaphase plate by an arrow. × 1500

abnormal diploid and 8(2.2%) hyperdiploid, besides 177(49.2%) normal diploid among 360 mitoses. These figures of mitoses in the three kinds of second-generation tadpoles total 645(53.8%) normal diploid and 554(46.2%) hypo-, hyper- or abnormal diploid among 1199 mitoses of 261 tadpoles. While normal diploid mitoses were more or less larger in number than abnormal ones in the tadpoles obtained by six of the twelve experimental matings, they were far fewer than abnormal mitoses in the tadpoles obtained by the other six matings. In the above three kinds of abnormalities, various chromosomal aberrations, such as deficiency, translocation, fragmentation, and existence of minute or ring chromosomes were included, too.

Of the 261 tadpoles with normal appearance in the second generations of the two nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8, only 93(35.6%) consisted of nothing but normal diploid cells, while 161(94.7%) of the 170 control tadpoles consisted of normal diploid cells alone. The other nine control tadpoles consisted of a mixture of normal diploid and abnormal cells. Among the remaining 168 second-generation offspring, there were 97(58.1%) tadpoles consisting of a mixture of normal diploid and abnormal cells, 14(8.3%), of a kind of abnromal diploid, one (0.6%), of hypo- or hyperdiploid, 41(24.4%), of more than two kinds of abnormal diploid, 15(8.9%), of a mixture of abnormal diploid and hypo- or hyperdiploid cells.

b. Chromosomes of third-generation offspring

Numerous chromosomal aberrations were found in third-generation offspring of the three nucleo-cytoplasmic hybrids. The results of observations on the chromosomes of third-generation offspring produced by 40 of the 46 experimental matings and of 80 control tadpoles produced by four matings are presented in Tables 11 and 12.

i) Edematous embryos

Out of the embryos produced by four experimental matings, edematous ones were selected for chromosomal observations. Two of these matings were made between a second-generation female No. 1 and a male *japonica* or a brother of the same generation. Sixty embryos obtained by these two matings were observed. Those obtained by the other two experimental matings were third-generation offspring of the two nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8. They were produced by mating of two second-generation males with female *japonica*. Chromosomes were examined in 40 embryos of the third generations.

Of 513 mitoses observed in 100 edematous embryos in total which were obtained

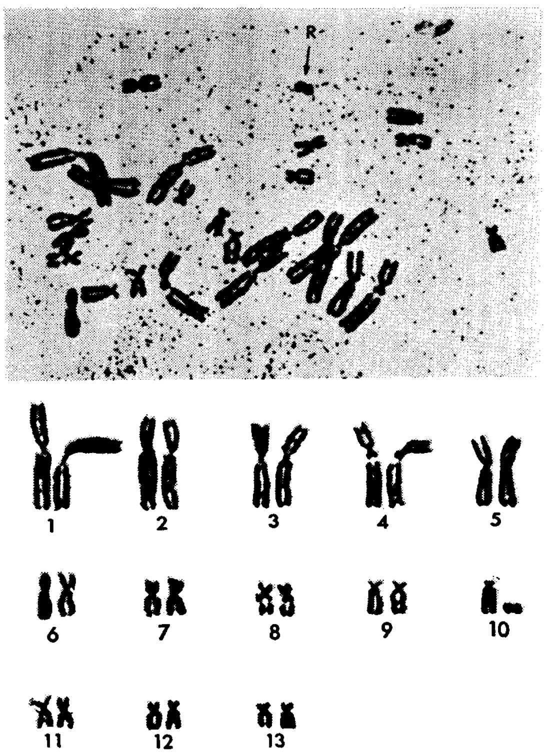

Fig. 10. Chromosomal aberration in an edematous embryo at the stage 17 among the third-generation offspring produced by mating, J.L64♀ Nos. 7∼8×J{J(61OT2)}$_1$♂ No. 3. A small ring chromosome (R) at No. 10 is indicated in the metaphase plate by an arrow. ×1500

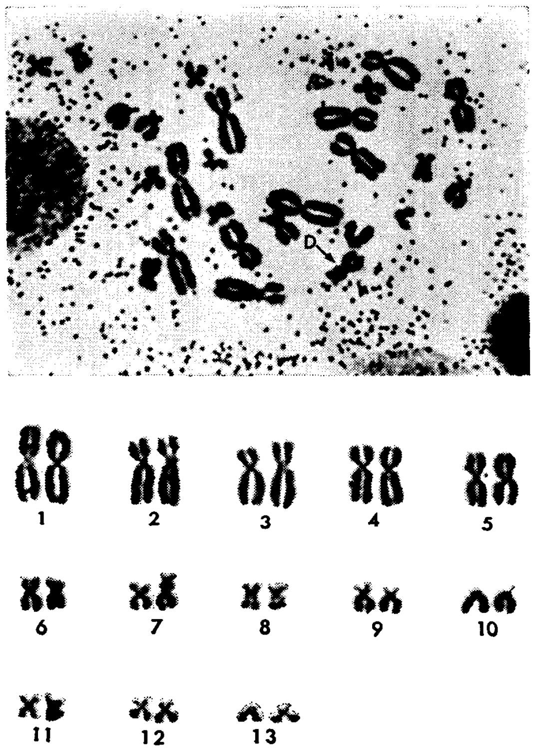

Fig. 11. Chromosomal aberration in an edematous embryo at the stage 20 among the third-generation offspring produced by mating, J{J(61AT8)}$_4$♀ No. 1×J.W64♂ No. 1. A dicentric chromosome (D) at No. 7 is indicated in the metaphase plate by an arrow. ×1500

TABLE 11

Mitoses with chromosomal aberrations in third-generation offspring of the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12

Parents		Analysed embroys or tadpoles		No. of analysed mitoses					No. of mitoses	
				Total	$2n-\alpha$	2n		$2n+\alpha$	With dicentric chrom.	With ring chrom.
Female no.	Male no.	Stage	No.			Abnorm.	Norm.			
J{J(61OT2)}$_{9}$.1	J.W64.3	III~V n	17	66	1	1	64	0	0	0
	J{J(61OT2)}$_{9}$.1	III~V n	21	96	10	13	72	1	0	0
J{J(61OT2)}$_{8}$.1	J.W64.3	III~IV n	9	54	10	41	2	1	0	0
	J{J(61OT2)}$_{1}$.1	III~IV n	4	21	5	15	1	0	0	0
J{J(61OT2)}$_{8}$.2	J.W64.3	III~IV n	10	54	7	36	7	4	0	0
	J{J(61OT2)}$_{1}$.2	III~IV n	1	3	0	3	0	0	0	0
J{J(61OT2)}$_{8}$.3	J.W64.3	III~V n	25	110	13	64	21	12	0	0
	J{J(61OT2)}$_{1}$.3	III~V n	3	16	1	14	1	0	0	0
J{J(61OT2)}$_{8}$.4	J.W64.3	III~V n	11	60	8	40	9	3	0	0
J{J(61OT2)}$_{8}$.5	J.W64.3	III~V n	30	185	21	42	117	5	0	0
	J{J(61OT2)}$_{1}$.5	III~V n	40	232	35	19	175	3	0	0
J.L64.7~8	J.W64.3	IV~V n	20	87	1	2	84	0	0	0
	J{J(61OT2)}$_{1}$.1	IV~V n	28	143	21	37	80	5	0	0
	J{J(61OT2)}$_{1}$.2	IV n	1	4	0	4	0	0	0	0
	J{J(61OT2)}$_{1}$.3	17~18 e	20	101	61	35	3	2	22	10
		IV n	1	4	0	4	0	0	0	0
	J{J(61OT2)}$_{1}$.4	IV~V n	3	18	3	1	14	0	0	0
	J{J(61OT2)}$_{1}$.5	IV~V n	24	93	7	26	58	2	0	0
	J{J(61OT2)}$_{1}$.6	V n	1	2	0	0	2	0	0	0
	J{J(61OT2)}$_{4}$.2	III~V n	2	7	0	0	7	0	0	0
	J{J(61OT2)}$_{9}$.1	III~V n	41	205	21	68	113	3	0	0
	J{J(61OT2)}$_{9}$.2	III~V n	6	30	4	23	2	1	0	0
J{J(61AT8)}$_{4}$.1	J.W64.1	20~21 e	30	161	112	12	31	6	76	4
	J{J(61AT8)}$_{4}$.1	20~21 e	30	127	87	14	24	2	43	5
J{J(61AT8)}$_{4}$.2	J.W64.1	III~IV n	20	86	4	36	45	1	0	0
	J{J(61AT8)}$_{4}$.2	III~IV n	20	76	5	22	48	1	0	0
J.W64.1~3	J.W64.1	III~V n	20	66	1	0	65	0	0	0
	J{J(61AT8)}$_{4}$.1	III~IV n	1	2	0	0	2	0	0	0
	J{J(61AT8)}$_{4}$.2	III~IV n	20	96	2	21	72	1	0	0

J.W64.4~6	J.L64.2	III~IV n	20	76	2	2	69	3	0	0
	J{J(61AT8)}$_{6}$.1	17 ~18 e	20	124	79	26	10	9	39	13
	J{J(61AT8)}$_{6}$.4	III~IV n	20	101	10	31	57	3	0	0
J{J(61AT12)}$_{3}$.1	J.L64.4	III~IV n	16	84	20	50	12	2	0	0
	J{J(61AT12)}$_{3}$.1	III~IV n	5	24	14	3	0	7	0	0
J{J(61AT12)}$_{3}$.2	J.L64.4	III~IV n	21	75	20	27	15	13	0	0
	J{J(61AT12)}$_{3}$.2	III~IV n	11	33	2	17	7	7	0	0
J{J(61AT12)}$_{6}$.1	J.L64.4	III~V n	20	93	3	2	87	1	0	0
	J{J(61AT12)}$_{6}$.1	III~IV n	49	117	20	20	73	4	0	0
J{J(61AT12)}$_{6}$.2	J.L64.4	III~V n	20	98	10	2	81	5	0	0
	J{J(61AT12)}$_{6}$.2	20 ~23 a	13	76	11	25	20	20	0	1
		III~IV n	30	130	23	11	89	7	0	0
J.L64.1~3	J.L64.4	III~V n	20	107	1	11	95	0	0	0
	J{J(61AT12)}$_{3}$.1	17 ~19 a	11	32	11	13	5	3	2	0
		20 ~22 a	7	33	10	13	5	5	0	0
		III~V n	29	127	5	49	70	3	0	0
	J{J(61AT12)}$_{3}$.2	III~IV n	3	14	0	2	12	0	0	0
	J{J(61AT12)}$_{6}$.1	20 ~22 a	7	19	6	5	5	3	1	1
		III~VI n	20	68	2	11	54	1	0	0
	J{J(61AT12)}$_{6}$.2	III~VI n	49	140	21	16	100	3	0	0

e, Edema   n, Normal   a, Abnormal $\alpha = 1$ or 2   Stage — Shumway 1940, Anat. Rec. 78: 139
Taylor and Kollros 1946, Anat. Rec. 94: 7.

TABLE 12

Numbers of embryos and tadpoles with chromosomal aberrations in third-generation offspring of the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12

Parents		Analysed embryos or tadpoles		With normal cells only	With abnormal cells only				With normal and abnormal cells
					Pure		Mosaic		
Female no.	Male no.	Stage	No.		2n	2n±α	2n	2n, 2n±α	
J{J(61OT2)}$_{9.1}$	J.W64.3	III~V n	17	16	0	0	0	0	1
	J{J(61OT2)}$_{9.1}$	III~V n	21	11	0	0	1	1	8
J{J(61OT2)}$_{6.1}$	J.W64.3	III~V n	9	0	2	0	5	0	2
	J{J(61OT2)}$_{1.1}$	III~IV n	4	0	1	0	1	1	1
J{J(61OT2)}$_{1.2}$	J.W64.3	III~IV n	10	0	1	0	5	1	3
	J{J(61OT2)}$_{8.2}$	III~IV n	1	0	0	0	1	0	0
J{J(61OT2)}$_{8.3}$	J.W64.3	III~V n	25	0	3	0	12	0	10
	J{J(61OT2)}$_{8.3}$	III~V n	3	0	0	0	2	0	1
J{J(61OT2)}$_{1.4}$	J.W64.3	III~V n	11	0	1	1	5	1	3
J{J(61OT2)}$_{8.5}$	J.W64.3	III~V n	30	9	2	0	5	0	14
	J{J(61OT2)}$_{1.5}$	III~V n	40	24	1	0	3	1	11
J.L64.7~8	J.W64.3	IV~V n	20	18	0	0	0	0	2
	J{J(61OT2)}$_{1.1}$	IV~V n	28	13	1	0	3	2	9
	J{J(61OT2)}$_{1.2}$	IV n	1	0	0	0	1	0	0
	J{J(61OT2)}$_{1.3}$	17~18 e	20	0	3	1	9	5	2
		IV~V n	1	0	0	0	1	0	0
	J{J(61OT2)}$_{1.4}$	IV~V n	3	1	0	0	0	0	2
	J{J(61OT2)}$_{1.5}$	IV~V n	24	10	1	0	7	0	6
	J{J(61OT2)}$_{1.6}$	V n	1	1	0	0	0	0	0
	J{J(61OT2)}$_{4.2}$	III~V n	2	2	0	0	0	0	0
	J{J(61OT2)}$_{9.1}$	III~V n	41	19	4	0	6	3	9
	J{J(61OT2)}$_{9.2}$	III~V n	6	0	1	0	3	1	1
J{J(61AT8)}$_{4.1}$	J.W64.1	20 ~21 e	30	0	0	0	0	17	13
	J{J(61AT8)}$_{4.1}$	20 ~21 e	30	0	0	0	0	11	19
J{J(61AT8)}$_{4.2}$	J.W64.1	III~IV n	20	4	2	0	7	2	5
	J{J(61AT8)}$_{4.2}$	III~IV n	20	7	0	0	3	0	10

J.W64.1~3	J.W64.1	III~V n	20	19	0	0	0	0	1
	J{J(61AT8)}$_4$.1	III~IV n	1	1	0	0	0	0	0
	J{J(61AT8)}$_4$.2	III~IV n	20	14	0	0	2	0	4
J.W64.4~6	J.L64.2	III~IV n	20	17	0	0	0	0	3
	J{J(61AT8)}$_6$.1	17 ~18 e	20	0	2	1	2	10	5
	J{J(61AT8)}$_6$.4	III~IV n	20	6	2	0	2	1	9
J{J(61AT12)}$_3$.1	J.L64.4	III~IV n	16	0	3	0	5	3	5
	J{J(61AT12)}$_3$.1	III~IV n	5	0	0	2	0	3	0
J{J(61AT12)}$_3$.2	J.L64.4	III~IV n	21	0	0	2	7	6	6
	J{J(61AT12)}$_3$.2	III~IV n	11	0	2	0	6	0	3
J{J(61AT12)}$_6$.1	J.L64.4	III~V n	20	15	0	0	0	0	5
	J{J(61AT12)}$_6$.1	III~V n	49	11	1	0	4	4	29
J{J(61AT12)}$_6$.2	J.L64.4	III~V n	20	13	0	1	0	0	6
	J{J(61AT12)}$_6$.2	20 ~23 a	13	0	1	2	2	2	6
		III~IV n	30	14	1	2	1	5	7
J.L64.1~3	J.L64.4	III~V n	20	18	0	0	1	0	1
	J{J(61AT12)}$_3$.1	17 ~19 a	11	0	1	0	4	4	2
		20 ~22 a	7	0	0	0	2	3	2
		III~V n	29	10	1	1	8	0	9
	J{J(61AT12)}$_3$.2	III~IV n	3	2	0	0	0	0	1
	J{J(61AT12)}$_6$.1	20 ~22 a	7	0	1	1	1	2	2
		III~VI n	20	11	1	0	3	0	5
	J{J(61AT12)}$_6$.2	III~VI n	49	27	1	0	2	0	19

by the above four experimental matings, 68(13.3%) were normal diploid, while the other 445(86.7%) had abnormal chromosome complements. Among the latter, there were 339(76.2%) hypodiploid, 87(19.6%) abnormal diploid and 19 (4.3%) hyperdiploid mitoses. On the other hand, there were 180 mitoses with dicentric chromosomes and 32 mitoses with ring ones.

It was found that there were no embryos with nothing but normal cells. Of these edematous embryos, 39 consisted of a mixture of normal and abnormal cells, while 43 others were mosaics of abnormal diploid and hypo- or hyperdiploid cells. Five embryos consisted of a kind of abnormal diploid, two, of a kind of hypo- or hyperdiploid, and 11, of more than two kinds of abnormal diploid cells.

ii) Embryos with an abnormal appearance

Chromosomal observations were made on 38 embryos of third-generation offspring of the nucleo-cytoplasmic hybrid No. 61AT12. They were produced from second-generation offspring by brother and sister mating as well as from two male second-generation offspring by mating with female *japonica*.

There were 35 normal diploid among 160 mitoses of the embryos with abnormal appearance. Among the other abnormal mitoses, there were 38 hypodiploid, 56 abnormal diploid and 31 hyperdiploid. On the other hand, three mitoses had

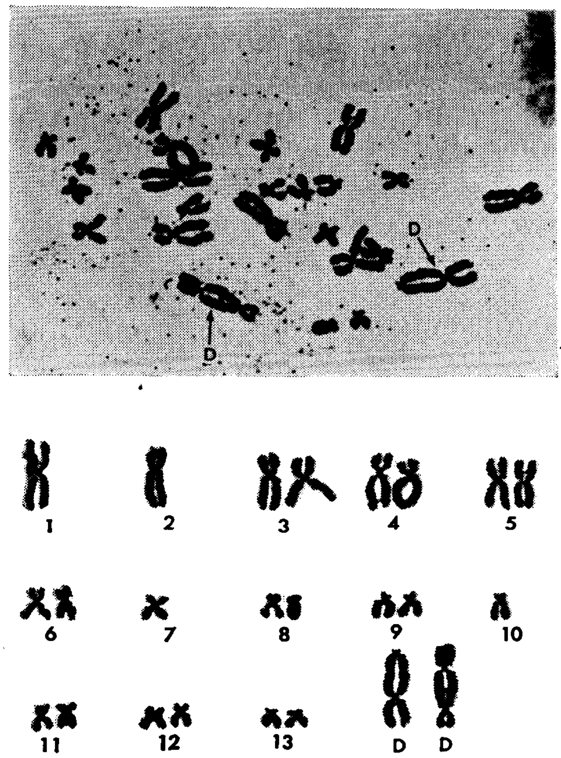

Fig. 12. Chromosomal aberration in an abnormal embryo at the stage 17 among the third-generation offspring produced by mating, J.L64♀ Nos. 1~3× J{J(61AT12)}$_3$♂ No. 1. Dicentric chromosomes (D) are indicated in the metaphase plate by arrows. ×1500

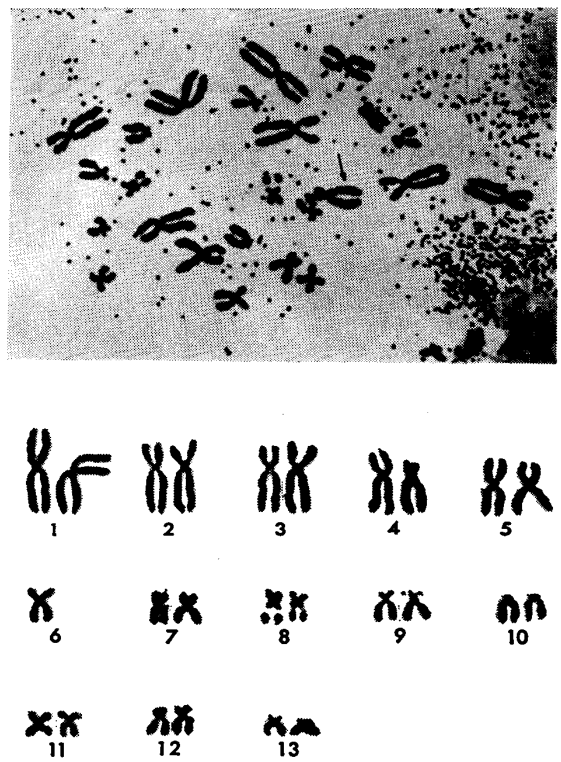

Fig. 13. Chromosomal aberration in an abnormal embryo at the stage 17 among the third-generation offspring produced by mating, J.L64♀ Nos. 1~3× J{J(61AT12)}$_3$♂ No. 1. A deletion in one of No. 4 chromosomes is indicated in the metaphase plate by an arrow. ×1500

dicentric and two had ring chromosomes.

Among the 38 abnormal embryos, there were no individuals which were constructed of normal diploid cells alone. Twelve embryos consisted of a mixture of normal and abnormal cells, three, of a kind of abnormal diploid, three, of a kind of hypo- or hyperdiploid, nine, of more than two kinds of abnormal diploid and the remaining eleven, of a mixture of abnormal diploid and hypo- or hyperdiploid cells.

iii) Tadpoles with a normal appearance

By examining chromosomes in clipped tail-tips, it was found that there were numerous abnormal mitoses in most of 632 third-generation offspring of the three nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12, differing from the situation in 80 control tadpoles obtained by four matings between eight female and four male *japonica*.

In the control tadpoles, 313 of 336 mitoses were normal diploid, while five were hypodiploid, 15, abnormal diploid and three, hyperdiploid. There was neither dicentric nor ring chromosome. In the third-generation tadpoles, 1569 (56.7%) of 2767 mitoses were normal diploid, while the other 1198 were abnormal: 328 were hypodiploid, 771, abnormal diploid and 99, hyperdiploid. However, there were no mitoses with dicentric or ring chromosomes. When these mitoses were assorted into three groups according to the kind of nucleo-cytoplasmic hybrid from which the tadpoles were descended, there were 224(62.0%) normal diploid, 21 hypodiploid, 110 abnormal diploid and 6 hyperdiploid among 361 mitoses of 81 third-generation offspring of No. 61AT8, 745(53.1%) normal diploid, 167 hypodiploid, 451 abnormal diploid and 40 hyperdiploid among 1403 mitoses of 278 third-generation offspring of No. 61OT2, and 600(59.8%) normal diploid, 140 hypodiploid, 210 abnormal diploid and 53 hyperdiploid among 1,003 mitoses of 273 third-generation offspring of No. 61AT12.

Of the 80 tadpoles produced by four control matings, 72(90.0%) consisted of normal diploid cells alone, while seven, of a mixture of normal and abnormal cells and one, of more than two kinds of abnormal diploid cells. In contrast with this, only 241 (38.1%) of the 632 third-generation offspring produced by 37 experimental matings consisted of normal diploid cells alone. Of the other tadpoles, 204 were constructed of a mixture of normal and abnormal cells, 32, of a kind of abnormal diploid, nine, of a kind of hypo- or hyperdiploid, 111, of more than two kinds of abnormal diploid and 35, of a mixture of abnormal diploid and hypo- or hyperdiploid. It was noteworthy that no individuals constructed of normal diploid cells alone were found among 124 tadpoles obtained by 14 experimental matings.

4. Metamorphosed frogs

a. Second-generation offspring

As stated above, it was very clear that the male and female first-generation offspring of the two nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8 were distinctly inferior to the control frogs in reproductive capacity (Table 3). No normal tadpoles were produced from three of the six female first-generation offspring by mating with male *japonica* or male first-generation offspring of the same nucleo-cytoplasmic hybrids. Furthermore, no normal metamorphosed frogs were obtained from two of the other three females by mating with the male *japonica* or the male first-generation offspring, although some abnormal frogs were produced from these females. From the remaining female, however, 163(38.2%) of total 427 eggs developed into normal frogs. Two of the six first-generation males also produced no normal, metamorphosed frogs by mating with female *japonica*,

TABLE 13

Shape, size and sex of metamorphosed second-generation offspring of the two

Set	(Mating)	Parents: Female no.	Parents: Male no.	Age at the time of landing (Mean) (days)	No. of frogs: Total	No. of frogs: Normal	No. of frogs: With abnormal forelegs
1	(3)	J.L64.12	J.L64.8	82~94 (88.2)	52	52	0
	(4)		J(61OT2).10	82~92 (87.5)	40	40	0
2	(1)	J(61OT2).2	J.W64.9	85~97 (91.5)	67	67	0
	(2)		J(61OT2).11	82~91 (86.4)	94	94	0
	(3)	J.W64.13	J.W64.9	82~93 (86.5)	57	57	0
	(4)		J(61OT2).11	82~95 (88.6)	102	102	0
3	(1)	J(61OT2).3	J.W64.10	90~91 (90.5)	2	0	2
	(2)		J(61OT2).12	90~93 (91.8)	4	0	4
	(3)	J.W64.14	J.W64.10	90~93 (91.7)	123	123	0
	(4)		J(61OT2).12	91~92 (91.5)	4	0	4
4	(3)	J.L64.9	J.W64.5	82~93 (87.3)	125	125	0
	(4)		J(61AT8).8	83~91 (86.5)	15	15	0
5	(3)	J.L64.10	J.W64.6	83~94 (86.6)	78	78	0
	(4)		J(61AT8).9	90~95 (91.5)	4	0	4
6	(1)	J(61AT8).3	J.L64.7	90~95 (92.3)	15	0	15
	(2)		J(61AT8).10	91~96 (92.5)	7	0	7
	(3)	J.L64.11	J.L64.7	83~94 (87.5)	49	49	0
	(4)		J(61AT8).10	83~86 (84.6)	5	5	0

although they did a few abnormal ones. By mating of the other four males with female *japonica*, 5～78% of eggs became normal metamorphosed frogs, differing from 93～95% in the control matings between six male and six female *japonica*.

All the metamorphosed frogs of the second-generation of the two nucleo-cytoplasmic hybrids and the controls are listed in Table 13 to show their shape, size and sex. In the six control matings, 484 tadpoles completed their metamorphosis 82～94 days after the insemination and became quite normal frogs, while in the experimental matings, 359 tadpoles did 82～97 days after the insemi-

male nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8

Body length immediately after metamorphosis (mm.)	Body length at the age of one year (mm.)	Sex of young frogs shortly after metamorphosis						Sex of matured frogs		
		Total	♀$_{N}$	⚥$_{1}$	⚥$_{2}$	⚥$_{3}$	♂$_{N}$	Total	♀	♂
14.6±0.09		52	27	1	0	0	24			
13.6±0.12		40	23	0	0	0	17			
15.5±0.07	38.4±0.17	46	12	5	1	2	26	19	1	18
14.2±0.11	35.0±0.35	64	20	6	3	6	29	25	1	24
16.2±0.08	36.7±0.33	37	19	1	0	0	17	18	10	8
16.0±0.08	39.1±0.75	89	46	2	1	0	40	12	0	12
13.5～14.0		2	0	0	0	1	1			
13.0～14.5		4	2	0	0	0	2			
14.6±0.07		123	61	0	0	0	62			
13.0～14.0		4	3	0	0	0	1			
16.2±0.06		125	60	0	0	0	65			
15.7±0.07		15	8	0	0	0	7			
14.0±0.12		78	40	0	0	0	38			
13.5～14.5		4	2	0	0	0	2			
13.2±0.15		15	6	0	0	0	9			
13.5±0.73		7	3	0	0	0	4			
13.6±0.07		49	25	0	0	0	24			
13.5±1.06		5	1	1	0	0	3			

nation, that is, nearly at the same time as a whole as the control tadpoles did, in spite of the fact that there were 36 frogs with abnormal forelegs. The latter were produced from two male and two female first-generation offspring by mating with female or male *japonica* as well as by brother and sister mating. They were rather retarded in the completion of metamorphosis: they were 90.5～92.5 days in the mean age at the time of metamorphosis in six matings, in contrast to 84.6～88.6 days in five of the other six experimental matings and 86.5～88.2 days in five of the six control. In the body length immediately after metamorphosis, they were also somewhat smaller as a whole than those of the other experimental as well as the control matings, while there were scarcely any differences between the frogs of the latter two.

In order to examine the sex of the second-generation offspring of the two nucleo-cytoplasmic hybrids, all the frogs belonging to 14 of the 18 experimental and control matings as well as most of the frogs belonging to the other four were killed and their gonads were observed microscopically. The remaining frogs of the latter four matings, consisting of three experimental and one control, were reared continuously.

At the stage soon after the completion of metamorphosis, there were nearly equal number of males and females in each of the six control matings. Moreover, there were no hermaphrodites but two of Type 1 among 464 frogs. In the second generations of six first-generation males mated with female *japonica,* there were 83 females, 4 hermaphrodites and 70 males, while in the second generations of three first-generation females mated with male *japonica,* there were 18 females, 9 hermaphrodites and 36 males. In the second generations obtained by brother and sister matings between three male and three female first-generation offspring, there were 25 females, 15 hermaphrodites and 35 males.

Matured frogs were obtained in four matings using a male and a female first-generation offspring and a male and female *japonica.* Among 18 frogs of the control mating, there were 10 females and 8 males. In contrast with this, nearly all the frogs of the three experimental matings were males: there were no females in 12 second-generation offspring of a first-generation male mated with a female *japonica* and only a female in each of two kinds of 19 and 25 second-generation offspring produced from a first-generation female by mating with a male *japonica* or first-generation offspring. It was reasonably presumed that sex-reversal had occurred in nearly half of the matured males of each experimental mating, because there were already a considerable number of hermaphrodites together with many females at the time soon after metamorphosis, besides, the mortality during the period of time from the completion of metamorphosis to sexual maturity was extremely low.

b. Third-generation offspring

i) Offspring of the male nucleo-cytoplasmic hybrid No. 61OT2

Normal metamorphosed frogs were produced from two second-generation females by mating with a male *japonica* or second-generation males as well as from six second-generation males by mating with female *japonica* (Table 6). However, the percentages of frogs produced by these ten matings were generally low as compared with that in the controls: while 93% of eggs became normal frog in the latter, 2～59% did in the former. Although no normal frogs were produced by the other 14 matings, a few or occasionally many frogs with abnormal forelegs appeared by ten of the latter. By the remaining four matings neither normal frog nor abnormal one was obtained.

The metamorphosed frogs, both normal and abnormal, produced from the second-generation offspring are presented in Table 14. In the control mating, 37 tadpoles made their metamorphosis 77～85 (mean 81.5) days after the insemination, while 334 tadpoles belonging to three kinds of third-generation offspring did 61～98 (mean 80.6) days after the insemination. In three of the six matings between second-generation females and male *japonica*, 50 tadpoles made their metamorphosis at the ages of 77～92 (mean 82.2) days, while 28 tadpoles produced from the same three females by mating with three third-generation males did at the ages of 61～82 (mean 68.3) days. In another mating between second-generation females and male *japonica*, the metamorphosis of 65 tadpoles occurred at the ages of 77～92 (mean 84.0) days, while those of 71 tadpoles produced from the same female by mating with a second-generation male did at the ages of 87～98 (mean 92.5) days. By the other two matings between male and female second-generation offspring, only one frog with abnormal forelegs was produced. In the nine matings between female *japonica* and second-generation males, 103 tadpoles made their metamorphosis at the ages of 66～91 (mean 81.4) days.

The frogs of all the experimental matings were rather larger in the mean body length of the frogs of each mating than those of the controls, as shown in Table 14. It was found that frogs with abnormal forelegs were not always smaller in body length immediately after metamorphosis than the latter. They were not always delayed in the time of metamorphosis as compared with normal frogs, too.

Among 26 control frogs, immediately after metamorphosis, there were 14 females and 12 males. In the three kinds of third-generation offspring, there were 116 females, 21 hermaphrodites and 141 males among 278 young frogs. The sex of each kind was as follows: 48 females, 9 hermaphrodites and 52 males in the third-generation offspring of second-generation females mated with male *japonica*, 29 females, 5 hermaphrodites and 42 males in those of second-generation females mated with second-generation males, and 39 females, 7 hermaphrodites and 47 males in those of second-generation males mated with female *japonica*.

The most distinct preponderance of males was found in three matings using a second-generation female No. 5 or a second-generation male No. 5 at least. There were 12 females, 4 hermaphrodites and 27 males among 43 third-generation offspring produced from the second-generation females by mating with a male *japonica*, while there were 19 females, 4 hermaphrodites and 39 males among 62 third-generation offspring produced from the above female by mating with a second-generation male No. 5. Among 14 third-generation offspring produced from the latter male mated with female *japonica*, there were a female, two

TABLE 14

Shape, size and sex of metamorphosed third-generation offspring of the

Set	(Mating)	Parents: Female no.	Parents: Male no.	Age at the time of landing (Mean) (days)	No. of frogs: Total	No. of frogs: Normal	No. of frogs: With abnormal forelegs
1	(1)	J{J(61OT2)}$_9$.1	J.W64.3	82~92 (87.0)	17	17	0
	(2)		J{J(61OT2)}$_9$.1	64~82 (70.5)	21	21	0
	(3)	J.L64.7~8	J{J(61OT2)}$_9$.1	82~91 (87.5)	37	37	0
	(4)		J{J(61OT2)}$_9$.2	91 (91.0)	6	1	5
2	(1)	J{J(61OT2)}$_8$.1	J.W64.3	85~90 (89.3)	9	0	9
	(2)		J{J(61OT2)}$_1$.1	61~63 (62.0)	4	0	4
	(3)	J{J(61OT2)}$_8$.2	J.W64.3	62~66 (62.3)	8	0	8
	(4)		J{J(61OT2)}$_1$.2	63	1	0	1
	(5)	J{J(61OT2)}$_8$.3	J.W64.3	77~85 (80.3)	24	0	24
	(6)		J{J(61OT2)}$_1$.3	61~62 (61.3)	3	0	3
	(7)	J{J(61OT2)}$_8$.4	J.W64.3	63~66 (64.3)	8	0	8
	(9)	J{J(61OT2)}$_8$.5	J.W64.3	77~92 (84.0)	65	65	0
	(10)		J{J(61OT2)}$_1$.5	87~98 (92.5)	71	71	0
	(11)	J.L64.7~8	J.W64.3	77~85 (81.5)	37	37	0
	(12)		J{J(61OT2)}$_1$.1	66~82 (80.0)	28	28	0
	(13)		J{J(61OT2)}$_1$.2	77	1	0	1
	(14)		J{J(61OT2)}$_1$.3	79	1	0	1
	(15)		J{J(61OT2)}$_1$.4	68 (68.0)	3	3	0
	(16)		J{J(61OT2)}$_1$.5	82~90 (86.3)	24	24	0
	(17)		J{J(61OT2)}$_1$.6	76	1	1	0
3	(2)	J.L64.7~8	J{J(61OT2)}$_4$.2	82 (82.0)	2	2	0

hermaphrodites and 11 males. Some of the frogs produced by each of these three experimental matings were reared continuously. Besides, some of the third-generation offspring produced from a second-generation female No. 1 by brother and sister mating as well as the control frogs were also reared continuously. After one year, all these frogs attained their sexual maturity and it was found that all but two of the 51 frogs in the four experimental mating groups were males, male nucleo-cytoplasmic hybrid No. 61OT2

Body length immediately after meta-morphosis (mm.)	Body length at the age of one year (mm.)	Sex of the young frogs shortly after metamorphosis						Sex of matured frogs		
		Total	♀N	⚥1	⚥2	⚥3	♂N	Total	♀	♂
15.0±0.07		17	8	1	0	0	8			
15.0±0.07	39.3±0.12	6	2	1	0	0	3	15	0	15
14.8±0.07		37	18	2	0	0	17			
15.0±0.00		6	1	1	0	0	4			
14.6±0.15		9	4	0	0	0	5			
14.5~15.5		4	4	0	0	0	0			
15.1±0.09		8	5	1	0	0	2			
17.0		1	1	0	0	0	0			
16.2±0.07		24	12	1	1	0	10			
15.2~16.5		3	3	0	0	0	0			
15.5±0.13		8	7	1	0	0	0			
13.8±0.07	45.4±0.44	43	12	2	1	1	27	19	2	17
13.6±0.07	32.3±0.73	62	19	1	1	2	39	7	0	7
13.0±0.07	40.5±0.55	26	14	0	0	0	12	10	5	5
14.5±0.06		28	14	0	0	0	14			
16.5		1	1	0	0	0	0			
15.6		1	1	0	0	0	0			
14.3~15.2		3	1	1	0	0	1			
15.2±0.12	37.5±0.75	14	1	1	0	1	11	10	0	10
14.5		1	1	0	0	0	0			
14.5~15.0		2	1	1	0	0	0			

while there were five females and five males among the control frogs. As the mortality was very low in these experimental matings and the controls, it was assumed that nearly half of the matured third-generation males were sex-reversed genetic females.

ii) Offspring of the male nucleo-cytoplasmic hybrid No. 61AT8

All the six male and two female second-generation offspring of the female

TABLE 15

Shape, size and sex of metamorphosed third-generation offspring of the

Set (Mating)	Parents		Age at the time of landing (Mean) (days)	No. of frogs		
	Female no.	Male no.		Total	Normal	With abnormal forelegs
1 (3)	J{J(61AT8)}$_{4}$.2	J.W64.1	83～90 (86.9)	51	51	0
(4)		J{J(61AT8)}$_{4}$.2	85～93 (89.2)	46	46	0
(5)	J.L64.1～3	J.W64.1	82～91 (86.3)	65	65	0
(6)		J{J(61AT8)}$_{4}$.1	81	1	1	0
(7)		J{J(61AT8)}$_{4}$.2	82～93 (87.1)	34	34	0
2 (1)	J.W64.4～6	J.L64.1	80～91 (85.4)	63	63	0
(3)		J{J(61AT8)}$_{6}$.2	77～81 (77.8)	5	5	0
(5)		J{J(61AT8)}$_{6}$.4	88～95 (90.4)	24	24	0

TABLE 16

Shape, size and sex of metamorphosed third-generation offspring of the

Set (Mating)	Parents		Age at the time of landing (Mean) (days)	No. of frogs		
	Female no.	Male no.		Total	Normal	With abnormal forelegs
1 (1)	J{J(61AT12)}$_{3}$.1	J.L64.4	78～88 (83.0)	7	0	7
(3)	J{J(61AT12)}$_{3}$.2	J.L64.4	84～94 (89.4)	12	0	12
(4)		J{J(61AT12)}$_{3}$.2	81～90 (85.5)	11	0	11
(5)	J.L64.1～3	J.L64.4	75～79 (77.2)	50	50	0
(6)		J{J(61AT12)}$_{3}$.1	79～82 (80.7)	29	29	0
(7)		J{J(61AT12)}$_{3}$.2	82	3	3	0
2 (1)	J{J(61AT12)}$_{6}$.1	J.L64.4	83～92 (88.1)	51	51	0
(2)		J{J(61AT12)}$_{6}$.1	83～85 (84.0)	14	14	0
(3)	J{J(61AT12)}$_{6}$.2	J.L64.4	75～82 (79.4)	45	45	0
(4)		J{J(61AT12)}$_{6}$.2	78～82 (80.3)	27	27	0
(5)	J.L64.1～3	J{J(61AT12)}$_{6}$.1	82～87 (84.5)	44	44	0
(6)		J{J(61AT12)}$_{6}$.2	80～85 (83.5)	44	44	0

male nucleo-cytoplasmic hybrid No. 61AT8

Body length immediately after meta-morphosis (mm.)	Body length at the age of one year (mm.)	Sex of young frogs shortly after metamorphosis						Sex of matured frogs		
		Total	♀$_{N}$	⚥$_{1}$	⚥$_{2}$	⚥$_{3}$	♂$_{N}$	Total	♀	♂
15.2±0.07	35.2±0.17	47	30	2	1	0	14	4	0	4
15.0±0.12	36.6±0.74	36	17	2	1	1	15	10	0	10
15.5±0.08	37.7±0.54	45	21	0	0	0	24	20	9	11
15.0		1	0	0	0	0	1			
15.3±0.07	34.3±0.91	30	15	5	2	1	7	4	0	4
14.9±0.11		63	31	2	0	0	30			
14.3~14.5		5	2	0	0	0	3			
13.4±0.04		24	7	1	0	0	16			

male nucleo-cytoplasmic hybrid No. 61AT12

Body length immediately after meta-morphosis (mm.)	Body length at the age of one year (mm.)	Sex of young frogs shortly after metamorphosis						Sex of matured frogs		
		Total	♀$_{N}$	⚥$_{1}$	⚥$_{2}$	⚥$_{3}$	♂$_{N}$	Total	♀	♂
16.2±0.04		7	2	1	0	1	3			
15.5±0.06		12	5	4	0	0	3			
14.5±0.05		11	8	3	0	0	0			
15.0±0.06	39.6±0.13	30	17	0	0	0	13	17	8	9
14.9±0.08		29	14	0	0	0	15			
14.5~15.2		3	2	0	0	0	1			
15.3±0.07		51	31	2	2	1	15			
14.3±0.07		14	6	2	1	1	4			
16.0±0.07	38.5±0.73	35	19	1	1	2	12	10	1	9
14.7±0.06	33.0±0.11	20	8	2	1	0	9	7	0	7
14.5±0.07		44	17	4	2	1	20			
14.3±0.07	32.3±0.46	34	17	3	1	1	12	9	0	9

nucleo-cytoplasmic hybrid No. 61AT8 were remarkably inferior in reproductive capacity to the controls (Table 7). No metamorphosed frogs were obtained from one of the two females mated with a male *japonica* or a second-generation male, while 40.7 and 53.7% of eggs of the other female grew into normal metamorphosed frogs by mating with the same male *japonica* and another second-generation male, respectively. By mating with these two second-generation males, 1.6 and 44.2% of *japonica* eggs became metamorphosed frogs, respectively. Such percentages of metamorphosed frogs obtained from second-generation males or females were very low in comparison with 83.3% in the control mating. By mating with two of four other second-generation males no *japonica* eggs cleaved or developed passing over the tail-bud stage, while 3.9 and 38.7% of *japonica* eggs became metamorphosed frogs by mating with the other two second-generation males, respectively. In the control mating between these female *japonica* and the above-stated male *japonica*, 88.7% of eggs grew into metamorphosed frogs.

The shape, size and sex of all metamorphosed frogs of the experimental and the control matings are shown in Table 15. While 128 tadpoles of the two control matings made metamorphosis 80～91 (mean 85.9) days after the insemination, 161 tadpoles belonging to three kinds of third-generation offspring did 77～95 (mean 87.8) days after the insemination. All these metamorphosed frogs were quite normal in shape. Except for a group of tadpoles produced from a second-generation male by mating with female *japonica*, all the metamorphosed frogs of the experimental matings were nearly the same in body length as those of the control. The exceptional group of tadpoles was somewhat smaller in mean body length.

Concerning the sex of the third-generation offspring, stated above, there were 71 females, 16 hermaphrodites and 56 males among 143 young frogs which were examined soon after metamorphosis, while there were 52 females, two hermaphrodites of Type 1 and 54 males in the two control matings (Table 15). However, 18 matured third-generation offspring produced from a female and a male second-generation offspring by brother and sister mating or by mating with male or female *japonica*, were all males, differing from the control frogs among which there were nine females and eleven males. It was quite clear that about half of the matured third-generation males were sex-reversed females, as there was no mortality in these experimental mating series.

iii) Offspring of the male nucleo-cytoplasmic hybrid No. 61AT12

In the control mating, 50(96.2%) of 52 eggs cleaved normally and developed into normal metamorphosed frogs. In contrast with the male and female *japonica*, all the second-generation offspring of the male nucleo-cytoplasmic hybrid were distinctly low in reproductive capacity (Table 8). No normal metamorphosed frogs were produced from two of the four second-generation females by mating with a male *japonica* or by brother and sister mating with two second-generation males, although a small number of abnormal frogs were obtained. From the other two second-generation females some normal frogs were produced, that is, 14.9～60.0% of eggs developed into normal tadpoles which completed their metamorphosis by mating with the above male *japonica* or by brother and sister mating with two second-generation males. By mating with the four second-generation males, 4.0~42.7% of *japonica* eggs became normal metamorphosed frogs.

All the normal and abnormal metamorphosed frogs are listed in Table 16. In the control mating, 50 frogs were 75~79 (mean 77.2) days old at the time of metamorphosis, while the frogs in each of the 11 experimental mating groups were 79.4~89.4 days in the mean age at the time of metamorphosis. Although all the 30 frogs produced from two second-generation females were abnormal in the development of forelegs, they were not always delayed in metamorphosis, as compared with the normal frogs produced from the other two second-generation females or from the four second-generation males. Moreover, these frogs with abnormal forelegs were nearly the same in body length at the stage immediately after metamorphosis as the normal frogs in the other experimental and the control matings.

The gonads of most of the third-generation offspring and the control frogs were examined immediately after metamorphosis. As the result, there were 129 females, 37 hermaphrodites and 94 males among 260 frogs produced by the 11 experimental matings, while there were 17 females and 13 males among 30 control frogs. Twenty-six frogs obtained by three experimental matings, between a second-generation female and a male *japonica* or a second-generation male, and between a second-generation male and female *japonica*, were reared continuously, together with 17 control frogs. When they attained their sexual maturity, all but one frog of the experimental matings were males, while there were eight females and nine males in the control mating.

II. Results of mating experiments performed in 1965

1. Parents used for producing third- and fourth-generation offspring

In the breeding season of the year 1965, mating experiments were performed to produce third- and fourth-generation offspring of nucleo-cytoplasmic hybrids. For the purpose of producing third-generation offspring, 11 males of three kinds of second-generation offspring of the male nucleo-cytoplasmic hybrid No. 61OT2 were mated with female *japonica*.

The 11 males were all one year old and 33~37.5 mm. in body length. Their testes were 1.6 mm. in length and 1.1 mm. in width, on the average. In the inner structures of testes, only one of the 11 males was of Type 1 and there were no males of Type 2. Six males were of Type 3, three, of Type 4 and the remaining one, of Type 5 (Table 17).

Fourth-generation offspring were produced from 32 males of ten kinds of third-generation offspring of the nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12 by mating with female *japonica*. All these males were one year old and 31.5~37 mm. in body length, except for one which was 40 mm. Their testes were 2.1 mm. in length and 1.4 mm. in width, on the average. Seventeen of the 32 males had testes which were quite normal, that is, of Type 1 in inner structure. Of the remaining 15, two were of Type 2, nine, of Type 3, three, of

TABLE 17

Male second-generation offspring of a nucleo-cytoplasmic hybrid used in the mating experiments performed in 1965

Offspring				Size of the testes		Inner structure	
Kind	Individual no.	Age (year)	Body length (mm.)	Left (mm.)	Right (mm.)	Type	Ploidy
{J(61OT2)}₂J	1	1	37.5	1.5×1.0	1.5×1.0	type 1	2n
	2	1	36.0	1.5×1.0	1.5×1.0	type 3	2n
	3	1	35.0	2.0×1.0	1.5×1.0	type 5	2n
	4	1	34.0	2.0×1.5	1.5×1.0	type 4	2n
{J(61OT2)}₂ {J(61OT2)}₁₁	1	1	35.5	1.5×1.0	1.5×1.0	type 3	2n
	2	1	33.0	1.5×1.0	1.0×1.0	type 4	2n
	3	1	34.0	1.5×1.0	1.5×1.0	type 3	2n
J{J(61OT2)}₁₁	1	1	34.5	2.0×1.0	2.0×1.5	type 3	2n
	2	1	33.5	1.5×1.0	1.5×1.5	type 4	2n
	3	1	34.5	1.5×1.0	1.5×1.0	type 3	2n
	4	1	35.0	1.5×1.0	1.5×1.0	type 3	2n

J —— *Rana japonica*

$\{J(61OT2)\}_2J$ —— Offspring obtained by (J♀ × ♂No. 61OT2) ♀No. 2 × J♂

$\{J(61OT2)\}_2\{J(61OT2)\}_{11}$ —— Offspring obtained by (J♀ × ♂No. 61OT2) ♀No. 2 × (J♀ × ♂No. 61OT2) ♂No. 11

$J\{J(61OT2)\}_{11}$ —— Offspring obtained by J♀ × (J♀ × ♂No. 61OT2) ♂No. 11

Type 4 and one, of Type 5 in the inner structures of testes (Table 18). As the control of these male third-generation offspring, five male *japonica* were utilized for mating with female *japonica*. They belonged to a laboratory strain and were reared for one year since the egg stage in our laboratory. They were 37～46 mm. in body length. Their testes were 2.5 mm. in length and 1.7 mm. in width, on the average. All of them were of Type 1 in inner structure.

Thirteen female *japonica* were utilized for mating with the above male second- and third-generation offspring. Five of the 13 female *japonica* were of laboratory strains and had been reared for a year since the egg stage in our laboraotry, while the other eight were collected from the field. They were 35.5～46.5 mm. in body length. Their eggs were 1.52～1.67 mm. in the mean diameter of 50 eggs; there were scarcely any differences in egg size among these females (Table 19).

2. Production of third-generation offspring

Four of the 11 male second-generation offspring were obtained from a female No. 2 of first-generation offspring between a female *japonica* and the male nucleo-cytoplasmic hybrid by mating with a male *japonica*. Three other males were obtained from the same female of first-generation offspring by mating with a brother No. 11. The remaining four males were obtained from the latter male No. 11 by mating with a female *japonica*. The first-generation offspring were those which had been produced by mating of the male nucleo-cytoplasmic hybrid No. 61OT2 with a female *japonica* (Table 20).

Set 1. Control mating. J.L65♀ Nos. 12 and 13 × J.L65♂ No. 5

There were 92(97.9%) normally cleaved eggs. Two of them died of abnormal

TABLE 18

Male third-generation offspring of three nucleo-cytoplasmic hybrids and the control frog used in the mating experiments performed in 1965

Offspring				Size of the testes		Inner structure	
Kind	Individual no.	Age (year)	Body length (mm.)	Left (mm.)	Right (mm.)	Type	Ploidy
J.L65	1	1	46.0	3.0×2.0	3.0×2.0	type 1	2n
	2	1	45.0	3.0×2.0	3.0×2.0	type 1	2n
	3	1	37.0	2.5×2.0	2.5×2.0	type 1	2n
	4	1	45.5	2.0×1.0	2.0×1.0	type 1	2n
	5	1	41.0	2.0×1.5	2.0×1.5	type 1	2n
[J{J(61OT2)}$_8$]$_5$J	1	1	34.0	2.0×1.0	2.0×1.0	type 1	2n
	2	1	35.0	2.5×1.5	2.5×1.5	type 1	2n
[J{J(61OT2)}$_9$]$_1$[J{J(61OT2)}$_9$]$_1$	1	1	34.5	2.0×1.5	2.0×2.0	type 2	4n
	2	1	34.0	2.0×1.5	2.0×1.5	type 3	2n
[J{J(61OT2)}$_8$]$_5$[J{J(61OT2)}$_1$]$_5$	1	1	33.0	2.0×1.5	2.0×1.5	type 3	2n
	2	1	32.5	2.0×1.0	2.0×1.5	type 1	2n
	3	1	34.5	2.5×1.5	2.0×1.5	type 3	2n
	4	1	33.5	2.0×1.0	2.0×1.5	type 3	2n
	5	1	34.0	2.5×2.0	2.5×2.0	type 3	2n
J[J{J(61OT2)}$_1$]$_5$	1	1	40.0	2.5×2.0	2.5×2.0	type 1	2n
	2	1	32.0	2.0×1.0	2.0×1.5	type 1	2n
	3	1	32.5	2.0×1.0	1.5×1.0	type 5	2n
[J{J(61AT8)}$_4$]$_2$J	1	1	35.0	2.0×1.5	2.0×1.0	type 1	2n
	2	1	33.0	2.0×1.5	2.0×1.5	type 1	2n
	3	1	35.5	2.5×2.0	2.5×2.0	type 2	2n
	4	1	33.0	2.0×1.5	2.0×1.0	type 1	2n
[J{J(61AT8)}$_4$]$_2$[J{J(61AT8)}$_4$]$_2$	1	1	32.5	2.0×1.0	2.0×1.0	type 4	2n
	2	1	32.0	2.0×1.0	2.0×1.0	type 4	2n
	3	1	31.5	1.5×1.0	2.0×1.0	type 3	2n
	4	1	33.0	2.0×1.5	2.5×2.0	type 3	2n
J[J{J(61AT8)}$_4$]$_2$	1	1	32.5	2.0×1.5	2.0×1.5	type 1	2n
	2	1	33.5	2.5×1.0	2.5×1.5	type 1	2n
[J{J(61AT12)}$_6$]$_2$J	1	1	37.0	2.0×1.5	2.0×2.0	type 1	2n
	2	1	35.5	2.0×1.0	2.0×1.0	type 1	2n
	3	1	36.0	2.0×1.0	1.5×1.0	type 3	2n
[J{J(61AT12)}$_6$]$_2$[J{J(61AT12)}$_6$]$_2$	1	1	35.0	2.0×1.0	2.0×1.0	type 3	2n
	2	1	35.5	2.0×1.5	2.0×1.5	type 1	2n
	3	1	34.5	2.0×1.0	2.0×1.5	type 1	2n
	4	1	33.0	2.0×1.0	2.0×1.5	type 4	2n
J[J{J(61AT12)}$_6$]$_2$	1	1	34.5	1.5×1.0	1.5×1.0	type 1	2n
	2	1	34.0	2.0×1.0	2.0×1.0	type 1	2n
	3	1	35.0	2.0×1.5	2.0×1.0	type 1	2n

J —— *Rana japonica*

L —— Laboratory strain

[J{J(61AT8)}$_4$]$_2$J —— Offspring obtained by {J♀ × (J♀ × ♂No. 61AT8) ♂No. 4} ♀No. 2 × J♂

[J{J(61AT8)}$_4$]$_2$[J{J(61AT8)}$_4$]$_2$ —— Offspring obtained by {J♀ × (J♀ × ♂No. 61AT8) ♂No. 4} ♀No. 2 × {J♀ × (J♀ × ♂No. 61AT8) ♂No. 4} ♂No. 2

J[J{J(61AT8)}$_4$]$_2$ —— Offspring obtained by J♀ × {J♀ × (J♀ × ♂No. 61AT8) ♂No. 4} ♂No. 2

TABLE 19

Female control frogs used in the mating experiments performed in 1965

Offspring		Age (year)	Body length (mm.)	Mean diameter of 50 eggs
Kinds	Individual no.			
J.W65	1		45.5	1.62±0.05
	2		46.5	1.60±0.04
	3		42.0	1.55±0.04
	4		40.5	1.53±0.04
	5		40.0	1.52±0.03
J.L65	6	1	40.0	1.65±0.03
	7	1	35.5	1.67±0.03
	8	1	37.0	1.66±0.04
J.W65	9		45.0	1.64±0.03
	10		46.0	1.60±0.03
	11		46.5	1.65±0.03
J.L65	12	1	40.5	1.53±0.03
	13	1	40.0	1.60±0.03

J.L —— Laboratory strain of *Rana japonica*
J.W —— *Rana japonica* collected from the field

TABLE 20

Reproductive capacities of male second-generation offspring of the

Set (Mating)	Parents		No. of eggs	No. of cleaved eggs	
	Female no.	Male no.		Normal	Abnormal
1 (Control)	J.L65.12～13	J.L65.5	94	92 (97.9%)	0
2 (1)	J.L65.12～13	{J(61OT2)}$_2$J.1	125	121 (96.8%)	0
(2)		{J(61OT2)}$_2$J.2	130	116 (89.2%)	0
(3)		{J(61OT2)}$_2$J.3	144	3 (2.1%)	12 (8.3%)
(4)		{J(61OT2)}$_2$J.4	126	32 (25.4%)	8 (6.3%)
3 (1)	J.L65.12～13	{J(61OT2)}$_2${J(61OT2)}$_{11}$.1	135	34 (25.2%)	0
(2)		{J(61OT2)}$_2${J(61OT2)}$_{11}$.2	137	127 (92.7%)	0
(3)		{J(61OT2)}$_2${J(61OT2)}$_{11}$.3	141	138 (97.9%)	0
4 (1)	J.L65.12～13	J{J(61OT2)}$_{11}$.1	210	116 (55.2%)	3 (1.4%)
(2)		J{J(61OT2)}$_{11}$.2	163	5 (3.1%)	0
(3)		J{J(61OT2)}$_{11}$.3	251	57 (22.7%)	4 (1.6%)
(4)		J{J(61OT2)}$_{11}$.4	342	115 (33.6%)	0

structures at the tail-bud or hatching stage. The other 90 developed normally into tadpoles and completed their metamorphosis except for three which died during the metamorphosis.

Set 2. Four males Nos. 1 ~ 4 produced from a female first-generation offspring No. 2 mated with a male *japonica*

Mating 1. J.L65♀ Nos. 12 and 13 × $\{J(61OT2)\}_2J$♂ No. 1

Normally cleaved eggs were 121(96.8%) in number. Four, four and five of them died of various types of abnormalities at the gastrula, neurula and the tail-bud stage, respectively. Nineteen at the hatching and four at the XII stage died of edema. The other 69 developed normally and completed their metamorphosis. However, there were 17 frogs whose legs were ill-developed or abnormal. The remaining 52 frog were quite normal.

Mating 2. J.L65♀ Nos. 12 and 13 × $\{J(61OT2)\}_2J$♂ No. 2

There were 116(89.2%) normally cleaved eggs. Two at the neurula, seven at the tail-bud and 16 at the hatching stage died of edema, respectively. At the V ~ XIII stages 58 tadpoles became edematous and died. These tadpoles had a few white flecks of aragonite crystal in the dorsal skin of the body. At the XX ~ XXII stages three tadpoles died. Although the remaining 30 completed metamorphosis, only seven of them were normal frogs. Eleven had abnormal or

male nucleo-cytoplasmic hybrid No. 61OT2

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.
92 (97.9%)	0	92 (97.9%)	0	91 (96.8%)	1 (1.1%)	90 (95.7%)	1 (1.1%)	87 (92.6%)	0
101 (80.8%)	4 (3.2%)	97 (77.6%)	4 (3.2%)	92 (73.6%)	5 (4.0%)	73 (58.4%)	19 (15.2%)	52 (41.6%)	17 (13.6%)
116 (89.2%)	0	114 (87.7%)	2 (1.5%)	107 (82.3%)	7 (5.4%)	91 (70.0%)	16 (12.3%)	7 (5.4%)	23 (17.7%)
2 (1.4%)	0	2 (1.4%)	0	1 (0.7%)	1 (0.7%)	0	1 (0.7%)	0	0
32 (25.4%)	0	27 (21.4%)	5 (4.0%)	21 (16.7%)	6 (4.8%)	15 (11.9%)	6 (4.8%)	4 (3.2%)	2 (1.6%)
34 (25.2%)	0	34 (25.2%)	0	34 (25.2%)	0	34 (25.2%)	0	5 (3.7%)	23 (17.0%)
127 (92.7%)	0	127 (92.7%)	0	119 (86.9%)	8 (5.8%)	68 (49.6%)	51 (37.2%)	3 (2.2%)	0
138 (97.9%)	0	138 (97.9%)	0	138 (97.9%)	0	101 (71.6%)	37 (26.2%)	24 (17.0%)	0
116 (55.2%)	0	107 (50.9%)	9 (4.3%)	97 (46.2%)	10 (4.8%)	21 (10.0%)	76 (36.2%)	0	0
5 (3.1%)	0	5 (3.1%)	0	5 (3.1%)	0	3 (1.8%)	2 (1.2%)	3 (1.8%)	0
55 (21.9%)	2 (0.8%)	50 (19.9%)	5 (2.0%)	36 (14.3%)	14 (5.6%)	26 (10.4%)	10 (4.0%)	8 (3.2%)	7 (2.8%)
115 (33.6%)	0	115 (33.6%)	0	95 (27.8%)	20 (5.8%)	82 (24.0%)	13 (3.8%)	53 (15.5%)	0

ill-developed fore and hind legs. Eight had no forelegs. The remaining four had forelegs and were edematous. They had deposition of aragonite crystals in the dorsal skin of the body.

Mating 3. J.L65♀ Nos. 12 and 13×$\{J(61OT2)\}_2$J♂ No. 3

The rate of fertilization was very low: only three eggs cleaved normally. These eggs died by the hatching stage.

Mating 4. J.L65♀ Nos. 12 and 13×$\{J(61OT2)\}_2$J♂ No. 4

There were 32(25.4%) normally cleaved eggs. Seventeen eggs died by the hatching stage and 15 developed normally into tadpoles. By the XII stage nine tadpoles died of edema. Although six tadpoles completed their metamorphosis, two died of edema soon afterwards. The other four became quite normal frogs.

Set 3. Three males Nos. 1~3 produced by brother and sister mating of first-generation offspring

Mating 1. J.L65♀ Nos. 12 and 13×$\{J(61OT2)\}_2$ $\{J(61OT2)\}_{11}$♂ No. 1

All the normally cleaved eggs, 34(25.2%) in number, developed normally into tadpoles. At the XVII stage six tadpoles died of edema. These tadpoles had deposition of aragonite crystals in the dorsal skin. Although 28 tadpoles completed their metamorphosis, only 5 were quite normal. Sixteen had no forelegs. Seven were abnormal in the development of fore and hind legs. Some frogs had deposits of aragonite crystals in the dorsal skin of the body as a few white flecks.

Mating 2. J.L65♀ Nos. 12 and 13×$\{J(61OT2)\}_2$ $\{J(61OT2)\}_{11}$♂ No. 2

The rate of fertilization was very high, that is, 127(92.7%) eggs cleaved normally. However, 59 of them died of edema by the hatching stage. During the time from the II stage to the XVII, 65 tadpoles died of edema or some other types of abnormalities. Only three tadpoles completed their metamorphosis and became quite normal frogs.

Mating 3. J.L65♀ Nos. 12 and 13×$\{J(61OT2)\}_2\{J(61OT2)\}_{11}$♂ No. 3

There were 138(97.9%) normally cleaved eggs. At the hatching stage 37 of them died of edema. The others developed normally into tadpoles. But 52 of the latter died of edema or some other types of abnormalities at the XII~XVII stages and 25 died during metamorphosis. The remaining 24 tadpoles completed their metamorphosis.

Set 4. Four males Nos. 1~4 produced from a male first-generation offspring No. 11 mated with a female *japonica*

Mating 1. J.L65♀ Nos. 12 and 13×J$\{J(61OT2)\}_{11}$♂ No. 1

There were 116(55.2%) normally cleaved eggs. However, 95 of them died of edema by the hatching stage and the remaining 21 died also of edema at the XII~XVII stages.

Mating 2. J.L65♀ Nos. 12 and 13×J$\{J(61OT2)\}_{11}$♂ No. 2

The rate of fertilization was very low and only five (3.1%) eggs cleaved normally. Two of them died of edema at the hatching stage and the other three completed their metamorphosis.

Mating 3. J.L65♀ Nos. 12 and 13×J$\{J(61OT2)\}_{11}$♂ No. 3

Normally cleaved eggs were 57(22.7%) in number. Thirty-one of them died of various types of abnormalities by the hatching stage. Although the others developed normally into tadpoles, six of the latter died of edema at the XII～XVII stages and five others died during metamorphosis. The remaining 15 tadpoles completed their metamorphosis. While eight of them were quite normal, the other seven had ill-developed forelegs which could not support the body.

Mating 4. J.L65♀ Nos. 12 and 13×J{J(61OT2)}$_{11}$♂ No. 4

Of 115(33.6%) normally cleaved eggs 33 died of edema by the hatching stage and the others developed into tadpoles. At the II～III stages 29 tadpoles died of various types of abnormalities. The remaining 53 completed their metamorphosis and became normal frogs.

3. Production of fourth-generation offspring

Fourth-generation offspring of the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12 were produced by mating of male third-generation offspring obtained by mating experiments performed in the year 1964, with female *japonica*.

a. Mating experiments by using male third-generation offspring of the male nucleo-cytoplasmic hybrid No. 61OT2

Three females (Nos. 6～8) raised since the egg stage in our laboratory were mated with four kinds of male third-generation offspring of the male nucleo-cytoplasmic hybrid No. 61OT2(Table 21). The eggs of each of these females were divided into 13 groups. The eggs of one group were utilized as the control (Set 1) by insemination with sperm of a male *japonica* which had been reared since the egg stage in our laboratory. The eggs of two groups (Set 2) were inseminated with sperm of two male third-generation offspring which had been produced from a female second-generation offspring No. 5 by mating with a male *japonica*. This female was obtained from a male first-generation offspring No. 8. The eggs of two other groups (Set 3) were inseminated with sperm of two males of third-generation offspring which had been produced by brother and sister mating between a female No. 1 and a male No. 1 of the second-generation obtained from a male first-generation offspring No. 9. The eggs of five groups (Set 4) were inseminated with sperm of five male third-generation offspring which had been produced by mating between a female second-generation offspring No. 5 obtained from a male first-generation offspring No. 8 and a male second-generation offspring No. 5 obtained from a male first-generation offspring No. 1. The eggs of the remaining three groups (Set 5) were inseminated with sperm of three male third-generation offspring which had been produced by the above-stated mating between the male second generation offspring No. 5 and the female *japonica*.

Set 1. Control mating. J. L65♀ Nos. 6～8×J.L65♂ No. 3

There were 130(97.0%) normally cleaved eggs. While seven of them died of ill-development at the tadpole stage, the others developed normally into metamorphosed frogs.

TABLE 21

Reproductive capacities of male third-generation offspring of

Set (Mating)	Parents		No. of eggs	No. of cleaved eggs	
	Female no.	Male no.		Normal	Abnormal
1 (Control)	J.L65.6∼8	J.L65.3	134	130 (97.0%)	0
2 (1)	J.L65.6∼8	$[J\{J(61OT2)\}_8]_5J.1$	132	125 (94.7%)	2 (1.5%)
(2)		$[J\{J(61OT2)\}_8]_5J.2$	221	210 (95.0%)	1 (0.5%)
3 (1)	J.L65.6∼8	$[J\{J(61OT2)\}_9]_1$ $[J\{J(61OT2)\}_9]_1.1$	354	250 (70.6%)	14 (4.0%)
(2)		$[J\{J(61OT2)\}_9]_1$ $[J\{J(61OT2)\}_9]_1.2$	406	89 (21.9%)	36 (8.9%)
4 (1)	J.L65.6∼8	$[J\{J(61OT2)\}_8]_5$ $[J\{J(61OT2)\}_1]_5.1$	103	100 (97.1%)	2 (1.9%)
(2)		$[J\{J(61OT2)\}_8]_5$ $[J\{J(61OT2)\}_1]_5.2$	137	124 (90.5%)	0
(3)		$[J\{J(61OT2)\}_8]_5$ $[J\{J(61OT2)\}_1]_5.3$	165	152 (92.1%)	11 (6.7%)
(4)		$[J\{J(61OT2)\}_8]_5$ $[J\{J(61OT2)\}_1]_5.4$	142	71 (50.0%)	53 (37.3%)
(5)		$[J\{J(61OT2)\}_8]_5$ $[J\{J(61OT2)\}_1]_5.5$	86	50 (58.1%)	0
5 (1)	J.L65.6∼8	$J[J\{J(61OT2)\}_1]_5.1$	180	174 (96.7%)	6 (3.3%)
(2)		$J[J\{J(61OT2)\}_1]_5.2$	299	273 (91.3%)	15 (5.0%)
(3)		$J[J\{J(61OT2)\}_1]_5.3$	253	0	0

Set 2. Two males Nos. 1 and 2 produced from a female second-generation offspring No. 5 mated with a male *japonica*

Mating 1. J.L65♀ Nos. 6∼8 × $[J\{J(61OT2)\}_8]_5J$♂ No. 1

Normally cleaved eggs were 125(94.7%) in number. They developed normally into tadpoles, except for three which died at the hatching stage. Two tadpoles died of edema at the XII stage. The other 120 completed their metamorphosis.

Mating 2. J.L65♀ Nos. 6∼8 × $[J\{J(61OT2)\}_8]_5J$♂ No. 2

Normally cleaved eggs were 210(95.0%) in number. Eight and 13 of them died of ill-development or abnormal structures at the hatching and the feeding tadpole stage, respectively. The remaining 189 tadpoles completed their metamorphosis.

Set 3. Two males Nos. 1 and 2 produced by brother and sister mating of second-generation offspring

Mating 1. J.L65♀ Nos. 6∼8 × $[J\{J(61OT2)\}_9]_1[J\{J(61OT2)\}_9]_1$♂ No. 1

There were 250(70.6%) normally cleaved eggs. While nine and ten of them the male nucleo-cytoplasmic hybrid No. 61OT2

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.
130 (97.0%)	0	130 (97.0%)	0	130 (97.0%)	0	130 (97.0%)	0	123 (91.8%)	0
125 (94.7%)	0	125 (94.7%)	0	125 (94.7%)	0	122 (92.4%)	3 (2.3%)	120 (90.9%)	0
210 (95.0%)	0	210 (95.0%)	0	210 (95.0%)	0	202 (91.4%)	8 (3.6%)	189 (85.5%)	0
250 (70.6%)	0	250 (70.6%)	0	250 (70.6%)	0	241 (68.1%)	9 (2.5%)	231 (65.3%)	0
89 (21.9%)	0	89 (21.9%)	0	89 (21.9%)	0	74 (18.2%)	15 (3.7%)	45 (11.1%)	1 (0.2%)
97 (94.2%)	3 (2.9%)	97 (94.2%)	0	95 (92.2%)	2 (1.9%)	89 (86.4%)	6 (5.8%)	5 (4.9%)	0
124 (90.5%)	0	124 (90.5%)	0	122 (89.1%)	2 (1.5%)	117 (85.4%)	5 (3.6%)	4 (2.9%)	0
149 (90.3%)	3 (1.8%)	149 (90.3%)	0	138 (83.6%)	11 (6.7%)	118 (71.5%)	20 (12.1%)	20 (12.1%)	0
61 (43.0%)	10 (7.0%)	55 (38.7%)	6 (4.2%)	51 (35.9%)	4 (2.8%)	44 (31.0%)	7 (4.9%)	4 (2.8%)	0
50 (58.1%)	0	25 (29.1%)	25 (29.1%)	16 (18.6%)	9 (10.5%)	3 (3.5%)	13 (15.1%)	3 (3.5%)	0
174 (96.7%)	0	174 (96.7%)	0	174 (96.7%)	0	163 (90.6%)	11 (6.1%)	161 (89.4%)	0
273 (91.3%)	0	273 (91.3%)	0	273 (91.3%)	0	267 (89.3%)	6 (2.0%)	242 (80.9%)	0
0	0	0	0	0	0	0	0	0	0

died of edema at the hatching and the feeding tadpole stage, respectively, the other 231 developed normally into metamorphosed frogs.

Mating 2. J.L65♀ Nos. 6～8 × $[J\{J(61OT2)\}_9]_1[J\{J(61OT2)\}_9]_1$♂ No. 2

Normally cleaved eggs were 89(21.9%) in number. Fifteen and 28 of them died of ill-development or abnormal structures at the hatching and the feeding tadpole stage, respectively. The remaining 46 developed normally into metamorphosed frogs, except for one which was edematous at the time of metamorphosis and died soon afterwards.

Set 4. Five male Nos. 1～5 produced by mating between a female second-generation offspring No. 5

Mating 1. J.L65♀ Nos. 6～8 × $[J\{J(61OT2)\}_8]_5[J\{J(61OT2)\}_1]_5$♂ No. 1

Of 100(97.1%) normally cleaved eggs 11 died of abnormal structures by the hatching stage and the other 89 developed into feeding tadpoles. Most of the latter died of ill-development or some other abnormalities at the II～III stages. However, five tadpoles grew rapidly and were of extraordinarily large size. They completed normally their metamorphosis.

Mating 2. J.L65♀ Nos. 6～8 × $[J\{J(61OT2)\}_8]_5[J\{J(61OT2)\}_1]_5$♂ No. 2

Seven of 124(90.5%) normally cleaved eggs died of abnormal structures by the hatching stage and 117 developed normally into tadpoles. Only four of the latter became extraordinarily large and completed their metamorphosis, while the other 113 gradually died of ill-development during the period of the II～VI stages.

Mating 3. J.L65♀ Nos. 6～8 × $[J\{J(61OT2)\}_8]_5[J\{J(61OT2)\}_1]_5$♂ No. 3

There were 152(92.1%) normally cleaved eggs. By the hatching stage 34 of them died of abnormal structures. Although the other 118 developed normally into tadpoles, 98 of the latter gradually died of ill-development during the period of the II～V stages. The remaining 20 tadpoles became remarkably large and completed their metamorphosis.

Mating 4. J.L65♀ Nos. 6～8 × $[J\{J(61OT2)\}_8]_5[J\{J(61OT2)\}_1]_5$♂ No. 4

Of 71(50.0%) normally cleaved eggs 27 died of abnormal structures by the hatching stage and the other 44 developed normally into tadpoles. Forty of the latter gradually died of ill-development or edema at the II～V stages. The

TABLE 22

Reproductive capacities of male third-generation offspring of the

Set (Mating)	Parents: Female no.	Parents: Male no.	No. of eggs	No. of cleaved eggs: Normal	No. of cleaved eggs: Abnormal
1 (Control)	J.W65.1～3	J.L65.1	127	96 (75.6%)	0
2 (1)	J.W65.1～3	$[J\{J(61AT8)\}_4]_2J.1$	220	180 (81.8%)	0
(2)		$[J\{J(61AT8)\}_4]_2J.2$	112	90 (80.4%)	6 (5.4%)
(3)		$[J\{J(61AT8)\}_4]_2J.3$	308	262 (85.1%)	2 (0.6%)
(4)		$[J\{J(61AT8)\}_4]_2J.4$	306	211 (69.0%)	2 (0.7%)
3 (Control)	J.W65.4～5	J.L65.2	43	42 (97.7%)	0
4 (1)	J.W65.4～5	$[J\{J(61AT8)\}_4]_2[J\{J(61AT8)\}_4]_2.1$	183	141 (77.0%)	6 (3.3%)
(2)		$[J\{J(61AT8)\}_4]_2[J\{J(61AT8)\}_4]_2.2$	116	0	2 (1.7%)
(3)		$[J\{J(61AT8)\}_4]_2[J\{J(61AT8)\}_4]_2.3$	294	257 (87.4%)	10 (3.4%)
(4)		$[J\{J(61AT8)\}_4]_2[J\{J(61AT8)\}_4]_2.4$	236	223 (94.5%)	11 (4.7%)
5 (1)	J.W65.4～5	$J[J\{J(61AT8)\}_4]_2.1$	235	217 (92.3%)	14 (6.0%)
(2)		$J[J\{J(61AT8)\}_4]_2.2$	214	171 (79.9%)	25 (11.7%)

remaining four were extremely good in growth and completed their metamorphosis.

Mating 5. J.L65♀ Nos. 6~8×$[J\{J(61OT2)\}_8]_5[J\{J(61OT2)\}_1]_5$♂ No. 5

Fifty (58.1%) eggs cleaved normally. By the hatching stage 47 of them died of edema and the other three developed normally into tadpoles. The latter completed their metamorphosis.

Set 5. Three males Nos. 1~3 produced from a male second-generation offspring No. 5 mated with a female *japonica*

Mating 1. J.L65♀ Nos. 6~8×$J[J\{J(61OT2)\}_1]_5$♂ No. 1

There were 174(96.7%) normally cleaved eggs. Eleven of them died of edema at the hatching stage and two died of ill-development at the feeding tadpole stage. The remaining 161 completed normally their metamorphosis.

Mating 2. J.L65♀ Nos. 6~8×$J[J\{J(61OT2)\}_1]_5$♂ No. 2

There were 273(91.3%) normally cleaved eggs. At the hatching stage six of them died of edema and the others developed into tadpoles. While 21 of the latter died of ill-development at the feeding tadpole stage and four died during the metamorphosis, the remaining 242 became normal, metamorphosed frogs.

male nucleo-cytoplasmic hybrid No. 61AT8

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.
94 (74.0%)	2 (1.6%)	94 (74.0%)	0	94 (74.0%)	0	94 (74.0%)	0	93 (73.2%)	1 (0.8%)
179 (81.4%)	1 (0.5%)	179 (81.4%)	0	178 (80.9%)	1 (0.5%)	167 (75.9%)	11 (5.0%)	97 (44.1%)	58 (26.4%)
90 (80.4%)	0	90 (80.4%)	0	90 (80.4%)	0	89 (79.5%)	1 (0.9%)	54 (48.2%)	26 (23.2%)
261 (84.7%)	1 (0.3%)	250 (81.2%)	11 (3.6%)	250 (81.2%)	0	239 (77.6%)	11 (3.6%)	82 (26.6%)	130 (42.2%)
208 (68.0%)	3 (1.0%)	208 (68.0%)	0	208 (68.0%)	0	208 (68.0%)	0	133 (43.5%)	75 (24.5%)
42 (97.7%)	0	42 (97.7%)	0	42 (97.7%)	0	42 (97.7%)	0	39 (90.7%)	0
141 (77.0%)	0	141 (77.0%)	0	141 (77.0%)	0	4 (2.2%)	137 (74.9%)	4 (2.2%)	0
0	0	0	0	0	0	0	0	0	0
247 (84.0%)	0	246 (83.7%)	1 (0.3%)	245 (83.3%)	1 (0.3%)	1 (0.3%)	244 (83.0%)	0	1 (0.3%)
212 (89.8%)	0	212 (89.8%)	0	207 (87.8%)	5 (2.1%)	8 (3.4%)	199 (84.3%)	6 (2.5%)	2 (0.8%)
217 (92.3%)	0	217 (92.3%)	0	217 (92.3%)	0	213 (90.6%)	4 (1.7%)	213 (90.6%)	0
171 (79.9%)	0	165 (77.1%)	6 (2.8%)	152 (71.0%)	13 (6.1%)	140 (65.4%)	12 (5.6%)	14 (6.5%)	59 (27.6%)

Mating 3. J.L65♀ Nos. 6～8 ×J[J{J(61OT2)}$_1$]$_5$♂ No. 3
No eggs were fertilized.

b. Mating experiments by using male third-generation offspring of the male nucleo-cytoplasmic hybrid No. 61AT8

Five females Nos. 1～5 collected from the field were used for mating experiments to produce fourth-generation offspring of this nucleo-cytoplasmic hybrid (Table 22). They were divided into two parts, three females Nos. 1～3 and two females Nos. 4 and 5. The eggs of each of Nos. 1～3 were divided into five groups. One group was utilized as the control (Set 1) of the following mating experiments, by inseminating with sperm of a male *japonica* raised since the egg stage in our laboratory. The eggs of the other four groups (Set 2) were inseminated with sperm of four male third-generation offspring which had been produced by mating of a female No. 2 of the second-generation offspring obtained from J♀ ×{J♀ ×(61AT8)♂}♂No. 4 with a male *japonica*.

The eggs of each of the two females Nos. 4 and 5 of the other part were divided into seven groups. The eggs of one group were inseminated with sperm of a male *japonica* raised in our laboratory and utilized as the control (Set 3). The eggs of four other groups (Set 4) were inseminated with sperm of four male third-generation offspring produced by mating between a female No. 2 and a male No. 2 of the above-stated second-generation offspring. The eggs of each of the remaining two groups (Set 5) were inseminated with sperm of two male third-generation offspring which had been produced by mating of a female *japonica* with the male No. 2 of the above second-generation offspring.

Set 1. Control mating. J.W65♀ Nos. 1～3 ×J.L65♂ No. 1

There were 96(75.6%) normally cleaved eggs. All these developed normally into tadpoles, except for two which died of abnormalities at the gastrula stage. All the tadpoles completed their metamorphosis. While one frog had abnormal hind legs, the others were quite normal in external structure.

Set 2. Four males Nos. 1～4 produced from a female second-generation offspring No. 2 mated with a male *japonica*

Mating 1. J.W65♀ Nos. 1～3×[J{J(61AT8)}$_4$]$_2$J♂ No. 1

Normally cleaved eggs were 180(81.8%) in number. Thirteen of them died of abnormalities by the hatching stage and the others developed into tadpoles. Nine tadpoles died at the VII～XVII stages and three others died during their metamorphosis. Although the remaining 155 tadpoles completed their metamorphosis, 58 metamorphosed frogs had ill-developed fore and hind legs. The other 97 frogs were quite normal in external characters.

Mating 2. J.W65♀ Nos. 1～3×[J{J(61AT8}$_4$]$_2$J♂ No. 2

Normally cleaved eggs were 90(80.4%) in number. One of them died of abnormalities at the tail-bud stage and the others developed into tadpoles. Seven and two of the latter died at the V～X and the XX～XXIII stages, respectively. The other 80 tadpoles completed their metamorphosis. However, 26 frogs had abnormal fore and hind legs and some of these frogs had deposits of aragonite crystals in the dorsal skin of the body. The remaining 54 frogs were quite normal.

Mating 3. J.W65♀ Nos. 1～3×[J{J(61AT8)}$_4$]$_2$J♂ No. 3

Normally cleaved eggs were 262(85.1%) in number. Twenty-three of them died of edema by the hatching stage. While twenty and seven tadpoles died at the XVII and the XX and XXIII stages, respectively, the other 212 completed their metamorphosis. Eighty-two frogs were quite normal, 127 had abnormal fore and hind legs (Fig. 4, e and f) and the remaining three were edematous.

Mating 4. J.W65♀ Nos. 1～3×[J{J(61AT8)}$_4$]$_2$J♂ No. 4

There were 211(69.0%) normally cleaved eggs. With the exception of three eggs which died of abnormalities at the gastrula stage, all of them developed normally into tadpoles and completed their metamorphosis. While 133 frogs were quite normal in external characters, the other 75 had ill-developed fore and hind legs. Some of the latter frogs had deposits of aragonite crystals in the dorsal skin of the body.

Set 3. Control mating. J.W65♀ Nos. 4 and 5×J.L65♂ No. 2

There were 42(97.7%) normally cleaved eggs. All of them developed normally into tadpoles. While three tadpoles died at the XX～XXII stages, the others completed their metamorphosis and became normal frogs.

Set 4. Four male Nos. 1～4 produced by brother and sister mating of second-generation offspring

Mating 1. J.W65♀ Nos. 4 and 5×[J{J(61AT8)}$_4$]$_2$[J{J(61AT8)}$_4$]$_2$♂ No. 1

Although normally cleaved eggs were 141(77.0%) in number, 137 of them died of various types of abnormalities at the hatching stage and only four developed normally into metamorphosed frogs.

Mating 2. J.W65♀ Nos. 4 and 5×[J{J(61AT8)}$_4$]$_2$[J{J(61AT8)}$_4$]$_2$♂ No. 2

No eggs cleaved normally.

Mating 3. J.W65♀ Nos. 4 and 5×[J{J(61AT8)}$_4$]$_2$[J{J(61AT8)}$_4$]$_2$♂ No. 3

There were 257(87.4%) normally cleaved eggs. Twelve of them died of abnormal structures by the tail-bud stage. At the hatching stage 244 embryos died simultaneously of edema and only one developed normally into a tadpole. The latter died of edema after the completion of metamorphosis.

Mating 4. J.W65♀ Nos. 4 and 5×[J{J(61AT8)}$_4$]$_2$[J{J(61AT8)}$_4$]$_2$♂ No. 4

Sixteen of 223(94.5%) normally cleaved eggs died by the tail-bud stage and 199 died simultaneously of various types of abnormalities at the hatching stage. The remaining eight developed normally into tadpoles and completed their metamorphosis. While two frogs died soon afterwards of edema, the other six showed especially good growth.

Set 5. Two males Nos. 1 and 2 produced from a male second-generation offspring No. 2 mated with a female *japonica*

Mating 1. J.W65♀ Nos. 4 and 5×J[J{J(61AT8)}$_4$]$_2$♂ No. 1

There were 217(92.3%) normally cleaved eggs. Four of them died of abnormal structures at the hatching stage. All the other 213 developed normally and completed their metamorphosis.

Mating 2. J.W65♀ Nos. 4 and 5×J[J{J(61AT8)}$_4$]$_2$♂ No. 2

There were 171(79.9%) normally cleaved eggs. By the hatching stage 31 of them died of various types of abnormalities. Although the other 140 developed normally into tadpoles, 67 died of edema at the X~XVII stages and 73 completed their metamorphosis. While 14 frogs were normal in external characters, the other 59 had ill-developed fore and hind legs.

c. Mating experiments by using male third-generation offspring of the male nucleo-cytoplasmic hybrid No. 61AT12

The eggs of each of three female *japonica* collected from the field were divided into 11 groups. The eggs of one group were inseminated with sperm of a male raised since the egg stage in our laboratory. This group (Set 1) was made as the control of the following experimental groups. The eggs of three groups (Set 2) were inseminated with sperm of three male third-generation offspring produced by mating between a female second-generation offspring No. 2 obtained from a male first-generation offspring No. 6 and a male *japonica*. The eggs of four other groups (Set 3) were inseminated with sperms of four male third-generation offspring produced by brother and sister mating of the same second-generation. The eggs of the remaining three groups (Set 4) were inseminated with sperms of

TABLE 23

Reproductive capacity of male third-generation offspring of the male

Set (Mating)	Parents: Female no.	Parents: Male no.	No. of eggs	No. of cleaved eggs: Normal	No. of cleaved eggs: Abnormal
1 (Control)	J.W65.9~11	J.L65.4	72	70 (97.2%)	0
2 (1)	J.W65.9~11	$[J\{J(61AT12)\}_6]_2$J.1	156	141 (90.4%)	5 (3.2%)
(2)		$[J\{J(61AT12)\}_6]_2$J.2	104	88 (84.6%)	5 (4.8%)
(3)		$[J\{J(61AT12)\}_6]_2$J.3	190	173 (91.1%)	0
3 (1)	J.W65.9~11	$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2$.1	324	279 (86.1%)	0
(2)		$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2$.2	549	392 (71.4%)	112 (20.4%)
(3)		$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2$.3	240	146 (60.8%)	59 (24.6%)
(4)		$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2$.4	460	241 (52.4%)	155 (33.7%)
4 (1)	J.W65.9~11	J$[J\{J(61AT12)\}_6]_2$.1	315	211 (67.0%)	16 (5.1%)
(2)		J$[J\{J(61AT12)\}_6]_2$.2	334	207 (62.0%)	13 (3.9%)
(3)		J$[J\{J(61AT12)\}_6]_2$.3	250	184 (73.6%)	0

three male third-generation offspring produced by mating of the same male of the second generation with a female *japonica* (Table 23).

Set 1. Control mating. J.W65♀ Nos. 9∼11×J.L65♂ No. 4

Seventy (97.2%) eggs cleaved normally. Except for one which became abnormal at the hatching stage, all these eggs developed normally into tadpoles and became frogs. While one frog had abnormal hind legs, the other 68 were quite normal.

Set 2. Three males Nos. 1∼3 produced from a female second-generation offspring No. 2 mated with a male *japonica*

Mating 1. J.W65♀ Nos. 9∼11×$[J\{J(61AT12)\}_6]_2J$♂ No. 1

There were 141 (90.4%) normally cleaved eggs. By the hatching stage 55 embryos became abnormal and died. The other 86 hatched normally. However, 22 of them died of under-development by the XV stage. Although the remaining 64 completed their metamorphosis, 41 had abnormal fore and hind legs.

Mating 2. J.W65♀ Nos. 9∼11×$[J\{J(61AT12)\}_6]_2J$♂ No. 2

Of 88 (84.6%) normally cleaved eggs 46 became abnormal or edematous by the hatching stage. The other 42 hatched normally and grew into normal tad-

nucleo-cytoplasmic hybrid No. 61AT12

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.
70 (97.2%)	0	70 (97.2%)	0	70 (97.2%)	0	69 (95.8%)	1 (1.4%)	68 (94.4%)	1 (1.4%)
122 (78.2%)	7 (4.5%)	111 (71.2%)	11 (7.1%)	96 (61.5%)	15 (9.5%)	86 (55.1%)	10 (6.4%)	23 (14.7%)	41 (26.3%)
85 (81.7%)	3 (2.9%)	81 (77.9%)	4 (3.8%)	76 (73.1%)	5 (4.8%)	42 (40.4%)	34 (32.7%)	40 (38.5%)	0
173 (91.1%)	0	170 (89.5%)	3 (1.6%)	162 (85.3%)	8 (4.2%)	151 (79.5%)	11 (5.8%)	4 (2.1%)	0
279 (86.1%)	0	279 (86.1%)	0	279 (86.1%)	0	255 (78.7%)	24 (7.4%)	11 (4.4%)	0
383 (69.8%)	4 (0.7%)	383 (69.8%)	0	342 (62.3%)	41 (7.5%)	309 (56.3%)	33 (6.0%)	19 (3.5%)	8 (1.5%)
131 (54.6%)	9 (3.8%)	112 (46.7%)	19 (7.9%)	90 (37.5%)	22 (9.2%)	69 (28.8%)	21 (8.8%)	13 (5.4%)	23 (9.6%)
231 (50.2%)	4 (0.9%)	189 (41.1%)	42 (9.1%)	155 (33.7%)	34 (7.4%)	112 (24.3%)	43 (9.3%)	8 (1.7%)	2 (0.4%)
175 (55.6%)	3 (1.0%)	163 (51.7%)	12 (3.8%)	138 (43.8%)	25 (7.9%)	104 (33.0%)	34 (10.8%)	50 (15.9%)	39 (12.4%)
202 (60.5%)	0	202 (60.5%)	0	147 (44.0%)	55 (16.5%)	86 (25.8%)	61 (18.3%)	24 (7.2%)	52 (15.6%)
182 (72.8%)	0	180 (72.0%)	2 (0.8%)	171 (68.4%)	9 (3.6%)	151 (60.4%)	20 (8.0%)	150 (60.0%)	0

poles. While two of them died at the XX∼XXII stages, the remaining 40 tadpoles completed normally their metamorphosis.

Mating 3. J.W65♀ Nos. 9∼11 × $[J\{J(61AT12)\}_6]_2J$♂ No. 3

There were 173(91.1%) normally cleaved eggs; 22 died by the hatching stage and 151 hatched normally. At the II∼V stages most of the latter died of under-development or other abnormalities. Eventually, only four tadpoles completed normally their metamorphosis.

Set 3. Four males Nos. 1∼4 produced by brother and sister mating of second-generation offspring

Mating 1. J.W65♀ Nos. 9∼11 × $[J\{J(61AT12)\}_6]_2[J\{J(61AT12)\}_6]_2$♂ No. 1

All the 279(86.1%) normally cleaved eggs attained the hatching stage. However, 24 embryos became edematous at this stage. At the III∼XVII stages 244 tadpoles became edematous one after another and died. Only 11 tadpoles grew normally and completed their metamorphosis.

Mating 2. J.W65♀ Nos. 9∼11 × $[J\{J(61AT12)\}_6]_2[J\{J(61AT12)\}_6]_2$♂ No. 2

Normally cleaved eggs were 392(71.4%) in number, while there were 112 abnormally cleaved ones. By the hatching stage 83 of the former died of various kinds of abnormalities. While the other 309 hatched normally, most of them died one after another at the II∼VIII stages. Eventually, 27 tadpoles completed their metamorphosis; 19 of them were quite normal and eight had abnormal fore and hind legs.

Mating 3. J.W65♀ Nos. 9∼11 × $[J\{J(61AT12)\}_6]_2[J\{J(61AT12)\}_6]_2$♂ No. 3

There were 146(60.8%) normally and 59 abnormally cleaved eggs. Seventy-seven of the former became abnormal and died by the hatching stage. The others hatched normally. While 23 tadpoles died of under-development at the II∼X stages, the remaining 46 attained the metamorphosing stage. However, 10 tadpoles died at the XX∼XXIII stages. Eventually, 36 tadpoles completed their metamorphosis; 13 of them were quite normal frogs, while the other 23 had abnormal fore and hind legs.

Mating 4. J.W65♀ Nos. 9∼11 × $[J\{J(61AT12)\}_6]_2[J\{J(61AT12)\}_6]_2$♂ No. 4

As many as 155 eggs cleaved abnormally, while 241(52.4%) did normally. By the hatching stage, 129 of the latter died of abnormalities of various types. The other 112 hatched normally and became tadpoles. However, 99 of them died one after another of under-development during the period of the II∼XVII stages. Three others died at the XX∼XXII stages. The remaining ten tadpoles completed their metamorphosis; eight of them were quite normal and two died of edema soon afterwards.

Set 4. Three males Nos. 1∼3 produced from mating between a male second-generation offspring No. 2 and a female *japonica*

Mating 1. J.W65♀ Nos. 9∼11 × $J[J\{J(61AT12)\}_6]_2$♂ No. 1

There were 211(67.0%) normally cleaved eggs. However, 104 of them hatched normally and grew into tadpoles, while 107 died of abnormalities of various types by the hatching stage. Although 15 tadpoles became abnormal and died at the II∼V stages, the other 89 completed their metamorphosis. Fifty frogs were quite normal, while 39 had abnormal fore and hind legs and became edematous.

Mating 2. J.W65♀ Nos. 9～11×J[J{J(61AT12)}$_6$]$_2$♂ No. 2

Of 207(62.0%) normally cleaved eggs, 116 died of edema at the tail-bud and the hatching stages, while 86 hatched normally. Ten tadpoles died of edema or some other abnormalities during the period of the II～XVII stages. Although 76 tadpoles completed their metamorphosis, only 24 frogs were quite normal. The other 52 frogs had abnormal fore and hind legs or became edematous.

Mating 3. J.W65♀ Nos. 9～11×J[J{J(61AT12)}$_6$]$_2$♂ No. 3

There were 184(73.6%) normally cleaved eggs; 151 of them hatched normally and became normal tadpoles. Except for one tadpole which died at the XX stage, all of them normally completed their metamorphosis.

4. Chromosomal aberrations

a. Chromosomes of third-generation offspring

Chromosomal aberrations were examined in edematous embryos as well as externally normal tadpoles of third generations derived from the nucleo-cytoplasmic hybrid No. 61OT2. These third-generation offspring were obtained by mating of male second-generation offspring with two female *japonica*. The results of observations are presented in Tables 24 and 25.

i) Edematous embryos

The embryos used for microscopical observations were those produced by matings between two female *japonica* and six male second-generation offspring. Two of the latter six males were those which had been obtained from a female first-generation offspring No. 2 mated with a male *japonica*, two others were from the same female by brother and sister mating with a male first-generation offspring, and the remaining two were from the latter male mated with a female *japonica*. All these edematous embryos were of the stages 20～23.

Chromosome analyses were made in 457 mitoses obtained from 109 edematous embryos, and it was found that nearly all the mitoses were abnormal in chromosome complement. Only six mitoses had normal diploid chromosomes, while 344 were hypodiploid, 90 were abnormal diploid and 17 were hyperdiploid. Moreover, 114 mitoses had dicentric and 28 had ring chromosomes.

There were no edematous embryos which consisted of normal diploid cells alone. All the 109 edematous embryos produced by six experimental matings contained mitoses with abnormal chromosome complements. Of these embryos, four consisted of a mixture of normal diploid and abnormal cells, while two, of a kind of abnormal diploid, 15, of hypo- or hyperdiploid cells, 21, of a mixture of more than two kinds of abnormal diploid and 67, of a mixture of abnormal diploid and hypo- or hyperdiploid cells.

ii) Tadpoles with a normal appearance

The tadpoles used for examining chromosomes were 145 in number. Of these tadpoles, 71 were produced by the above two matings between the two female *japonica* and the two male second-generation offspring which had been obtained from the female first-generation offspring No. 2 mated with a male *japonica*.

TABLE 24

Mitoses with chromosomal aberrations in third-generation offspring

Parents		Analysed embryos or tadpoles		
Female no.	Male no.	Stage	Remark	No.
J.L65.12～13	J.L65.5	III～IV	n	20
	$\{J(61OT2)\}_2J.1$	20～23	e	19
		III～IV	n	32
	$\{J(61OT2)\}_2J.2$	20～23	e	17
		III～IV	n	39
J.L65.12～13	$\{J(61OT2)\}_2\{J(61OT2)\}_{11}.1$	III～V	n	28
	$\{J(61OT2)\}_2\{J(61OT2)\}_{11}.2$	20～23	e	20
		III～IV	n	3
	$\{J(61OT2)\}_2\{J(61OT2)\}_{11}.3$	20～21	e	20
		III～IV	n	23
J.L65.12～13	$J\{J(61OT2)\}_{11}.1$	20～21	e	20
	$J\{J(61OT2)\}_{11}.2$	20～22	e	13
		III～IV	n	20

e, Edema n, Normal $\alpha=1$ or 2

TABLE 25

Numbers of embryos and tadpoles with chromosomal aberrations in third-generation offspring

Parents		Analysed embryos or tadpoles		
Female no.	Male no.	Stage	Remark	No.
J.L65.12～13	J.L65.5	III～IV	n	20
	$\{J(61OT2)\}_2J.1$	20～23	e	19
		III～IV	n	32
	$\{J(61OT2)\}_2J.2$	20～23	e	17
		III～IV	n	39
J.L65.12～13	$\{J(61OT2)\}_2\{J(61OT2)\}_{11}.1$	III～V	n	28
	$\{J(61OT2)\}_2\{J(61OT2)\}_{11}.2$	20～23	e	20
		III～IV	n	3
	$\{J(61OT2)\}_2\{J(61OT2)\}_{11}.3$	20～21	e	20
		III～IV	n	23
J.L65.12～13	$J\{J(61OT2)\}_{11}.1$	20～21	e	20
	$J\{J(61OT2)\}_{11}.2$	20～22	e	13
		III～IV	n	20

of the male nucleo-cytoplasmic hybrid No. 61OT2

No. of analysed mitoses					No. of mitoses	
Total	2n−α	2n		2n+α	With dicentric chrom.	With ring chrom.
		Abnorm.	Normal			
55	2	0	52	1	0	0
91	82	7	0	2	20	0
124	11	38	72	3	0	0
70	57	10	2	1	24	3
147	8	77	57	5	0	0
106	7	69	28	2	0	0
96	90	3	1	2	25	12
10	0	1	9	0	0	0
84	75	5	0	4	20	11
58	4	0	54	0	0	0
76	20	48	3	5	11	2
40	20	17	0	3	14	0
40	4	0	34	2	0	0

Stage — Shumway 1940, Anat. Rec. 78: 139
Taylor and Kollros 1946, Anat. Rec. 94: 7

of the male nucleo-cytoplasmic hybrid No. 61OT2

With normal cells only	With abnormal cells only				With normal and abnormal cells
	Pure		Mosaic		
	2n	2n±α	2n	2n, 2n±α	
18	0	0	0	0	2
0	0	3	2	14	0
16	1	0	6	4	5
0	0	3	1	12	1
12	2	0	18	2	5
6	4	0	13	2	3
0	0	3	1	15	1
2	0	0	0	0	1
0	0	3	1	16	0
21	0	0	0	0	2
0	1	1	11	5	2
0	1	2	5	5	0
17	0	0	0	0	3

Fifty-four other tadpoles were produced from three male second-generation offspring which had been obtained by brother and sister mating of first-generation offspring. The remaining 20 tadpoles were produced from a male second-generation offspring which had been obtained by mating between a male first-generation offspring and a female *japonica*. Besides these third-generation offspring, 20 control tadpoles were examined. All the tadpoles were of the stages III～V.

In the control tadpoles, 52 of 55 analysed mitoses had normal diploid chromosomes, while there were two hypodiploid and one hyperdiploid mitoses. Differing from the control tadpoles, there were numerous abnormal mitoses in the externally normal tadpoles of the third generation. Of 485 mitoses found in the 145 tadpoles, 231 had abnormal chromosome complements, that is, 34 were hypodiploid, 185 were abnormal diploid and 12 were hyperdiploid, while 254 were normal diploid. There were no mitoses with dicentric or ring chromosomes.

It was remarkable that 71 of the 145 tadpoles with a normal appearance in the third-generation offspring of the nucleo-cytoplasmic hybrid No. 61OT2 contained abnormal mitoses, differing from control tadpoles. The other 74(51%) tadpoles consisted of nothing but normal diploid cells, while 18 of 20 control tadpoles were such individuals. Of the third-generation offspring containing abnormal cells, 19 consisted of a mixture of normal diploid and abnormal cells, seven, of a kind of abnormal diploid, 37, of a mixture of more than two kinds of abnormal diploid and eight, of a mixture of normal diploid and hypo- or hyperdiploid cells.

b. Chromosomes of fourth-generation offspring

Chromosomal aberrations were examined in edematous or externally abnormal embryos and externally normal tadpoles of fourth generations derived from the three nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12. These fourth-generation offspring were produced by mating of 30 male third-generation offspring with 11 female *japonica*. Among the 30 male third-generation offspring, there were eleven of No. 61OT2, nine of No. 61AT8 and ten of 61AT12. In each group of the third-generation offspring, there were three kinds of frogs in respect of origin. The first kind included males produced from a female second-generation offspring by mating with a male *japonica*. The second kind consisted of males obtained from a female second-generation offspring by mating with her brother or another male second-generation offspring. The third kind included males produced from a male second-generation offspring by mating with a female *japonica*. The results of observations are presented in Table 26 and 27.

i) Edematous embryos

Observations were made upon 648 mitoses of 164 edematous embryos of fourth-generations produced by eight experimental matings between female *japonica* and male third-generation offspring. Of these embryos, 144 were of the stages 20～22, that is, of nearly the hatching stage, while the remaining 20 were of the stages 18～19. In one of the eight experimental matings, the male parent was obtained from a female second-generation offspring of the nucleo-cytoplasmic hybrid No. 61OT2 by mating with a male cousin. The male parent of another experimental mating was obtained from a male and a female second-generation offspring of the nucleo-cytoplasmic hybrid No. 61AT8 by brother and sister mating. The six male parents of the other six experimental matings were third-generation offspring of the nucleo-cytoplasmic hybrid No. 61AT12. Two of the six males were obtained from a female second-generation offspring mated with a male *japonica*, another was from the same female by brother and sister mating with a male second-generation offspring, and the other three were from the latter male mated with a female *japonica*.

Of 648 analysed mitoses, only 25 were normal diploid. Among the other 623, there were 468 hypodiploid, 48 abnormal diploid, 91 hyperdiploid, 13 approximately triploid and 3 approximately tetraploid mitoses. There were, moreover, 200 mitoses with dicentric and 92 mitoses with ring chromosomes.

There were no edematous embryos consisting of normal diploid cells alone. Fourteen of the 164 embryos were constructed of a mixture of normal diploid and abnormal cells, while the others contained no normal diploid mitoses. Of the latter embryos, one consisted of a kind of abnormal diploid, ten, of hypo- or hyperdiploid, 139, of various kinds of mosaics. Of these mosaics, three consisted of a mixture of more than two kinds of abnormal diploid, 131, of a mixture of abnormal diploid and hypo- or hyperdiploid, four, of a mixture of triploid and hypo- or hypertriploid, and one, of a mixture of tetraploid and hypo- or hypertetraploid cells.

ii) Embryos with an abnormal appearance

Chromosomes were examined in 76 externally abnormal embryos of fourth generations obtained by four experimental matings between female *japonica* and four male third-generation offspring of the three nucleo-cytoplasmic hybrids. These embryos were of the stages 20～23 and externally revealed various kinds of abnormalities. One of the four male third-generation offspring was that obtained from a female second-generation offspring of the nucleo-cytoplasmic hybrid No. 61OT2 by mating with a male cousin. Two other males were obtained from a female and a male second-generation offspring of the nucleo-cytoplasmic hybrid No. 61AT8 by brother and sister mating. The remaining male was obtained from a female and a male second-generation offspring of the nucleo-cytoplasmic hybrid No. 61AT12 by brother and sister mating.

Of 402 mitoses observed, only 56 were normal diploid. Among the others, there were 194 hypodiploid, 58 abnormal diploid, 35 hyperdiploid, 46 approximately triploid, and 13 approximately tetraploid mitoses. Moreover, these were 63 mitoses with dicentric and 41 with ring chromosomes.

Only one individual consisted of normal diploid cells alone among the 76 embryos with an abnormal appearance, while 30 did of a mixture of normal diploid and abnormal cells. Of the other 45, three consisted of a kind of abnormal diploid, two, of hypo- or hyperdiploid, four, of triploid or tetraploid, seven, of a mixture of more than two kinds of abnormal diploid, 22, of a mixture of abnormal diploid and hypo- or hyperdiploid, five, of a mixture of triploid and hypo- or hypertriploid, and two, of a mixture of tetraploid and hypo- or hypertetraploid cells.

iii) Tadpoles with a normal appearance

In order to examine chromosomal aberrations in externally normal tadpoles of fourth generations of the three nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8

TABLE 26

Mitoses with chromosomal aberrations in fourth-generation offspring of

Parents		Analysed embryos or tadpoles			No. of
Female no.	Male no.	Stage	Remark	No.	Total
J.L65.6 ~ 8	J.L65.3	IV~ V	n	20	93
	$[J\{J(61OT2)\}_{8}]_{5}J.1$	IV~ V	n	40	183
	$[J\{J(61OT2)\}_{8}]_{5}J.2$	IV~ V	n	40	184
J.L65.6 ~ 8	$[J\{J(61OT2)\}_{9}]_{1}$ $[J\{J(61OT2)\}_{9}]_{1}.1$	III~ V	n	20	96
	$[J\{J(61OT2)\}_{9}]_{1}$ $[J\{J(61OT2)\}_{9}]_{1}.2$	III~ V	n	20	122
	$[J\{J(61OT2)\}_{8}]_{5}$ $[J\{J(61OT2)\}_{1}]_{5}.1$	20~23	a	6	40
		II~IV	n	15	42
	$[J\{J(61OT2)\}_{8}]_{5}$ $[J\{J(61OT2)\}_{1}]_{5}.2$	II~IV	n	24	81
	$[J\{J(61OT2)\}_{8}]_{5}$ $[J\{J(61OT2)\}_{1}]_{5}.3$	II~IV	n	30	81
	$[J\{J(61OT2)\}_{8}]_{5}$ $[J\{J(61OT2)\}_{1}]_{5}.4$	II~IV	n	24	85
	$[J\{J(61OT2)\}_{8}]_{5}$ $[J\{J(61OT2)\}_{1}]_{5}.5$	20~21	e	13	48
		II~IV	n	3	12
J.L65.6 ~ 8	$J[J\{J(61OT2)\}_{1}]_{5}.1$	III~IV	n	20	65
	$J[J\{J(61OT2)\}_{1}]_{5}.2$	III~IV	n	25	97
J.W65.1~ 3	J.L65.1	III~ V	n	20	86
	$[J\{J(61AT8)\}_{4}]_{2}J.1$	III~ V	n	40	179
	$[J\{J(61AT8)\}_{4}]_{2}J.2$	III~ V	n	50	228
	$[J\{J(61AT8)\}_{4}]_{2}J.3$	III~ V	n	60	244
	$[J\{J(61AT8)\}_{4}]_{2}J.4$	III~ V	n	29	90
J.W65.4~ 5	J.L65.2	III~ V	n	10	23
	$[J\{J(61AT8)\}_{4}]_{2}$ $[J\{J(61AT8)\}_{4}]_{2}.1$	20~22	a	30	138
		III~ V	n	4	16
	$[J\{J(61AT8)\}_{4}]_{2}$ $[J\{J(61AT8)\}_{4}]_{2}.2$	20~21	e	20	80
	$[J\{J(61AT8)\}_{4}]_{2}$ $[J\{J(61AT8)\}_{4}]_{2}.3$	20~23	a	20	107
		III~ V	n	8	31
J.W65.4~ 5	$J[J\{J(61AT8)\}_{4}]_{2}.1$	III~ V	n	20	71
	$J[J\{J(61AT8)\}_{4}]_{2}.2$	III~ V	n	30	95
J.W65.9~11	J.L65.4	III~IV	n	20	67
	$[J\{J(61AT12)\}_{6}]_{2}J.1$	III~IV	n	20	107
	$[J\{J(61AT12)\}_{6}]_{2}J.2$	20~21	e	20	68
		III~IV	n	20	74
	$[J\{J(61AT12)\}_{6}]_{2}J.3$	20~22	e	11	57
		II~IV	n	24	41
J.W65.9~11	$[J\{J(61AT12)\}_{6}]_{2}$ $[J\{J(61AT12)\}_{6}]_{2}.1$	20~22	e	20	91
		II~IV	n	20	85
	$[J\{J(61AT12)\}_{6}]_{2}$ $[J\{J(61AT12)\}_{6}]_{2}.2$	20~23	a	20	117
		II~IV	n	27	109
	$[J\{J(61AT12)\}_{6}]_{2}$ $[J\{J(61AT12)\}_{6}]_{2}.3$	II~IV	n	30	141
	$[J\{J(61AT12)\}_{6}]_{2}$ $[J\{J(61AT12)\}_{6}]_{2}.4$	II~IV	n	10	60
J.W65.9~11	$J[J\{J(61AT12)\}_{6}]_{2}.1$	20~22	e	20	46
		II~ V	n	30	126
	$J[J\{J(61AT12)\}_{6}]_{2}.2$	18~19	e	20	98
		20~22	e	20	91
		II~ V	n	20	100
	$J[J\{J(61AT12)\}_{6}]_{2}.3$	20~22	e	20	69
		III~IV	n	20	78

e, Edema n, Normal a, Abnromal

the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12

analysed mitoses						No. of mitoses	
2n−α	2n		2n+α	3n, 3n±α	4n, 4n±α	With dicentric chromosome	With ring chromosome
	Abnorm.	Norm.					
4	2	87	0	0	0	0	0
16	32	132	3	0	0	0	0
14	11	157	2	0	0	0	0
8	4	11	0	68	5	0	5
13	43	61	5	0	0	1	5
10	13	2	9	6	0	0	1
10	8	20	4	0	0	0	0
13	19	46	3	0	0	0	0
7	17	56	1	0	0	0	0
5	18	57	5	0	0	0	0
39	3	4	2	0	0	20	6
0	1	11	0	0	0	0	0
1	20	33	11	0	0	0	0
25	21	34	9	8	0	0	2
1	2	82	1	0	0	0	0
9	45	122	3	0	0		0
9	80	137	2	0	0	0	0
6	62	24	4	145	3	0	2
9	36	44	1	0	0	0	0
1	0	22	0	0	0	0	0
49	27	33	17	10	2	21	14
3	4	9	0	0	0	0	0
70	5	0	5	0	0	34	6
64	15	11	7	10	0	27	15
1	2	27	1	0	0	0	0
2	3	66	0	0	0	0	0
9	48	35	3	0	0	0	0
1	0	65	1	0	0	0	0
15	42	47	3	0	0	0	0
62	2	1	3	0	0	30	7
4	10	59	1	0	0	0	0
55	1	0	1	0	0	13	26
9	13	18	1	0	0	0	0
48	5	0	28	7	3	15	12
5	2	70	3	5	0	0	4
71	3	10	2	20	11	15	11
6	29	66	2	5	1	0	0
19	71	47	4	0	0	0	0
5	15	39	1	0	0	0	0
40	2	1	3	0	0	21	10
6	48	69	3	0	0	0	0
58	4	6	24	6	0	34	12
55	11	1	24	0	0	12	11
12	40	46	2	0	0	0	0
41	15	12	1	0	0	21	2
5	7	60	6	0	0	0	0

Stage —— SHUMWAY 1940, Anat. Rec. 78: 139
TAYLOR and KOLLROS 1946, Anat. Rec. 94: 7

TABLE 27

Embryos and tadpoles with chromosomal aberrations in fourth-generation offspring of

Parents		Analysed embryos or tadpoles			With normal cells only
Female no.	Male no.	Stage	Remark	No.	
J.L65.6 ~ 8	J.L65.3	IV~ V	n	20	17
	$[J\{J(61OT2)\}_8]_5J.1$	IV~ V	n	40	24
	$[J\{J(61OT2)\}_8]_5J.2$	IV~ V	n	40	31
J.L65.6 ~ 8	$[J\{J(61OT2)\}_9]_1$ $[J\{J(61OT2)\}_9]_1.1$	III~ V	n	20	2
	$[J\{J(61OT2)\}_9]_1$ $[J\{J(61OT2)\}_9]_1.2$	III~ V	n	20	9
	$[J\{J(61OT2)\}_8]_5$ $[J\{J(61OT2)\}_1]_5.1$	20~23	a	6	0
		II~IV	n	15	6
	$[J\{J(61OT2)\}_8]_5$ $[J\{J(61OT2)\}_1]_5.2$	II~IV	n	24	12
	$[J\{J(61OT2)\}_8]_5$ $[J\{J(61OT2)\}_1]_5.3$	II~IV	n	30	21
	$[J\{J(61OT2)\}_8]_5$ $[J\{J(61OT2)\}_1]_5.4$	II~IV	n	24	15
	$[J\{J(61OT2)\}_8]_5$ $[J\{J(61OT2)\}_1]_5.5$	20~21	e	13	0
		II~IV	n	3	2
J.L65.6 ~ 8	$J[J\{J(61OT2)\}_1]_5.1$	III~IV	n	20	9
	$J[J\{J(61OT2)\}_1]_5.2$	III~IV	n	25	11
J.W65.1~ 3	J.L65.1	III~ V	n	20	18
	$[J\{J(61AT8)\}_4]_2J.1$	III~ V	n	40	21
	$[J\{J(61AT8)\}_4]_2J.2$	III~ V	n	50	27
	$[J\{J(61AT8)\}_4]_2J.3$	III~ V	n	60	5
	$[J\{J(61AT8)\}_4]_2J.4$	III~ V	n	29	14
J.W65.4~ 5	J.L65.2	III~ V	n	10	9
	$[J\{J(61AT8)\}_4]_2$ $[J\{J(61AT8)\}_4]_2.1$	20~22	a	30	1
		III~ V	n	4	1
	$[J\{J(61AT8)\}_4]_2$ $[J\{J(61AT8)\}_4]_2.2$	20~21	e	20	0
	$[J\{J(61AT8)\}_4]_2$ $[J\{J(61AT8)\}_4]_2.3$	20~23	a	20	0
		III~ V	n	8	6
J.W65.4~ 5	$J[J\{J(61AT8)\}_4]_2.1$	III~ V	n	20	17
	$J[J\{J(61AT8)\}_4]_2.2$	III~ V	n	30	5
J.W65.9~11	J.L65.4	III~IV	n	20	19
	$[J\{J(61AT12)\}_6]_2J.1$	III~IV	n	20	8
	$[J\{J(61AT12)\}_6]_2J.2$	20~21	e	20	0
		III~IV	n	20	11
	$[J\{J(61AT12)\}_6]_2J.3$	20~22	e	11	0
		II~IV	n	24	4
J.W65.9~11	$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2.1$	20~22	e	20	0
		II~IV	n	20	15
	$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2.2$	20~23	a	20	0
		II~IV	n	27	13
	$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2.3$	II~IV	n	30	8
	$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2.4$	II~IV	n	10	6
J.W65.9~11	$J[J\{J(61AT12)\}_6]_2.1$	20~22	e	20	0
		II~ V	n	30	17
	$J[J\{J(61AT12)\}_6]_2.2$	18~19	e	20	0
		20~22	e	20	0
		II~ V	n	20	7
	$J[J\{J(61AT12)\}_6]_2.3$	20~22	e	20	0
		III~IV	n	20	15

the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12

With abnormal cells only							With normal and abnormal cells
Pure			Mosaic				
2n	2n±α	3n, 4n,	2n	2n, 2n±α	3n, 3n±α	4n, 4n±α	
0	0	0	0	0	0	0	3
2	0	0	2	2	0	0	10
0	0	0	0	0	0	0	9
0	0	10	0	0	4	0	4
2	0	0	5	2	0	0	2
0	0	0	1	4	0	0	1
0	1	0	0	7	0	0	1
0	0	0	3	5	0	0	4
0	0	0	2	3	0	0	4
0	1	0	2	0	0	0	6
0	0	0	0	11	0	0	2
0	0	0	0	0	0	0	1
3	2	0	0	3	0	0	3
2	2	2	3	4	1	0	0
0	0	0	0	0	0	0	2
3	0	0	5	6	0	0	5
4	0	0	12	2	0	0	5
4	0	10	10	1	29	1	0
3	0	0	5	3	0	0	4
0	0	0	0	0	0	0	1
3	2	1	4	4	1	1	13
1	0	0	0	0	0	0	2
0	0	0	1	19	0	0	0
0	0	0	2	4	2	0	12
0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	3
6	0	0	9	4	0	0	6
0	0	0	0	0	0	0	1
2	0	0	4	3	0	0	3
0	2	0	0	17	0	0	1
1	0	0	0	0	0	0	8
0	0	0	0	11	0	0	0
3	0	0	0	15	0	0	2
0	3	0	0	14	2	1	0
0	0	0	0	0	1	0	4
0	0	3(1)	0	10	2	1	4
3	0	0	4	2	1	0	4
2	0	0	11	3	0	0	6
1	0	0	1	2	0	0	0
0	0	0	0	19	0	0	1
3	0	0	1	3	0	0	6
0	3	0	0	12	2	0	3
0	2	0	1	16	0	0	1
2	0	0	3	3	0	0	5
1	0	0	1	12	0	0	6
1	0	0	0	4	0	0	0

and 61AT12, 2923 mitoses were observed in the tail tips of 723 individuals. These tadpoles were produced by 29 matings between female *japonica* and male third-generation offspring, as stated above. Besides them, 70 control tadpoles obtained by four matings between the same females and four male *japonica* were examined. All these experimental and the control tadpoles were of the stages II~V.

In the control tadpoles, 256 of 269 mitoses were normal diploid, while the other thirteen (5%) were abnormal, that is, seven were hypodiploid, four were abnormal diploid and two were hyperdiploid. Neither dicentric nor ring chromosome was found in the mitoses of the control tadpoles. Differing from the status in the control tadpoles, there were 1603 normal diploid and 1320(45%) abnormal mitoses in the fourth-generation offspring of the three nucleo-cytoplasmic hybrids. The abnormal mitoses included 246 hypodiploid, 751 abnormal diploid, 83 hyperdiploid, 231 approximately triploid and 9 approximately tetraploid. One mitosis had a dicentric, and 18 had ring chromosomes. When these mitoses of the fourth-generation offspring were assorted into three groups, according to the kind of the nucleo-cytoplasmic hybrids, the numbers of mitoses

TABLE 28

Shape, size and sex of metamorphosed third-generation offspring of the male nucleo-

Set (Mating)	Parents		Age at the time of landing (Mean) (days)	No. of frogs			Body length immediately after metamorphosis (mm.)
	Female no.	Male no.		Total	Normal	Abnorm.	
1 (Control)	J.L65.12~13	J.L65.5	95~113 (101.0)	87	87	0	17.0± 0.3
2 (1)	J.L65.12~13	{J(61OT2)}$_{2}$J.1	91~113 (101.5)	69	52	17	17.5± 0.7
(2)		{J(61OT2)}$_{2}$J.2	94~102 (97.0)	30	7	23	17.3± 0.7
(3)		{J(61OT2)}$_{2}$J.3					
(4)		{J(61OT2)}$_{2}$J.4	92~ 97 (95.0)	6	4	2	17.5± 0.9
3 (1)	J.L65.12~13	{J(61OT2)}$_{2}$ {J(61OT2)}$_{11}$.1	95~107 (97.3)	28	5	23	17.8± 1.2
(2)		{J(61OT2)}$_{2}$ {J(61OT2)}$_{11}$.2	92~ 97 (94.6)	3	3	0	16.0~16.7
(3)		{J(61OT2)}$_{2}$ {J(61OT2)}$_{11}$.3	91~103 (97.3)	24	24	0	16.8± 0.8
4 (1)	J.L65.12~13	J{J(61OT2)}$_{11}$.1					
(2)		J{J(61OT2)}$_{11}$.2	95~ 97 (96.0)	3	3	0	16.0~16.2
(3)		J{J(61OT2)}$_{11}$.3	95~ 99 (97.5)	15	8	7	16.5~17.0
(4)		J{J(61OT2)}$_{11}$.4	95~112 (100.3)	53	53	0	17.2± 0.9

with normal or abnormal chromosome complements in each group were as follows. In 261 fourth-generation offspring of No. 61OT2, there were 618 normal diploid and 430(41%) abnormal mitoses, including 112 hypodiploid, 194 abnormal diploid, 43 hyperdiploid, 76 approximately triploid and five approximately tetraploid mitoses; in 241 fourth-generation offspring of No. 61AT8, there were 464 normal diploid and 490(51%) abnormal mitoses, including 48 hypodiploid, 280 abnormal diploid, 14 hyperdiploid, 145 approximately triploid and three approximately tetraploid mitoses; in 221 fourth-generation offspring of No. 61AT12, there were 521 normal diploid and 400(43%) abnormal mitoses, including 86 hypodiploid, 277 abnormal diploid, 26 hyperdiploid, 10 approximately triploid and one approximately tetraploid mitoses.

When an assortment of the mitoses of the fourth-generation offspring was made according to the type of matings which produced their male parents, the numbers of mitoses with normal or abnormal chromosome complements in each of three types were as follows. In the type of males obtained by mating between female second-generation offspring and male *japonica*, there were 740 normal diploid and 590(44%) abnormal mitoses, including 91 hypodiploid, 331 abnormal di-

cytoplasmic hybrid No. 61OT2

Body length of 9-month-old frogs (mm.)	Sex of young frogs shortly after metamorphosis								Sex of matured frogs		
	Total	♀N	♀U	⚥1	⚥2	⚥3	♂R	♂N	Total	♀	♂
27.5~28.0	77	39	0	0	0	0	0	38	10	5	5
25.5~27.5	59	32	2	1	1	2	1	20	10	2	8
	30	21	0	0	0	0	0	9			
	6	3	0	0	0	0	0	3			
	28	17	0	2	0	3	0	6			
	3	1	0	1	0	0	0	1			
	24	17	0	0	0	0	0	7			
	3	1	0	0	0	0	0	2			
	15	7	0	0	0	0	0	8			
26.0~27.5	43	20	0	0	0	0	0	23	9	1	8

ploid, 20 hyperdiploid, 145 nearly triploid and three approximately tetraploid among 1330 mitoses of 323 fourth-generation offspring; in the type of males obtained by brother and sister mating of second-generation offspring, there were 520 normal diploid and 441(46%) abnormal mitoses, including 95 hypodiploid, 233 abnormal diploid, 29 hyperdiploid, 78 nearly triploid and six nearly tetraploid among 961 mitoses of 235 fourth-generation offspring; in the type of males obtained by mating between female *japonica* and male second-generation offspring, there were 343 normal diploid and 289(46%) abnormal mitoses, including 60 hypodiploid, 187 abnormal diploid, 34 hyperdiploid and eight nearly triploid among 632 mitoses of 165 fourth-generation offspring.

It was remarkable that numerous triploid mitoses were found in the tadpoles produced from a male third-generation offspring which had been obtained by mating between a female second-generation offspring of No. 61AT8 and a male *japonica* as well as from a male third-generation offspring obtained between a female and a male second-generation offspring of No. 61OT2 by brother and

TABLE 29

Shape, size and sex of metamorphosed fourth-generation offspring of

Set (Mating)	Parents: Female no.	Parents: Male no.	Age at the time of landing (Mean) (days)	No. of frogs: Total	No. of frogs: Normal	No. of frogs: Abnorm.	Body length immediately after metamorphosis (mm.)
1 (Control)	J.L65.6~8	J.L65.3	95~112 (101.6)	123	123	0	17.8± 0.3
2 (1)	J.L65.6~8	[J{J(61OT2)}$_8$]$_5$J.1	98~112 (102.7)	120	120	0	16.9± 0.7
(2)		[J{J(61OT2)}$_8$]$_5$J.2	98~112 (103.1)	189	189	0	17.5± 0.7
3 (1)	J.L65.6~8	[J{J(61OT2)}$_9$]$_1$ [J{J(61OT2)}$_9$]$_1$.1	98~112 (103.5)	231	231	0	17.1± 0.6
(2)		[J{J(61OT2)}$_9$]$_1$ [J{J(61OT2)}$_9$]$_1$.2	98~112 (102.9)	46	45	1	17.5± 0.9
4 (1)	J.L65.6~8	[J{J(61OT2)}$_8$]$_5$ [J{J(61OT2)}$_1$]$_5$.1	93~102 (96.0)	5	5	0	17.5~18.0
(2)		[J{J(61OT2)}$_8$]$_5$ [J{J(61OT2)}$_1$]$_5$.2	92~105 (97.5)	4	4	0	17.0~17.5
(3)		[J{J(61OT2)}$_8$]$_5$ [J{J(61OT2)}$_1$]$_5$.3	96~115 (101.3)	20	20	0	18.0± 0.9
(4)		[J{J(61OT2)}$_8$]$_5$ [J{J(61OT2)}$_1$]$_5$.4	95~105 (98.3)	4	4	0	17.5~18.0
(5)		[J{J(61OT2)}$_5$]$_8$ [J{J(61OT2)}$_1$]$_5$.5	97~100 (98.3)	3	3	0	17.5~18.0
5 (1)	J.L65.6~8	J[J{J(61OT2)}$_1$]$_5$.1	95~112 (102.5)	161	161	0	18.0± 0.6
(2)		J[J{J(61OT2)}$_1$]$_5$.2	93~112 (103.0)	242	242	0	17.3± 0.4
(3)		J[J{J(61OT2)}$_1$]$_5$.3					

sister mating.

Among the 723 tadpoles with normal appearance, there were 342(47%) consisting of normal diploid cells alone, while the other 381(53%) contained mitoses with abnormal chromosome complements. Of the latter tadpoles, 109 were constructed of a mixture of normal diploid and abnormal cells. The remaining 272 tadpoles contained no normal diploid mitoses; 48 consisted of a kind of abnormal diploid, six, of hypo- or hyperdiploid, 22, of triploid or tetraploid, 82, of more than two kinds of abnormal diploid, 77, of a mixture of abnormal diploid and hypo- or hyperdiploid, 36, of triploid and hypo- or hypertriploid, and one, of tetraploid and hypo- or hypertetraploid cells. These figures differed from those of the 70 control tadpoles, as 63(90%) of the latter were constructed of nothing but normal diploid cells, although the remaining seven contained some mitoses with abnormal chromosome complements besides normal diploid.

5. Metamorphosed frogs

a. Third-generation offspring of the nucleo-cytoplasmic hybrid No. 61OT2

the male nucleo-cytoplasmic hybrid No. 61OT2

Body length of 9-month-old frogs (mm.)	Sex of young frogs shortly after metamorphosis								Sex of matured frogs		
	Total	♀N	♀U	⚥1	⚥2	⚥3	♂R	♂N	Total	♀	♂
27.5~30.0	118	59	0	0	0	0	0	59	5	2	3
21.5~25.0	110	44	4	4	0	2	0	56	10	1	9
30.0~32.5	179	55	2	2	4	1	0	115	10	1	9
30.0~33.0	220	0	5	12	0	1	0	202	6	0	6
	46	0	0	13	10	4	0	19			
	5	0	0	0	0	0	0	5			
	4	1	0	0	0	0	0	3			
32.5~35.0	15	3	1	1	0	0	0	10	5	1	4
	4	0	0	0	0	0	0	4			
	3	0	0	1	0	0	0	2			
32.0~34.5	145	47	0	0	0	0	0	98	15	2	13
20.5~22.5	221	90	0	1	2	1	0	127	15	4	11

While 87(93%) of 94 *japonica* eggs developed into normal, metamorphosed frogs by insemination with *japonica* sperm, most eggs of the same females did not develop into normal frogs by insemination with sperms of three kinds of male second-generation offspring of the nucleo-cytoplasmic hybrid No. 61OT2 (Table 20). No or only a few normal frogs were produced by eight of the 11 experimental matings. By the other three matings, 16~42% of eggs became normal frogs. All the normal and abnormal frogs obtained by the experimental and the control matings are listed in Table 28.

In the experimental matings, 231 third-generation offspring made their metamorphosis at the age of 91~113 days, nearly as the 87 control frogs did. They were similar to the control frogs in the body length immediately after metamorphosis, too, although there were larger deviations.

Concerning the sex of young frogs soon after metamorphosis, there were 39 females and 38 males among 77 control frogs. Among 211 frogs produced by nine experimental matings, there were 121 females, 10 hermaphrodites and 80 males. However, there were three females and 16 males among 19 matured

TABLE 30

Shape, size and sex of metamorphosed fourth-generation offspring of

Set (Mating)	Parents		Age at the time of landing (Mean) (days)	No. of frogs			Body length immediately after metamorphosis (mm.)
	Female no.	Male no.		Total	Normal	Abnorm.	
1 (Control)	J.W65.1~3	J.L65.1	91~114 (102.3)	94	93	1	16.6± 0.7
2 (1)	J.W65.1~3	$[J\{J(61AT8)\}_4]_2J.1$	91~114 (100.4)	155	97	58	17.3± 0.7
(2)		$[J\{J(61AT8)\}_4]_2J,2$	92~114 (102.5)	80	54	26	17.0± 0.6
(3)		$[J\{J(61AT8)\}_4]_2J.3$	92~114 (108.2)	212	82	130	16.5± 0.6
(4)		$[J\{J(61AT8)\}_4]_2J.4$	92~114 (104.0)	208	133	75	17.0± 0.6
3 (Control)	J.W65.4~5	J.L65.2	89~100 (96.1)	39	39	0	17.5± 0.6
4 (1)	J.W65.4~5	$[J\{J(61AT8)\}_4]_2$ $[J\{J(61AT8)\}_4]_2.1$	92~101 (94.5)	4	4	0	16.5~17.5
(2)		$[J\{J(61AT8)\}_4]_2$ $[J\{J(61AT8)\}_4]_2.2$					
(3)		$[J\{J(61AT8)\}_4]_2$ $[J\{J(61AT8)\}_4]_2.3$	98	1	0	1	16.5
(4)		$[J\{J(61AT8)\}_4]_2$ $[J\{J(61AT8)\}_4]_2.4$	89~103 (96.3)	8	6	2	17.9~18.5
5 (1)	J.W65.4~5	$J[J\{J(61AT8)\}_4]_2.1$	98~112 (106.3)	213	213	0	17.0± 0.6
(2)		$J[J\{J(61AT8)\}_4]_2.2$	95~114 (105.2)	73	14	59	17.5± 0.7

third-generation offspring produced by two matings, differing from five females and five males among ten matured control frogs. It seemed certain that the preponderance of males in the former was due to sex-reveral of genetic females to males, as mortality scarcely occurred in third-generation offspring after the young frog stage.

b. Fourth-generation offspring of the nucleo-cytoplasmic hybrid No. 61OT2

By the control mating between three female and one male *japonica*, 123 (91.8%) of 134 eggs developed into normal metamorphosed frogs. In contrast with this, only 2.9~12.1% of eggs of the same females became normal metamorphosed frogs by mating with six third-generation males, while 65.3~90.9% of their eggs did by mating with the other five third-generation males (Table 21).

All the frogs obtained by the 11 experimental and the control mating are listed in Table 29. The control frogs made their metamorphosis at the ages of 95~112 (mean 101.6) days, while the frogs obtained by the experimental matings did at the ages of 92~115 (mean 102.3) days. At the stage immediately after metamorphosis, the fourth-generation offspring were nearly the same with the

the male nucleo-cytoplasmic hybrid No. 61AT8

Body length of 9-month-old frogs (mm.)	Sex of young frogs shortly after metamorphosis								Sex of matured frogs		
	Total	♀N	♀U	⚥1	⚥2	⚥3	♂R	♂N	Total	♀	♂
25.0~28.0	88	42	0	0	0	0	0	46	5	2	3
	155	91	0	1	1	0	0	62			
	79	50	0	1	1	2	0	25			
27.0~30.5	202	120	0	0	1	1	0	80	8	1	7
	208	129	0	2	4	3	1	69			
	39	17	0	0	0	0	0	22			
	4	0	0	1	0	0	0	3			
	1	0	0	1	0	0	0	0			
37.5~42.0	4	0	0	0	1	1	0	2	4	0	4
37.5~40.5	207	62	0	3	2	3	0	137	4	0	4
32.0~35.0	68	23	1	2	0	1	0	41	5	1	4

control frogs in the mean body length of the frogs obtained by each experimental mating. However, at the age of nine months, frogs produced by two experimental matings were smaller than the control. On the other hand, those obtained by four experimental matings were larger than the latter.

Concerning the sex of young frogs shortly after metamorphosis, there were 59 females and 59 males among the 118 control frogs. Among 952 frogs produced by the 11 experimental matings, there were 252 females, 59 hermaphrodites and 641 males. Of these females, 12 had under-developed ovaries, differing from the females obtained by the control mating. The existence of fairly many hermaphrodites and the remarkable preponderance of males among the frogs obtained by the experimental matings were the points differing from the state of affairs in the control frogs. It was noteworthy that 278 frogs produced by five experimental matings consisted of 232 males, 41 hermaphrodites and five females with under-developed ovaries, and that there were no females with normal ovaries. At the stage of sexual maturity, there were 9 females and 52 males among 61 fourth-generation offspring, while there were two females and three males among five control frogs. It seems certain that many of the male fourth-generation offspring were sex-reversed genetic females.

c. Fourth-generation offspring of the nucleo-cytoplasmic hybrid No. 61AT8

TABLE 31

Shape, size and sex of metamorphosed fourth- generation offspring of

Set (Mating)	Parents		Age at the time of landing (Mean) (days)	No. of frogs			Body length immediately after meta-morphosis (mm.)
	Female no.	Male no.		Total	Normal	Abnorm.	
1 (Control)	J.W65.9～11	J.L65.4	95～115 (103.3)	69	68	1	17.2± 0.3
2 (1)	J.W65.9～11	$[J\{J(61AT12)\}_6]_2J.1$	95～113 (100.5)	64	23	41	17.3± 0.7
(2)		$[J\}J(61AT12)\}_6]_2J.2$	95～115 (102.3)	40	40	0	17.5± 0.6
(3)		$[J\}J(61AT12)\}_6]J_2.3$	97～105 (101.3)	4	4	0	16.5～17.0
3 (1)	J.W65.9～11	$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2.1$	95～102 (99.0)	11	11	0	17.5～17.9
(2)		$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2.2$	95～112 (105.0)	27	19	8	18.2± 0.9
(3)		$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2.3$	94～103 (101.3)	36	13	23	16.5± 0.9
(4)		$[J\{J(61AT12)\}_6]_2$ $[J\{J(61AT12)\}_6]_2.4$	93～111 (100.5)	10	8	2	17.0～17.5
4 (1)	J.W65.9～11	$J[J\{J(61AT12)\}_6]_2.1$	91～102 (94.3)	89	50	39	16.9± 0.6
(2)		$J[J\{J(61AT12)\}_6]_2.2$	93～111 (97.0)	76	24	52	17.0± 0.6
(3)		$J[J\{J(61AT12)\}_6]_2.3$	90～106 (96.5)	150	150	0	17.0± 0.6

By one of the two control matings, 93(73%) of 127 *japonica* eggs developed into normal metamorphosed frogs, although there were 31 uncleaved eggs. By mating of the same female *japonica* with four third-generation males, 27~48% of eggs developed into normal metamorphosed frogs, and 23~42% became abnormal (Table 22). While 39(91%) of 43 *japonica* eggs developed into normal metamorphosed frogs by the other control mating, no or only a few eggs of the same female *japonica* became normal metamorphosed frogs by mating with five of six third-generation males. By the remaining male, 91% of *japonica* eggs became normal metamorphosed frogs. All the normal and abnormal frogs produced by the experimental and the control matings are listed in Table 30.

In the two control matings, 133 frogs made their metamorphosis at the ages of 89~114 days. Similarly, all the 954 fourth-generation offspring did at the ages of 89~114 days. The latter were similar in body length to the controls, in spite of the existence of numerous abnormal frogs. However, it was remarkable that at the age of 9 months, fourth-generation offspring produced from three experimental matings were distinctly larger than those produced from another experimental mating and one of the control matings.

Among 88 young frogs produced by one of the two control matings between female and male *japonica* there were 42 females and 46 males. In contrast with

the male nucleo-cytoplasmic hybrid No. 61AT12

Body length of 9-month-old frogs (mm.)	Sex of young frogs shortly after metamorphosis								Sex of matured frogs		
	Total	♀N	♀U	⚥1	⚥2	⚥3	♂R	♂N	Total	♀	♂
26.5~28.0	57	27	0	0	0	0	0	30	10	5	5
	64	31	0	0	0	0	0	33			
27.5~30.0	30	14	0	4	0	0	0	12	7	1	6
	4	1	0	2	0	0	0	1			
	11	3	0	1	1	0	0	6			
36.7~37.0	22	2	2	12	2	0	0	4	5	1	4
27.5~28.0	31	0	0	6	3	2	0	20	4	0	4
	10	1	1	2	0	2	0	4			
	89	54	0	4	0	1	0	30			
	76	47	0	0	0	0	0	29			
26.0~27.5	117	72	0	0	0	0	0	45	30	5	25

this, there were 390 females, 17 hermaphrodites and 237 males among 644 young frogs of fourth generations produced from the same female *japonica* by mating with four third-generation males. At the stage of sexual maturity, however, there were a female and seven males among eight fourth-generation offspring produced from the female *japonica* by mating with a third-generation male No. 3, while there were 120 females, two hermaphrodites and 80 males at the stage shortly after metamorphosis.

The other control mating between two females and a male *japonica* produced 17 females and 22 males among 39 young frogs. In fourth generations obtained by mating of the same females with six third-generation males, there were 86 females, 15 hermaphrodites and 183 males among 284 young frogs shortly after metamorphosis. At the stage of sexual maturity, there were a female and 12 males among 13 frogs obtained by matings between the female *japonica* and three of the six third-generation males.

d. Fourth-generation offspring of the nucleo-cytoplasmic hybrid No. 61AT12

While 68(94%) of 72 eggs obtained from three female *japonica* became normal metamorphosed frogs by mating with a male *japonica*, only 1.7～60.0% of eggs of the same females did by mating with ten male third-generation offspring of the nucleo-cytoplasmic hybrid No. 61AT12 (Table 23). Besides these normal frogs, a small number of abnormal ones were produced by six experimental matings, while there was only one abnormal among the control frogs.

The size and sex of all these normal and abnormal frogs obtained by the experimental and the control mating are presented in Table 31. The fourth-generation offspring made their metamorphosis nearly at the same time as the control frogs did. They were also nearly the same as the latter in body length immediately after metamorphosis, although there were somewhat larger deviations. At the age of nine months, 27 fourth-generation offspring obtained by one of four experimental matings were distinctly larger than those obtained by the others as well as the control frogs.

Among 57 control young frogs preserved shortly after metamorphosis, there were 27 females and 30 males. On the other hand, there were 228 females, 42 hermaphrodites and 184 males among 454 young fourth-generation offspring produced by ten experimental matings with male third-generation offspring. However, distinct differences were found in sexual ratio among the three sets of experimental matings. In the sets 2 and 4, there were 219 females, 11 hermaphrodites and 150 males among 380 fourth-generation offspring, while in the set 3, there were nine females, 31 hermaphrodites and 34 males among 74. Three of the nine females in the set 3 had under-developed ovaries, while the other six had normal ones, in contrast with the former 219 females all of which had normal ovaries.

While there were five females and five males among ten matured control frogs, there were seven females and 39 males among 46 matured fourth-generation offspring obtained by mating of three female *japonica* with four third-generation males. Seven frogs of the set 1 consisted of a female and six males, nine of the set

2, of a female and eight males and the remaining 30 of the set 3, of five females and 25 males. Accordingly, it was presumed that sex-reversal occurred earlier or later in most genetic females of the fourth generations.

III. Results of mating experiments performed in 1966

1. Parents used for producing fifth-generation offspring

Fifth-generation offspring of the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12 were produced by mating of 21 male fourth-generation offspring obtained in the previous year with six female *japonica* collected from the field. A part of the eggs of each female *japonica* were inseminated with sperms of nine male *japonica* raised since the egg stage in our laboratory as the control series.

The six female *japonica* were 42.5~49.0 mm. in body length and 1.53~1.65 mm. in the mean diameter of 50 eggs taken at random (Table 32). All the nine male *japonica* were one year old and 34.5~40.0 mm. in body length. Their testes were 2.3 mm. in length and 1.3 mm. in width, on the average. All of them were of Type 1 in inner structure (Table 33).

TABLE 32

Female control frogs used in the mating experiments performed in 1966

Kind	Individual no.	Body length (mm.)	Mean diameter of 50 eggs (mm.)
J.W66	1	42.5	1.53±0.03
	2	45.0	1.57±0.03
	3	49.0	1.65±0.04
	4	46.5	1.60±0.03
	5	45.0	1.61±0.03
	6	43.0	1.53±0.03

J.W — *Rana japonica* collected from the field

TABLE 33

Male control frogs used in the mating experiments performed in 1966

Kind	Individual no.	Age (year)	Body length (mm.)	Size of the testes		Inner structure	
				Left (mm.)	Right (mm.)	Type	Ploidy
J.L66	1	1	39.0	2.5×1.5	2.5×1.5	1	2n
	2	1	37.5	2.5×1.0	2.5×1.0	1	2n
	3	1	35.0	2.0×1.0	2.0×1.0	1	2n
J.L66	4	1	34.5	2.0×1.0	2.0×1.0	1	2n
	5	1	37.5	2.5×1.5	2.5×1.5	1	2n
	6	1	36.0	2.0×1.5	2.0×1.5	1	2n
J.L66	7	1	36.0	2.0×1.5	2.0×1.5	1	2n
	8	1	38.5	2.5×1.5	2.5×1.5	1	2n
	9	1	40.0	2.5×1.5	2.5×1.5	1	2n

J.L — Laboratory strain of *Rana japonica*

The 21 fourth-generation males used for producing the fifth generations belonged to six kinds. Two kinds were originated from the nucleo-cytoplasmic hybrid No. 61OT2, three from No. 61AT8 and the remaining one from No. 61AT12.

All these males were one year old and 34~41 mm. in body length, being quite the same as the above male *japonica*. Their testes were 2.6 mm. in length and 1.3 mm. in width, on the average. Accordingly, these testes were rather superior in size to those of the control males. However, they were not always normal in inner structure. While the testes of five males were of Type 1, those of seven others were of Type 2 and those of the other nine were of Type 3 (Table 34).

TABLE 34

Male fourth-generation offspring of three nucleo-cytoplasmic hybrids used in the mating experiments performed in 1966

Offspring		Age	Body length	Size of testes		Inner structure	
Kind	Individual no.	(year)	(mm.)	Left (mm.)	Right (mm.)	Type	Ploidy
J〔[J{J(61OT2)}$_8$]$_5$J〕$_2$	1	1	40.0	3.0×1.0	3.0×1.0	2	2n
	2	1	40.0	3.0×1.5	2.5×1.0	3	2n
	3	1	41.0	3.0×1.5	3.0×1.5	2	2n
	4	1	40.5	3.0×1.5	3.0×1.5	3	2n
J〔J[J{J(61OT2)}$_1$]$_5$〕$_1$	1	1	37.5	2.5×1.5	2.5×1.5	2	2n
	2	1	38.5	2.0×1.0	2.0×1.0	3	2n
	3	1	35.0	2.0×1.0	2.0×1.0	3	2n
	4	1	40.0	3.0×1.5	3.0×1.5	1	2n
	5	1	36.5	2.5×1.5	2.5×1.0	1	2n
J〔[J{J(61AT8)}$_4$]$_2$J〕$_3$	1	1	35.5	2.5×1.0	2.5×1.0	2	2n
	2	1	39.5	3.0×1.5	3.0×1.5	3	2n
J〔J[J{J(61AT8)}$_4$]$_2$〕$_1$	1	1	38.0	2.5×1.0	2.5×1.0	1	2n
	2	1	34.0	2.5×1.0	2.5×1.0	2	2n
	3	1	35.5	2.5×1.0	2.5×1.5	3	2n
	4	1	34.5	2.0×1.0	2.0×1.0	3	2n
J〔J[J{J(61AT8)}$_4$]$_2$〕$_2$	1	1	37.0	2.5×1.5	2.5×1.5	3	2n
	2	1	37.5	2.5×1.5	2.5×1.5	2	2n
J〔J[J{J(61AT12)}$_6$]$_2$〕$_3$	1	1	39.0	3.0×1.5	2.5×1.5	1	2n
	2	1	38.5	3.0×1.5	3.0×1.5	3	2n
	3	1	37.0	2.5×1.5	2.5×1.0	1	2n
	4	1	37.0	2.5×1.0	2.5×1.0	2	2n

J ——— *Rana japonica*

J〔[J{J(61OT2)}$_8$]$_5$J〕$_2$—Offspring obtained by J♀×[{J♀×(J♀×♂ No. 61OT2)♂No. 8}♀ No. 5×J♂]♂No. 2

J〔J[J{J(61OT2)}$_1$]$_5$〕$_1$—Offspring obtained by J♀×[J♀×{J♀×(J♀×♂No. 61OT2)♂No. 1}♂ No. 5]♂No. 1

2. Control series

The six female and nine male *japonica* were divided into three sets. In each set, eggs of two females were inseminated with sperm of each of three males (Table 35).

Set 1. J.W66♀ Nos. 1 and 2×J.L66♂ Nos. 1~3

There were 369(95.1%) normally cleaved eggs. Seven of them died of abnormal structures by the hatching stage. Seven tadpoles died at the XX~XXIII stages and 355 completed their metamorphosis. The latter were all normal, except for three which had ill-developed hind legs.

Set 2. J.W66♀ Nos. 3 and 4×J.L66♂ Nos. 4~6

There were 239(97.2%) normally cleaved eggs. While seven died of abnormal structures, all the others developed normally into metamorphosed frogs.

Set 3. J.W66♀ Nos. 5 and 6×J.L66♂ Nos. 7～9

Of 326(98.5%) normally cleaved eggs eight died of abnormal structures by the hatching stage. All the others developed normally into metamorphosed frogs.

3. Experimental series

a. Mating experiments by using male fourth-generation offspring of the male nucleo-cytoplasmic hybrid No. 61OT2

Eggs of two female *japonica* Nos. 3 and 4 were inseminated with sperms of nine males belonging to two kinds of fourth-generation offspring of the nucleo-cytoplasmic hybrid. The first kind of males was derived from a male first-generation offspring No. 8. The second generation was produced from this male by mating with a female *japonica.* While the third generation was obtained by mating of a female No. 5 of this second generation with a male *japonica,* fourth-generation offspring were produced from a male No. 2 of the third generation by mating with a female *japonica* (Table 36).

The second kind of males was derived from a male first-generation offspring No. 1. The second-generation offspring was obtained by mating of this male with a female *japonica* and the third-generation offspring was produced by mating of a male No. 5 of the second generation with a female *japonica.* Fourth-generation offspring used for the mating experiments in 1966 were produced from a male No. 1 of the third generation by mating with a female *japonica.*

The eggs of the two female *japonica* were divided into nine groups besides the control one. Four groups were utilized for mating experiments of the first kind of males, while the other five were for those of the second kind.

Set 1. Four males Nos. 1～4 produced from a male third-generation offspring No. 2 mated with a female *japonica*

Mating 1. J.W66♀ Nos. 3 and $4\times J[[J\{J(61OT2)\}_8]_5J]_2$♂ No. 1

There were 92(27.4%) normally cleaved eggs. Two, three and 42 of them died of edema or other types of abnormalities at the neurula, the tail-bud and the hatching stage, respectively. The other 45 eggs developed normally into tadpoles. However, all the latter gradually died at the V～XVIII stages and none attained the metamorphosing stage.

Mating 2. J.W66♀ Nos. 3 and $4\times J[[J\{J(61OT2)\}_8]_5J]_2$♂ No. 2

Of 42(34.7%) normally cleaved eggs 18 died of edema or other types of abnormalities by the hatching stage and the remaining 24 died of ill-development or edema at the V～XVIII stages.

Mating 3. J.W66♀ Nos. 3 and $4\times J[[J\{J(61OT2)\}_8]_5J]_2$♂ No. 3

Of 185(47.7%) normally cleaved eggs 22, 11 and 124 died of edema at the neurula, the tail-bud and the hatching stage, respectively. Although the other 28 eggs developed normally into tadpoles, ten died of ill-development or edema at the V～XVIII stages, 11 died during their metamorphosis, and the remaining seven became metamorphosed frogs. While five of the latter were quite normal,

TABLE 35
Reproductive capacity of

Parents		No. of eggs	No. of cleaved eggs		No. of neurulae	
Female no.	Male no.		Normal	Abnormal	Normal	Abnormal
J.W66.1～2	J.L66.1	87	73 (83.9%)	0	71 (81.6%)	2 (2.3%)
	J.L66.2	196	193 (98.5%)	0	192 (98.0%)	1 (0.5%)
	J.L66.3	105	103 (98.1%)	0	103 (98.1%)	0
	Total	388	369 (95.1%)	0	366 (94.3%)	3 (0.8%)
J.W66.3～4	J.L66.4	64	59 (92.2%)	2 (3.1%)	59 (92.2%)	0
	J.L66.5	50	50 (100%)	0	50 (100 %)	0
	J.L66.6	132	130 (98.5%)	2 (1.5%)	130 (98.5%)	0
	Total	246	239 (97.2%)	4 (1.6%)	239 (97.2%)	0
J.W66.5～6	J.L66.7	127	125 (98.4%)	0	125 (98.4%)	0
	J.L66.8	120	117 (97.5%)	0	117 (97.5%)	0
	J.L66.9	84	84 (100 %)	0	84 (100 %)	0
	Total	331	326 (98.5%)	0	326 (98.5%)	0

the other two had abnormal hind legs.

Mating 4. J.W66♀ Nos. 3 and $4\times J[[J\{J(61OT2)\}_{8}]_{5}J]_{2}$♂ No. 4

There were 82(19.7%) normally cleaved eggs. Two and 68 of them died of edema at the tail-bud and the hatching stage, respectively. The remaining 12 developed normally into metamorphosed frogs.

Set 2. Five males Nos. 1～5 produced from a male third-generation offspring No. 1 mated with a female *japonica*

Mating 1. J.W66♀ Nos. 3 and $4\times J[J[J\{J(61OT2)\}_{1}]_{5}]_{1}$♂ No. 1

Only 11(2.7%) eggs cleaved normally. Five and one of them died of abnormal structures at the hatching and the VII stage, respectively. The remaining five developed normally into metamorphosed frogs.

Mating 2. J.W66♀ Nos. 3 and $4\times J[J[J\{J(61OT2)\}_{1}]_{5}]_{1}$♂ No. 2

There were 164(46.5%) normally cleaved eggs. However, 163 of them died of edema by the time of metamorphosis: 12 by the tail-bud, 130 at the hatching, 17 at the V～XVIII and the four at the metamorphosing stage. A single tadpole completed its metamorphosis and became a normal frog.

Mating 3. J.W66♀ Nos. 3 and $4\times J[J[J\{J(61OT2)\}_{1}]_{5}]_{1}$♂ No. 3

Only one egg cleaved normally. But this egg died of edema at the hatching stage.

Mating 4. J.W66♀ Nos. 3 and $4\times J[J[J\{J(61OT2)\}_{1}]_{5}]_{1}$♂ No. 4

the control series

No. of tail-bud embryos		No. of hatched tadpoles		No. of St. V～XVIII tadpoles		No. of metamorphosed frogs	
Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
70 (80.5%)	1 (1.1%)	70 (80.5%)	0	70 (80.5%)	0	67 (77.0%)	2 (2.3%)
191 (97.4%)	1 (0.5%)	191 (97.4%)	0	191 (97.4%)	0	185 (94.4%)	0
103 (98.1%)	0	101 (96.2%)	2 (1.9%)	101 (96.2%)	0	100 (95.2%)	1 (1.0%)
364 (93.8%)	2 (0.5%)	362 (93.3%)	2 (0.5%)	362 (93.3%)	0	352 (90.7%)	3 (0.8%)
57 (89.1%)	2 (3.1%)	56 (87.5%)	1 (1.6%)	56 (87.5%)	0	56 (87.6%)	0
50 (100 %)	0	50 (100 %)	0	50 (100 %)	0	50 (100 %)	0
130 (98.5%)	0	126 (95.5%)	4 (3.0%)	126 (95.5%)	0	126 (95.5%)	0
237 (96.3%)	2 (0.8%)	232 (94.3%)	5 (2.0%)	232 (94.3%)	0	232 (94.3%)	0
121 (95.3%)	4 (3.1%)	117 (92.1%)	4 (3.1%)	117 (92.1%)	0	117 (92.1%)	0
117 (97.5%)	0	117 (97.5%)	0	117 (97.5%)	0	117 (97.5%)	0
84 (100 %)	0	84 (100 %)	0	84 (100 %)	0	84 (100 %)	0
322 (97.3%)	4 (1.2%)	318 (96.1%)	4 (1.2%)	318 (96.1%)	0	318 (96.1%)	0

Of 96(82.1%) normally cleaved eggs four and two died of abnormal structures at the hatching and the V stage, respectively. The other developed normally into metamorphosed frogs.

Mating 5. J.W66♀ Nos. 3 and 4×J〔J[J{J(61OT2)}$_1$]$_5$〕$_1$♂ No. 5

Normally cleaved eggs were 119(95.2%) in number. Twenty-one and 18 of them died of edema by the hatching and at the V～XVIII stages, respectively. All the remaining 80 tadpoles completed their metamorphosis.

b. Mating experiments by using male fourth-generation offspring of the male nucleo-cytoplasmic hybrid No. 61AT8

Eggs of two female *japonica* Nos. 1 and 2 were inseminated with sperms of eight males belonging to three kinds of fourth-generation offspring of the nucleo-cytoplasmic hybrid. The first kind of males was obtained from a male third-generation offspring No. 3 by mating with a female *japonica*. The third-generation offspring was produced from a female second-generation offspring No. 2, which had been obtained from a male first-generation offspring No. 4, by mating with a male *japonica*. The second kind of males was obtained from a male third-generation offspring No. 1, which had been produced from a male No. 2 of the above second generation and a female *japonica*, by mating with a female *japonica*. The third kind of males was obtained from another male No. 2 of

TABLE 36

Reproductive capacity of male fourth-generation offspring of the three male

Set	(Mating)	Parents: Female no.	Parents: Male no.	No. of eggs	No. of cleaved eggs: Normal	No. of cleaved eggs: Abnormal
1	(1)	J.W66.3～4	J〔[J{J(61OT2)}$_{8}$]$_{5}$J〕$_{2}$.1	336	92 (27.4%)	0
	(2)		J〔[J{J(61OT2)}$_{8}$]$_{5}$J〕$_{2}$.2	121	42 (34.7%)	0
	(3)		J〔[J{J(61OT2)}$_{8}$]$_{5}$J〕$_{2}$.3	388	185 (47.7%)	0
	(4)		J〔[J{J(61OT2)}$_{8}$]$_{5}$J〕$_{2}$.4	417	82 (19.7%)	3
2	(1)	J.W66.3～4	J〔J[J{J(61OT2)}$_{1}$]$_{5}$〕$_{1}$.1	405	11 (2.7%)	0
	(2)		J〔J[J{J(61OT2)}$_{1}$]$_{5}$〕$_{1}$.2	353	164 (46.5%)	0
	(3)		J〔J[J{J(61OT2)}$_{1}$]$_{5}$〕$_{1}$.3	116	1 (0.9%)	12 (10.3%)
	(4)		J〔J[J{J(61OT2)}$_{1}$]$_{5}$〕$_{1}$.4	117	96 (82.1%)	11 (9.5%)
	(5)		J〔J[J{J(61OT2)}$_{1}$]$_{5}$〕$_{1}$.5	125	119 (95.2%)	4 (3.2%)
1	(1)	J.W66.1～2	J〔[J{J(61AT8)}$_{4}$]$_{2}$J〕$_{3}$.1	209	124 (59.3%)	16 (7.7%)
	(2)		J〔[J{J(61AT8)}$_{4}$]$_{2}$J〕$_{3}$.2	242	55 (22.7%)	13 (5.4%)
2	(1)	J.W66.1～2	J〔J[J{J(61AT8)}$_{4}$]$_{2}$〕$_{1}$.1	210	166 (79.0%)	7 (3.3%)
	(2)		J〔J[J{J(61AT8)}$_{4}$]$_{2}$〕$_{1}$.2	170	78 (45.9%)	0
	(3)		J〔J[J{J(61AT8)}$_{4}$]$_{2}$〕$_{1}$.3	210	125 (59.5%)	12 (5.7%)
	(4)		J〔J[J{J(61AT8)}$_{4}$]$_{2}$〕$_{1}$.4	123	74 (60.2%)	3 (2.4%)
3	(1)	J.W66.1～2	J〔J[J{J(61AT8)}$_{4}$]$_{2}$〕$_{2}$.1	160	49 (30.6%)	3 (1.9%)
	(2)		J〔J[J{J(61AT8)}$_{4}$]$_{2}$〕$_{2}$.2	156	88 (56.4%)	14 (9.0%)
1	(1)	J.W66.5～6	J〔J[J{J(61AT12)}$_{6}$]$_{2}$〕$_{3}$.1	102	90 (88.2%)	3 (2.9%)
	(2)		J〔J[J{J(61AT12)}$_{6}$]$_{2}$〕$_{3}$.2	153	20 (13.1%)	7 (4.6%)
	(3)		J〔J[J{J(61AT12)}$_{6}$]$_{2}$〕$_{3}$.3	122	101 (82.8%)	0
	(4)		J〔J[J{J(61AT12)}$_{6}$]$_{2}$〕$_{3}$.4	117	56 (47.9%)	13 (11.1%)

the same third generation as described lastly by mating with a female *japonica.*

Set 1. Two males Nos. 1 and 2 produced from a male third-generation offspring No. 3 mated with a female *japonica*

Mating 1. J.W66♀ Nos. 1 and 2×J〔[J{J(61AT8)}$_{4}$]$_{2}$J〕$_{3}$♂ No. 1

There were 124(59.3%) normally cleaved eggs. At the hatching and the V～

nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of St. V∼XVIII tadpoles		No. of metamorphosed frogs	
Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.	Normal	Abnorm.
90 (26.8%)	2 (0.6%)	87 (25.9%)	3 (0.9%)	45 (13.4%)	42 (12.5%)	0	45 (13.4%)	0	0
41 (33.9%)	1 (0.8%)	40 (33.1%)	1 (0.8%)	24 (19.8%)	16 (13.2%)	0	24 (19.8%)	0	0
163 (42.0%)	22 (5.7%)	152 (39.2%)	11 (2.8%)	28 (7.2%)	124 (32.0%)	18 (4.6%)	10 (2.6%)	5 (1.3%)	2 (0.5%)
82 (19.7%)	0	80 (19.2%)	2 (0.5%)	12 (2.9%)	68 (16.3%)	12 (2.9%)	0	12 (2.9%)	0
11 (2.7%)	0	11 (2.7%)	0	6 (1.5%)	5 (1.2%)	5 (1.2%)	1 (0.2%)	5 (1.2%)	0
160 (45.3%)	4 (1.1%)	152 (43.1%)	8 (2.3%)	22 (6.2%)	130 (36.8%)	5 (1.4%)	17 (4.8%)	1 (0.3%)	0
1 (0.9%)	0	1 (0.9%)	0	0	1 (0.9%)	0	0	0	0
96 (82.1%)	0	96 (82.1%)	0	92 (78.6%)	4 (3.4%)	90 (76.9%)	2 (1.7%)	90 (76.9%)	0
117 (93.6%)	2 (1.6%)	103 (82.4%)	14 (11.2%)	98 (78.4%)	5 (4.0%)	80 (64.0%)	18 (14.4%)	80 (64.0%)	0
124 (59.3%)	0	124 (59.3%)	0	66 (31.6%)	58 (27.8%)	8 (3.8%)	58 (27.8%)	0	0
55 (22.7%)	0	55 (22.7%)	0	48 (19.8%)	7 (2.9%)	48 (19.8%)	0	47 (19.4%)	1 (0.4%)
166 (79.0%)	0	166 (79.0%)	0	157 (74.8%)	9 (4.3%)	157 (74.8%)	0	155 (73.8%)	2 (1.0%)
78 (45.9%)	0	78 (45.9%)	0	75 (44.1%)	3 (1.8%)	75 (44.1%)	0	75 (44.1%)	0
84 (40.0%)	3 (1.4%)	59 (28.1%)	25 (11.9%)	46 (21.9%)	13 (6.2%)	46 (21.9%)	0	46 (21.9%)	0
57 (46.3%)	12 (9.8%)	41 (33.3%)	16 (13.0%)	16 (13.0%)	25 (20.3%)	5 (4.1%)	11 (8.9%)	4 (3.7%)	1 (0.8%)
49 (30.6%)	0	49 (30.6%)	0	46 (30.6%)	3 (1.9%)	40 (25.0%)	6 (3.8%)	40 (25.0%)	0
86 (55.1%)	2 (1.3%)	75 (48.1%)	11 (8.7%)	56 (35.9%)	19 (12.2%)	40 (25.6%)	16 (10.3%)	40 (25.6%)	0
84 (82.4%)	2 (2.0%)	81 (79.4%)	3 (2.9%)	76 (74.5%)	5 (4.9%)	71 (69.6%)	5 (4.9%)	70 (68.6%)	1 (1.0%)
15 (9.8%)	3 (2.0%)	13 (8.5%)	2 (1.3%)	13 (8.5%)	0	6 (3.9%)	7 (4.6%)	6 (3.9%)	0
98 (80.3%)	1 (0.8%)	98 (80.3%)	0	98 (80.3%)	0	98 (80.3%)	0	98 (80.3%)	0
51 (43.6%)	5 (4.3%)	49 (41.9%)	2 (1.7%)	40 (34.2%)	9 (7.7%)	28 (23.9%)	12 (10.3%)	25 (21.4%)	3 (2.6%)

VIII stages 58 and 58 individuals died of edema or other abnormalities, respectively. As the remaining eight tadpoles died also of edema or other abnormalities immediately before metamorphosis, there were no frogs.

Mating 2. J.W66♀ Nos. 1 and 2×$J[[J\{J(61AT8)\}_4]_2J]_3$♂ No. 2

Normally cleaved eggs were 55(22.7%) in number. While seven of them died of abnormal structures at the hatching stage, the others developed normally into metamorphosed frogs. The latter were all normal in external structures, except for one which was abnormal in the growth of the hind legs.

Set 2. Four males Nos. 1～4 produced from a male third-generation offspring No. 1 mated with a female *japonica*

Mating 1. J.W66♀ Nos. 1 and 2×J〔J[J{J(61AT8)}$_4$]$_2$〕$_1$♂ No. 1

Nine of 166(79.0%) normally cleaved eggs died of abnormal structures at the hatching stage and the others developed normally into metamorphosed frogs. All these frogs were normal, except for two which were edematous and died immediately after metamorphosis.

Mating 2. J.W66♀ Nos. 1 and 2×J〔J[J{J(61AT8)}$_4$]$_2$〕$_1$♂ No. 2

There were 78(45.9%) normally cleaved eggs. At the hatching stage three died of abnormal structures. The other 75 developed normally into metamorphosed frogs.

Mating 3. J.W66♀ Nos. 1 and 2×J〔J[J{J(61AT8)}$_4$]$_2$〕$_1$♂ No. 3

Of 125(59.5%) normally cleaved eggs 79 died of various types of abnormalities by the hatching stage. The remaining 46 tadpoles developed normally and completed their metamorphosis.

Mating 4. J.W66♀ Nos. 1 and 2×J〔J[J{J(61AT8)}$_4$]$_2$〕$_1$♂ No. 4

Of 74(60.2%) normally cleaved eggs 58 and eleven died of various types of abnormalities by the hatching stage and at the V～XVIII stages, respectively. The remaining five tadpoles completed their metamorphosis. While four frogs were normal in external structures, the other had abnormal hind legs and became

TABLE 37

Shape, size and sex of metamorphosed

Parents		Age at the time of landing (Mean) (days)	Total	No. of frogs		Body length immediately after metamorphosis (mm.)
Female no.	Male no.			Normal	Abnormal	
J.W66.1～2	J.L66.1	82～91 (87.2)	69	67	2	16.2±0.5
	J.L66.2	82～90 (87.5)	185	185	0	15.7±0.4
	J.L66.3	82～92 (88.0)	101	100	1	15.5±0.3
J.W66.3～4	J.L66.4	81～91 (87.5)	56	56	0	15.7±0.3
	J.L66.5	81～90 (87.0)	50	50	0	16.0±0.6
	J.L66.6	82～91 (87.7)	126	126	0	16.2±0.3
J.W66.5～6	J.L66.7	82～90 (86.7)	117	117	0	15.9±0.4
	J.L66.8	83～90 (87.2)	117	117	0	16.2±0.4
	J.L66.9	82～91 (87.0)	84	84	0	15.6±0.6

edematous.

Set 3. Two males Nos. 1 and 2 produced from a male third-generation offspring No. 2 mated with a female *japonica*

Mating 1. J.W66♀ Nos. 1 and 2×$J[J[J\{J(61AT8)\}_4]_2]_2$♂ No. 1

There were 49(30.6%) normally cleaved eggs. Three and six of them died of abnormal structures at the hatching and the V～XIII stages, respectively. The remaining 40 tadpoles developed normally and completed their metamorphosis.

Mating 2. J.W66♀ Nos. 1 and 2×$J[J[J\{J(61AT8)\}_4]_2]_2$♂ No. 2

Of 88(56.4%) normally cleaved eggs 32 and 16 died of various types of abnormalities by the hatching stage and at the V～XVIII stages, respectively. The remaining 40 tadpoles became normal metamorphosed frogs.

c. Mating experiments by using male fourth-generation offspring of the male nucleo-cytoplasmic hybrid No. 61AT12

Eggs of two female *japonica,* J.W66 Nos. 5 and 6, were divided into four groups and inseminated with sperms of four males of fourth-generation offspring of the nucleo-cytoplasmic hybrid. The fourth-generation offspring were obtained by mating of a male third-generation offspring No. 3 with a female *japonica.* The third generation was produced by mating of a male of second-generation offspring No. 2 with a female *japonica,* while the second generation was by mating of a male first-generation offspring No. 6 with a female *japonica.*

Set 1. Four males Nos. 1～4 produced from a male third-generation offspring No. 3 mated with a female *japonica*

Mating 1. J.W66♀ Nos. 5 and 6×$J[J[J\{J(61AT12)\}_6]_2]_3$♂ No. 1

Rana japonica in the control series

Body length of 9-month-old frogs (mm.)	Sex of young frogs shortly after metamorphosis							Sex of matured frogs		
	Total	♀N	♀U	⚥1	⚥2	⚥3	♂N	Total	♀	♂
	69	34	0	2	1	0	32			
37.0～38.5	155	76	0	1	0	0	78	25	12	13
	101	52	0	1	0	1	47			
	56	27	0	0	0	0	29			
39.0～41.5	30	15	0	0	1	0	14	20	9	11
38.0～39.0	116	58	0	2	0	0	56	10	5	5
	117	57	0	0	0	0	60			
	117	55	0	0	0	0	62			
40.0～41.5	64	36	0	0	0	0	28	18	8	10

There were 90(88.2%) normally cleaved eggs. Fourteen of them died of abnormal structures by the hatching stage. At the X～XVII stages five tadpoles died of abnormal structures. The other tadpoles developed normally into metamorphosed frogs. While one of them had abnormal hind legs, the remaining were quite normal.

Mating 2. J.W66♀ Nos. 5 and 6×J〔J[J{J(61AT12)}$_6$]$_2$〕$_3$♂ No. 2

Twenty (13.1%) eggs cleaved normally. Seven and seven of them died of abnormal structures by the hatching and at the V～X stages, respectively. The other six tadpoles completed their metamorphosis.

TABLE 38

Shape, size and sex of metamorphosed fifth-generation offspring of the three male

Set	(Mating)	Parents: Female no.	Parents: Male no.	Age at the time of landing (Mean) (days)	No. of frogs: Total	Normal	Abnormal
1	(3)	J.W66.3～4	J〔[J{J(61OT2)}$_8$]$_5$J〕$_2$.3	85～89 (87.1)	7	5	2
	(4)		J〔[J{J(61OT2)}$_8$]$_5$J〕$_2$.4	83～90 (86.5)	12	12	0
2	(1)	J.W66.3～4	J〔J[J{J(61OT2)}$_1$]$_5$〕$_1$.1	84～91 (87.0)	5	5	0
	(2)		J〔J[J{J(61OT2)}$_1$]$_5$〕$_1$.2	86	1	1	0
	(4)		J〔J[J{J(61OT2)}$_1$]$_5$〕$_1$.4	83～91 (87.5)	90	90	0
	(5)		J〔J[J{J(61OT2)}$_1$]$_5$〕$_1$.5	82～90 (87.0)	80	80	0
1	(2)	J.W66.1～2	J〔[J{J(61AT8)}$_4$]$_2$J〕$_3$.2	82～94 (88.5)	48	47	1
2	(1)	J.W66.1～2	J〔J[J{J(61AT8)}$_4$]$_2$〕$_1$.1	81～90 (86.3)	157	155	2
	(2)		J〔J[J{J(61AT8)}$_4$]$_2$〕$_1$.2	81～91 (87.0)	75	75	0
	(3)		J〔J[J{J(61AT8)}$_4$]$_2$〕$_1$.3	82～93 (88.2)	46	46	0
	(4)		J〔J[J{J(61AT8)}$_4$]$_2$〕$_1$.4	90～94 (92.2)	5	4	1
3	(1)	J.W66.1～2	J〔J[J{J(61AT8)}$_4$]$_2$〕$_2$.1	84～93 (87.4)	40	40	0
	(2)		J〔J[J{J(61AT8)}$_4$]$_2$〕$_2$.2	83～93 (87.8)	40	40	0
1	(1)	J.W66.5～6	J〔J[J{J(61AT12)}$_6$]$_2$〕$_3$.1	86～94 (90.1)	71	70	1
	(2)		J〔J[J{J(61AT12)}$_6$]$_2$〕$_3$.2	83～89 (86.1)	6	6	0
	(3)		J〔J[J{J(61AT12)}$_6$]$_2$〕$_3$.3	84～93 (89.0)	98	98	0
	(4)		J〔J[J{J(61AT12)}$_6$]$_2$〕$_3$.4	82～91 (88.6)	28	25	3

Mating 3. J.W66♀ Nos. 5 and 6×J〔J[J{J(61AT12)}$_6$]$_2$〕$_3$♂ No. 3

Only three of 101(82.8%) normally cleaved eggs died of abnormal structures. All the others developed normally into metamorphosed frogs.

Mating 4. J.W66♀ Nos. 5 and 6×J〔J[J{J(61AT12)}$_6$]$_2$〕$_3$♂ No. 4

Of 56(47.9%) normally cleaved eggs 16 and 12 died of abnormal structures by the hatching and at the V~XVIII stages, respectively. The remaining 28 tadpoles completed their metamorphosis. While three of them had abnormal hind legs and were edematous, the other 25 were quite normal.

nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12

Body length immediately after metamorphosis (mm.)	Body length of 9-month-old frogs (mm.)	Sex of young frogs shortly after metamorphosis							Sex of matured frogs		
		Total	♀$_N$	♀$_U$	⚥$_1$	⚥$_2$	⚥$_3$	♂$_N$	Total	♀	♂
15.5~17.0		7	0	0	1	0	2	4			
15.3±1.1		12	3	1	0	1	1	6			
16.0~16.5		5	2	0	1	0	0	2			
14.5		1	0	0	1	0	0	0			
15.3± 0.7	34.0~37.5	70	21	1	10	4	4	30	17	1	16
15.0± 0.8	36.0~37.0	75	30	1	4	0	0	40	5	0	5
15.7± 0.4	31.0~35.0	40	3	2	2	3	11	19	5	0	5
16.1± 0.7	36.0~37.0	125	61	1	7	1	9	46	15	2	13
15.5± 0.7		74	20	2	6	1	5	40			
16.0± 0.7		46	12	0	11	0	2	21			
15.0~16.5		5	1	0	1	0	0	3			
15.7± 0.6		40	14	1	1	2	3	19			
16.1± 1.2		40	21	0	0	0	4	15			
15.2± 0.9	34.0~36.5	65	19	3	6	2	4	31	4	0	4
15.5~16.0		6	1	0	1	0	0	4			
15.2± 0.8	36.2~37.5	80	34	0	1	2	1	42	16	2	14
15.7± 1.2	32.5~34.0	22	11	0	0	0	1	10	3	0	3

4. Metamorphosed frogs

a. Control frogs

In the control series, 77.0 ~ 100% of eggs developed into normal metamorphosed frogs. They were produced by mating between six female and nine male *japonica* (Table 35). By seven of nine control matings, more than 90% of eggs really became normal frogs. All the frogs produced by the control matings are listed in Table 37. They made their metamorphosis at the ages of 81 ~ 92 days; they were 15.5 ~ 16.2 mm. in mean body length immediately after metamorphosis.

Most of the frogs were killed shortly after metamorphosis for examining their sex. The others were reared continuously. As presented in the table, there were 410 females, 406 males and nine hermaphrodites among 825 young frogs. Among the females there were no individuals with under-developed ovaries.

At the stage of sexual maturity, there were 34 females and 39 males among 73 frogs.

b. Fifth-generation offspring of the three nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12

No or only a few frogs were produced from 10 of the 21 male fourth-generation offspring of the three nucleo-cytoplasmic hybrids by mating with six female *japonica* (Table 36). By mating with the other 11 males, 19.4 ~ 80.3% of *japonica* eggs developed into normal metamorphosed frogs, although less than 25.6% of eggs did so by mating with each of five males.

All the frogs produced by mating of the fourth-generation males with the female *japonica* are listed in Table 38. They made their metamorphosis at the ages of 81 ~ 94 days. They were 15.0 ~ 16.1 mm. in mean body length immediately after metamorphosis, that is, they were similar to the control frogs, although the deviations were somewhat larger.

Most frogs were killed shortly after metamorphosis for examining their sex, while the others were reared continuously. As the results of observations, there were 265 females, 116 hermaphrodites and 332 males among 713 young frogs. Among the females there were 12 with under-developed ovaries. When the fifth-generation offspring were assorted into three groups according to the kind of nucleo-cytoplasmic hybrids, there were 59 females, 29 hermaphrodites and 82 males among 170 offspring of No. 61OT2, 138 females, 69 hermaphrodites and 163 males among 370 offspring of No. 61AT8 and 68 females, 18 hermaphrodites and 87 males among 173 offspring of No. 61AT12. Accordingly, it was found that there was a male preponderance in the fifth-generation offspring of each of the three nucleo-cytoplasmic hybrids.

At the stage of sexual maturity, there were 5 females and 60 males among 65 frogs. Such a ratio seemed to indicate that sex-reversal had occurred in most genetic females.

CONCLUSION AND DISCUSSION

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

The reproductive capacities of three male and two female nucleo-cytoplasmic hybrids constructed of *Rana ornativentris* cytoplasm and *Rana japonica* nuclei have been reported by KAWAMURA and NISHIOKA (1963c). By mating with the nuclear species, the males produced viable first-generation offspring, while the females did only inviable offspring which died by the hatching stage. In the same paper, the reproductive capacities of 22 male first-generation offspring have been reported, too. As the results of these experiments, it was clearly noticed that the low reproductive capacities of the nucleo-cytoplasmic hybrids were not improved in the next generation, in spite of the crosses with the nuclear species.

In the present research, six females and six males other than the above 22 of the first, 12 females and 22 males of the second, 32 males of the third and 21 males of the fourth generation of the male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12 were mated with male or female *japonica* to obtain their next-generation offspring. In Figs. 14, 15 and 16, the development and viabilities of all the individuals in the generations derived from the three male and two female nucleo-cytoplasmic hybrids are shown. Each of the rectangular frames standing in line in each figure represents the number of total eggs of one or two to five matings which were very similar to one another in the development and viability of individuals obtained. 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. A latticed and a diagonally crosshatching area in some frames show the percentages of metamorphosed frogs with abnormal fore and hind legs, respectively. Each of the control frames shows the average of all the results of control matings in each generation. It was quite clear from the three figures that the offspring of the nucleo-cytoplasmic hybrids in every generation were remarkably inferior to the control frogs in reproductive capacity.

a. Descendants of No. 61OT2

By mating with male *japonica*, two of the three female first-generation offspring produced no normal frogs, although one did a few frogs with abnormal forelegs. In the case of the remaining female, 34.4% of the eggs used became normal frogs. On the other hand, five of 12 male first-generation offspring produced no or only a few normal frogs by mating with female *japonica*, and another did a few frogs with abnormal forelegs. In contrast with these males, the other six produced more or less numerous frogs, that is, 34.5% of *japonica* eggs used became normal frogs by mating with a male and 65%, by mating with the remaining five males. On the average, 30.1% of the *japonica* eggs became normal frogs by mating with 12 male first-generation offspring. The results of brother and sister matings between the three female and three of the 12 male first-generation offspring were very similar to those of the matings between the same females and the male *japonica* stated above.

In order to obtain third-generation offspring of the male nucleo-cytoplasmic hybrid, six female and 23 male second-generation offspring were mated with their

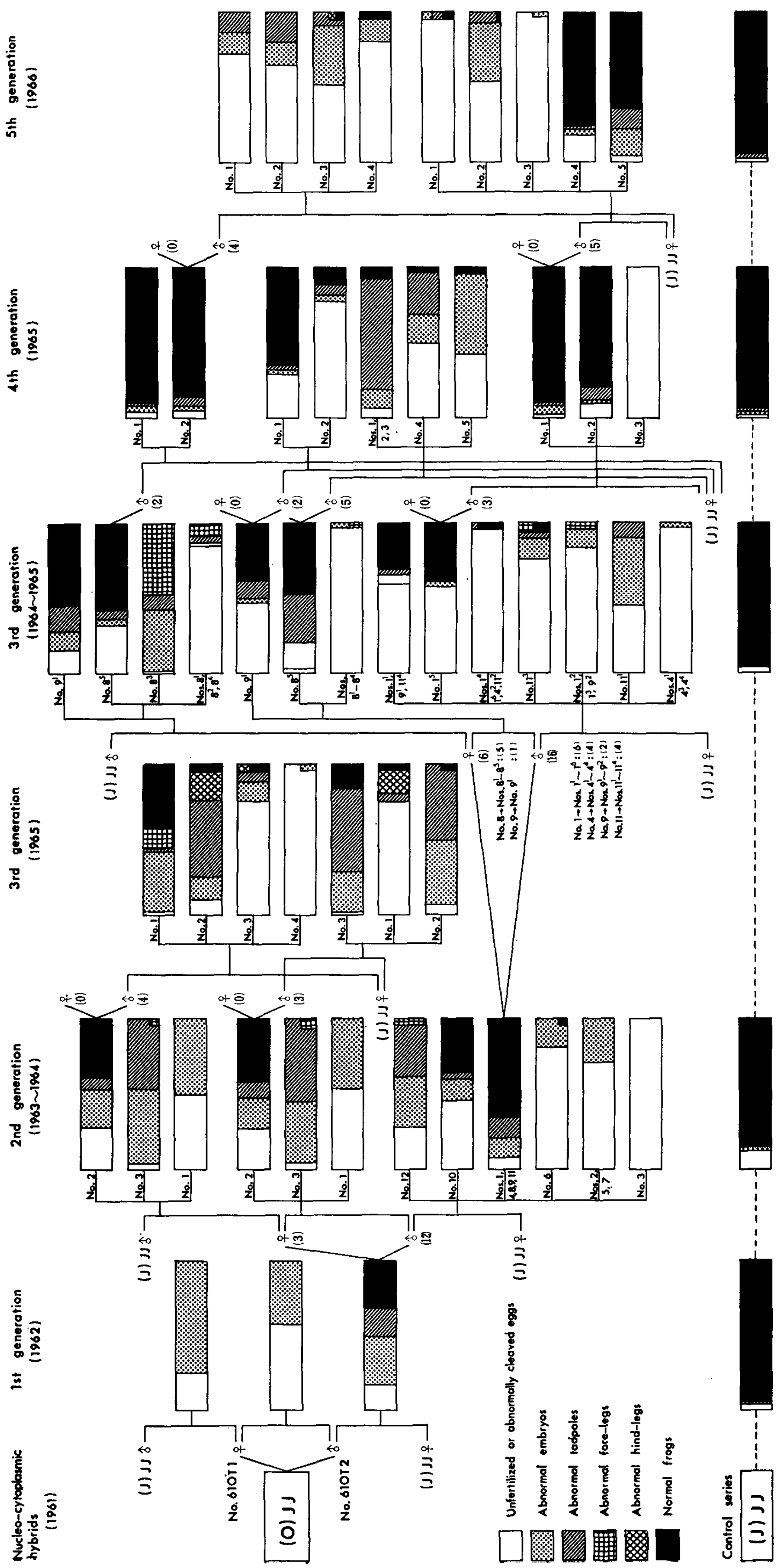

Fig. 14. Diagrammatic representation of the viabilities of the first- to fifth-generation offspring of the male nucleo-cytoplasmic hybrid No. 61OT2.

brothers, cousins or wild strains of *Rana japonica*. The six females were derived from two male first-generation offspring mated with female *japonica*. From four of them no normal frogs were obtained, although there were some frogs with abnormal forelegs. The other two females produced many normal frogs, corresponding to 36.8~59.1% of their total eggs used. Seven of the 23 male second-generation offspring were derived from female first-generation offspring mated with male *japonica* or by brother and sister mating. Five of them produced no or a few normal frogs, while another produced a small number and the remaining, many normal frogs, which corresponded to 17.0 and 41.6% of the eggs used, respectively. Besides these normal frogs, a few or a small number of frogs with abnormal fore or hind legs were produced from four females. The other 16 male second-generation offspring were derived from four male first-generation offspring mated with female *japonica*. Twelve of them produced no or only a few normal frogs by mating with female *japonica*, while the other four did fairly many frogs, corresponding to 15.5~49.3% of the eggs used. On the average, only 8.4% of the *japonica* eggs used became normal metamorphosed frogs by mating with 16 male second-generation offspring. A few frogs with abnormal forelegs were produced from four of these males.

Fourth-generation offspring were obtained from 12 male third-generation offspring which had been produced by three different ways. Two of them derived from a female second-generation offspring mated with a male *japonica*; they produced great many normal frogs, corresponding to 85.5 and 90.9% of the eggs used. Seven others derived from matings between female and male second-generation offspring; six of them produced a few or a small number of normal frogs, corresponding to 2.8~12.1% of the eggs used, while the other did many normal frogs, that is, 65.3% of the eggs used developed into normal frogs. The remaining three male third-generation offspring were derived from a male second-generation offspring mated with a female *japonica*. Two of them produced very numerous normal frogs, corresponding to 80.9 and 89.4% of the eggs used, while no eggs developed normally by mating with the other male. On the average, 56.8% of the *japonica* eggs developed into normal metamorphosed frogs by mating with the three male third-generation offspring.

Fifth-generation offspring were produced from nine male fourth-generation offspring by mating with female *japonica*. Four of them were derived from a female second-generation offspring mated with a male *japonica* by passing over a male third-generation offspring. From these four males no or only a few normal frogs were produced. The other five males were derived from a male second-generation offspring mated with a female *japonica* by passing over a male third-generation offspring. Three of them produced no or only a few normal frogs, while the other two did many, which corresponded to 64.0 and 76.9% of the eggs used. On the average, 28.5% of the *japonica* eggs developed into normal, metamorphosed frogs by mating with the five male fourth-generation offspring.

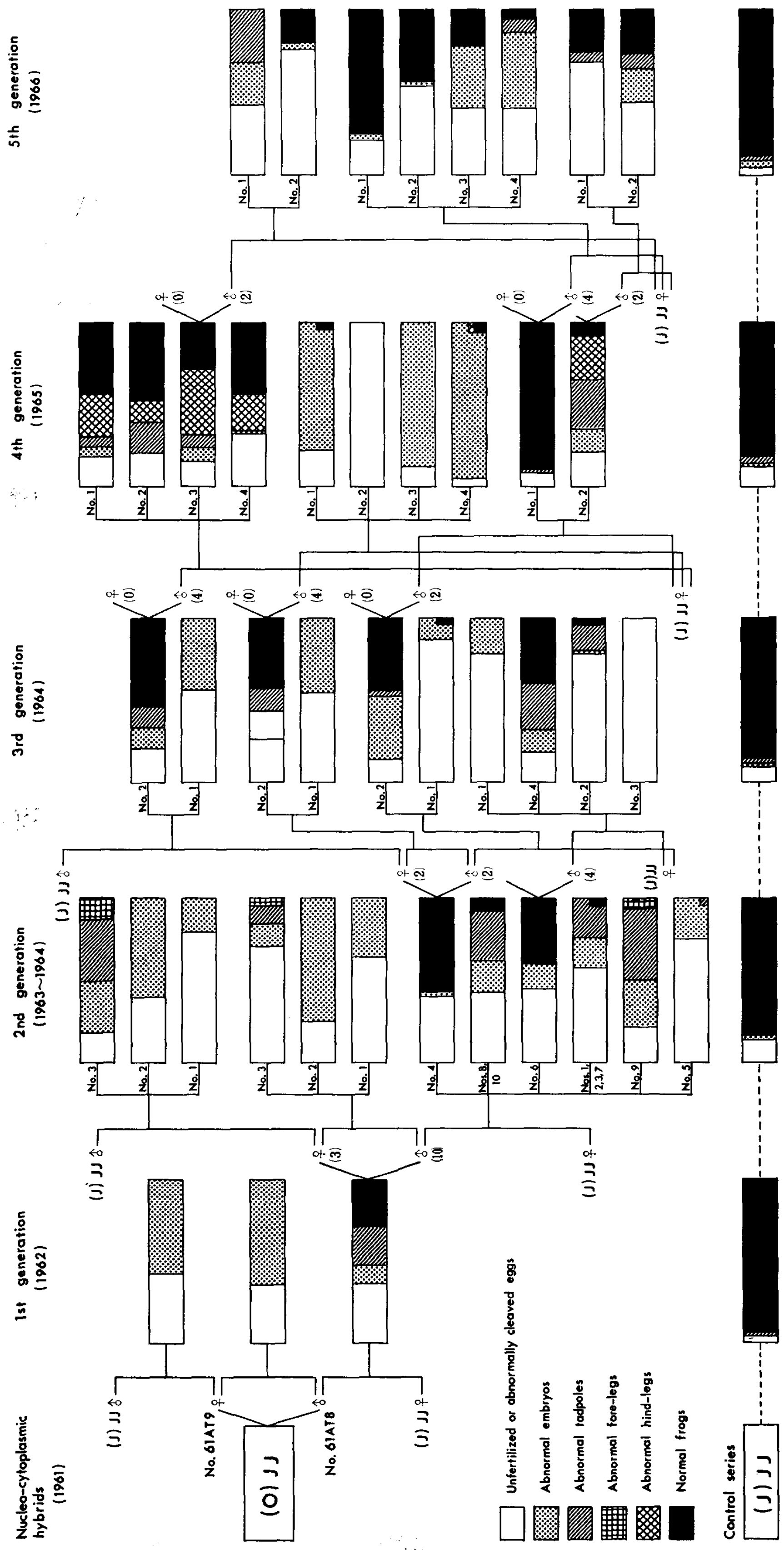

Fig. 15. Diagrammatic representation of the viabilities of the first- to fifth-generation offspring of the male nucleo-cytoplasmic hybrid No. 61AT8.

b. Descendants of No. 61AT8

Second-generation offspring were produced from three female and ten male first-generation offspring. The three females produced no normal frogs by mating with male *japonica* or by brother and sister mating, although a small number of frogs with abnormal forelegs were obtained from a female. The results of mating experiments by using seven of the ten males have been reported by KAWAMURA and NISHIOKA (1963c). By mating with female *japonica,* six males produced no normal frogs, although a few or a small number of frogs with abnormal forelegs were obtained. Two males produced a few or a small number of normal frogs, corresponding to 5.4 and 12.4% of the eggs used, respectively. By the other two males, 40.2 and 58.3% of the *japonica* eggs developed into normal frogs. On the average, 12.1% of the *japonica* eggs became normal metamorphosed frogs by mating with the ten male first-generation offspring.

Third-generation offspring were produced from two female and six male second-generation offspring. One of the two females produced no normal frogs by mating with male *japonica* or by brother and sister mating, while the other did many, which corresponded to 53.7 or 40.7% of the eggs used. Four of the six males produced no or only a few normal frogs, while the other two produced many normal frogs, corresponding to 38.7 and 44.2% of the eggs used. On the average, 14.7% of the *japonica* eggs developed into normal metamorphosed frogs by mating with the six male second-generation offspring.

Fourth-generation offspring were obtained from ten male third-generation offspring by mating with female *japonica.* Four of the males were derived from a female second-generation offspring mated with a male *japonica*; 26.6 ~ 48.2% of the *japonica* eggs used developed into normal frogs by mating with these males. Four other males were derived from second-generation offspring by brother and sister mating; they produced no or only a few normal frogs. The remaining two males were derived from a male second-generation offspring mated with a female *japonica.* By mating with one of these two males, 90.6% of the *japonica* eggs used, developed into normal frogs, while in the case of the other male, only 6.5% became normal frogs, although there were many frogs with abnormal hind legs. On the average, 48.6% of the *japonica* eggs became normal metamorphosed frogs by mating with the two male third-generation offspring.

Fifth-generation offspring were produced from eight male fourth-generation offspring by mating with female *japonica.* Two of the eight males were derived from a female second-generation offspring mated with a male *japonica* by passing over a male third-generation offspring. One of the two males produced no normal frogs, while the other did a small number of frogs, corresponding to 19.4% of the eggs used. The remaining six males were derived from a male second-generation offspring mated with a female *japonica* by passing over two male third-generation offspring. By mating with these males, 3.3 ~ 73.8%, 32.4% on the average, of the *japonica* eggs developed into normal metamorphosed frogs.

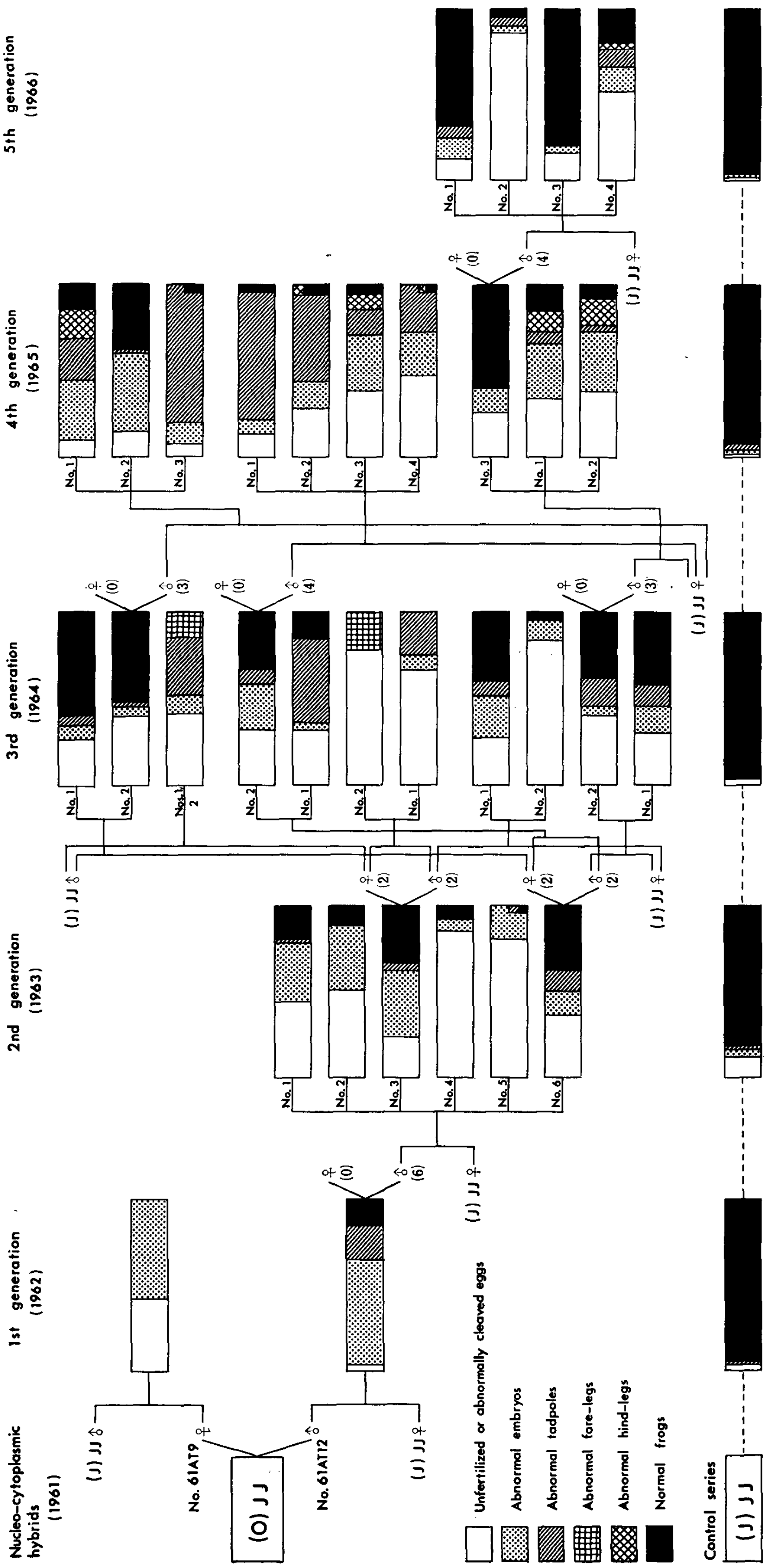

Fig. 16. Diagrammatic representation of the viabilities of the first- to fifth-generation offspring of the male nucleo-cytoplasmic hybrid No. 61AT12.

c. Descendants of No. 61AT12

Second-generation offspring were obtained from six male first-generation offspring by mating with female *japonica*. Three of them produced a few or a small number of normal frogs, while the other three did many, corresponding to 20.4~38.4% of the eggs used, although these percentages were remarkably lower than those in the control matings. The results of mating experiments producing the second-generation offspring have already been reported by KAWAMURA and NISHIOKA (1963c).

Third-generation offspring were produced from four female and four male second-generation offspring. Two of the four females produced no normal frogs by mating with male *japonica* or brother and sister mating, although they produced a small number of frogs with abnormal forelegs. From the other two females numerous normal frogs which corresponded to 51.1 and 60.0% of the eggs used were obtained by mating with male *japonica*, while 14.9 and 33.3% developed into normal frogs by brother and sister mating. Three of the four males produced many normal frogs corresponding to 37.9~42.7% of the eggs used by mating with female *japonica*, while the other one did only a few. On the average, 31.4% of the *japonica* eggs became normal metamorphosed frogs by mating with the four male second-generation offspring.

Fourth-generation offspring were obtained from ten male third-generation offspring by mating with female *japonica*. Three of the ten males were derived from a female second-generation offspring mated with a male *japonica*. One of them produced only a few normal frogs, another, a small number of normal frogs, besides a small number of frogs with abnormal hind legs and the remaining, many normal frogs corresponding to 38.5% of the eggs used. Four other frogs were derived from second-generation offspring by brother and sister mating; they produced a few normal frogs, although there were a few or a small number of frogs with abnormal hind legs in the offspring of three males. The remaining three males were derived from a male second-generation offspring mated with a female *japonica*. One of the three males produced numerous normal frogs, which corresponded to 60.0% of the eggs used, while the other two did a small number of normal frogs and a small number of frogs with abnormal hind legs. On the average, 27.7% of the *japonica* eggs developed into normal metamorphosed frogs by mating with the three male third-generation offspring.

Fifth-generation offspring were obtained from four male fourth-generation offspring by mating with female *japonica*. These males were derived from a male second-generation offspring mated with a female *japonica* by passing over a male third-generation offspring. One of them produced only a few normal frogs and another did a small number, together with a few frogs with abnormal hind legs. The remaining two males produced numerous normal frogs, corresponding to 68.6 and 80.3% of the eggs used. On the average, 43.6% of eggs became normal metamorphosed frogs by mating with the four male fourth-generation offspring.

As found in the figures, the males and females which were used for obtaining

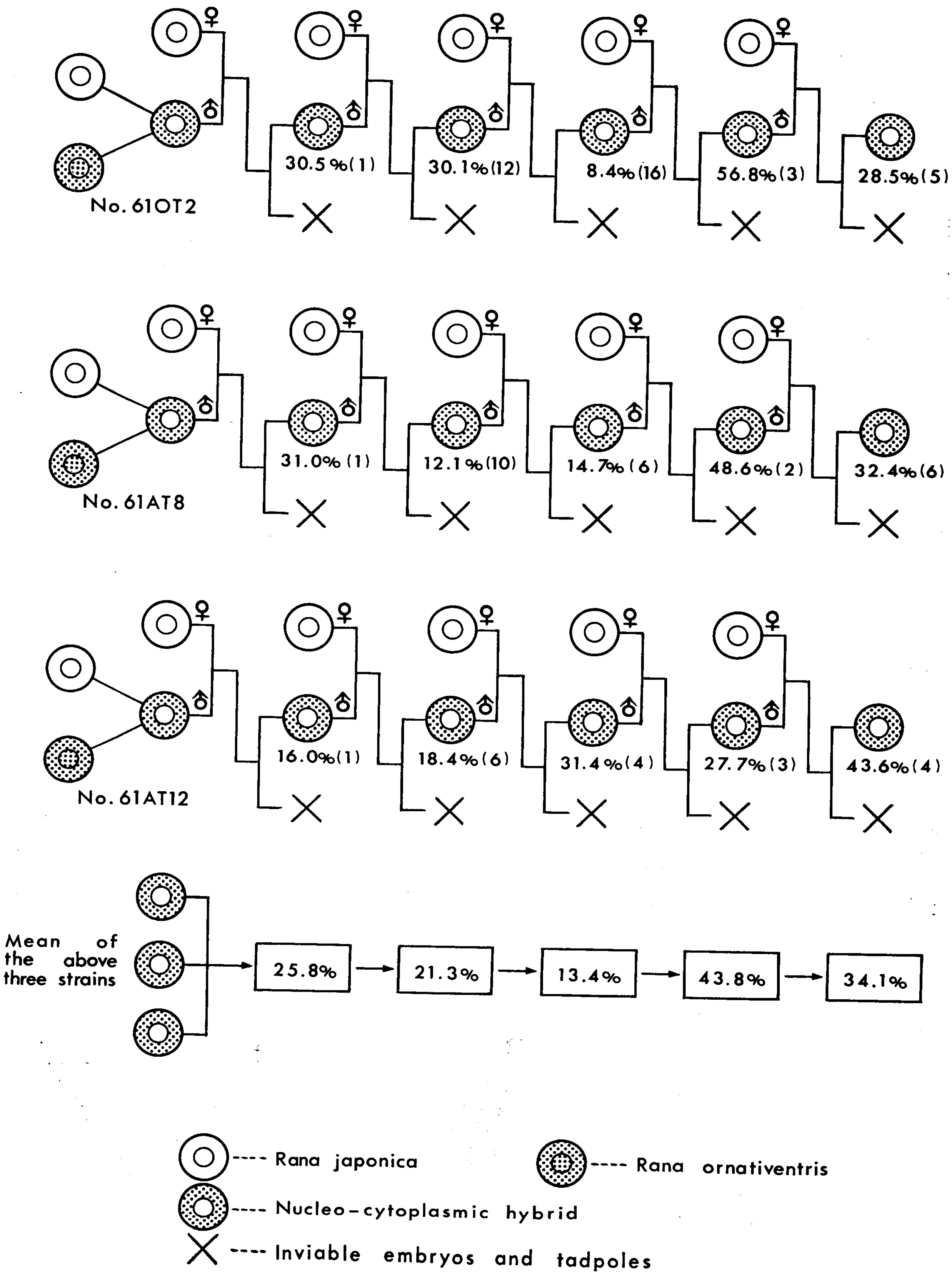

Fig. 17. Percentages of normal metamorphosed frogs in five consecutive generations derived from three male nucleo-cytoplasmic hybrids by repeated matings with females of the nuclear species. Parentheses show the number of male parents. The mean value of the three strains in each generation is the average of the percentages obtained by the individual male parents.

their offspring were selected from the groups in which comparatively numerous frogs were obtained. Such males and females were the most normal in appearance and behavior among their brothers and sisters. In other words, the best frogs were selected from generation to generation for obtaining their offspring. As a matter of course, it was expected that they would be nearly normal in reproductive capacity and their offspring would be almost normal, when they were mated with normal male or female *Rana japonica* at least. However, all the results of the voluminous mating experiments were contrary to the expection. As a whole, the low reproductive capacities of the offspring of the nucleo-cytoplasmic hybrids were not improved with the progress of generations. If the fact that the best males and females of each generation were used for producing their offspring was taken into consideration, the frogs of each generation seemed rather to become lower in reproductive capacity with the progress of generations. It was always recognized that the reproductive capacity of each frog was very labile and that a high capacity was not transmitted to the frogs of the next generation. There seemed to be no distinct differences in reproductive capacity between male and female first- or second-generation offspring of the nucleo-cytoplasmic hybrids. It was, however, noteworthy that there were some male third-generation offspring which were nearly normal in reproductive capacity, although the males of their next generation were mostly of very low reproductive capacity.

In order to follow up the reproductive capacities of the offspring of the three male nucleo-cytoplasmic hybrids from generation to generation, the mean percentages of normal metamorphosed frogs in each generation of the strains kept by passing over male offspring are shown in Fig. 17. It is quite clear that the low reproductive capacities of the male nucleo-cytoplasmic hybrids were not remarkably improved in their offspring during the four consecutive generations at least, although the third- and fourth-generation offspring as a whole seemed slightly better than the first- and second-generation offspring.

Such low reproductive capacities of male and female offspring of the nucleo-cytoplasmic hybrids can not be explained by dominant gene mutations or visible chromosomal aberrations which occurred in germ cells of the nucleo-cytoplasmic hybrids, because these gene mutations or chromosomal aberrations should certainly diminish rapidly with the progress of generations. Accordingly, it is very probable that the low reproductive capacities of male and female offspring were ascribed to chromosomal aberrations which newly occurred in germ cells of the offspring themselves. A similar state of affairs has been found in the offspring of nucleo-cytoplasmic hybrids between two Japanese pond frog species, *Rana nigromaculata* and *Rana brevipoda* by NISHIOKA (1972a).

2. Various abnormalities in the offspring of nucleo-cytoplasmic hybrids

a. External characters

As described in detail, a large majority of the offspring of each generation died after they had revealed various kinds of abnormalities in their external characters. These abnormalities were roughly divided into seven types. The first type was edema at the tail-bud stage. This kind of abnormality appeared in the next generations of male and female offspring derived from the male nucleo-cytoplasmic hybrids. All or most of the embryos produced from each male or female suddenly became edematous and died at the tail-bud stage. For example, in the next generation of a female No. 2 produced from the male nucleo-cytoplasmic hybrid No. 61AT8 by mating with a female *japonica* or in that of a male No. 1 produced from a brother No. 6 of the above female No. 2 by mating with a female *japonica*, all the embryos became edematous and died at the tail-bud stage (Tables 3, 6 and 7).

The second type was ascites at the hatching stage. They were frequently found in various generations derived from all the nucleo-cytoplasmic hybrids. All, most or a part of the embryos which were normally growing revealed severe ascites at the hatching stage and died. For example, in the next generations of the two female nucleo-cytoplasmic hybrids, Nos. 61OT1 and 61AT9, and in that of a female No. 1 obtained from the male nucleo-cytoplasmic hybrid No. 61OT2, all the embryos became ascitic and died at the hatching stage. In some cases a part of the embryos obtained became simultaneously ascitic and died, while the remaining hatched normally and grew into normal feeding tadpoles.

The third type was various kinds of abnormalities appearing during the embryonal stages. This type was frequently found in various generations derived from all the nucleo-cytoplasmic hybrids. A part of the embryos revealed morphological abnormalities and died one after another at various stages later than the gastrula, while the others hatched and grew into normal tadpoles.

The fourth type was under-development in the tadpole stages. The tadpoles of this type were usually defective in the development of teeth. Although they developed normally for a while after hatching, they suddenly stopped eating. They did not grow beyond the stages II$\sim$IV or VII$\sim$X; they became gradually thin and died. However, some tadpoles lived for longer than five months after fertilization. The fourth type of abnormalities was found in various generations derived from all the nucleo-cytoplasmic hybrids.

The fifth type was ascites appearing immediately before metamorphosis. Such ascites were usually found in the next generations of males which originated from the three male nucleo-cytoplasmic hybrids, although they rarely appeared in those of females.

The sixth type was ill-development of forelegs. This type appeared in the next generations of several females and a few males of the first or second generation of the three male nucleo-cytoplasmic hybrids. In these cases, all the frogs produced by each mating usually revealed this type of abnormality. In the mating groups which had contained some tadpoles with a few small white flecks of aragonite crystals in their dorsal skin, all the metamorphosing frogs had ill-developed forelegs which hardly extruded from their body walls. Abnormal frogs with such ill-developed forelegs were also frequently accompanied with the deposition of aragonite crystals in the dorsal skin.

The seventh type was ill-development of hind legs. Frogs or tadpoles with ill-developed hind legs appeared in the next generations of males which were second- or third-generation offspring of the three male nucleo-cytoplasmic hybrids. In these cases, there were always many normal frogs besides anbormal ones in each mating group.

b. Chromosome

Chromosomes were examined in numerous edematous (the first type), ascitic (the second type) and abnormal (the third type) embryos as well as normally shaped tadpoles at the III~VI stages (Table 39). Among 340 normally shaped tadpoles

TABLE 39

Number of embryos or tadpoles with chromosomal aberrations

Kind	Number of individuals									
	Total	With normal cells only	With abnormal cells only							With normal and abnorm. cells
			Pure			Mosaic				
			2n	2n±α	3n or 4n	2n	2n, 2n±α	3n, 3n±α	4n, 4n±α	
Control tadpoles	340	314 (92.4%)				1 (0.3%)				25 (7.4%)
1st type embryos	117		5 (4.3%)	5 (4.3%)		11 (9.4%)	56 (47.9%)	2 (1.7%)		38 (32.5%)
2nd type embryos	365		4 (1.1%)	34 (9.3%)		26 (7.1%)	240 (65.8%)	2 (0.5%)	1 (0.3%)	58 (15.9%)
3rd type embryos	114	1 (0.9%)	6 (5.3%)	5 (4.4%)	4 (3.5%)	16 (14.0%)	33 (28.9%)	5 (4.4%)	2 (1.8%)	42 (36.8%)
Normal tadpoles 2nd gen.	261	93 (35.6%)	14 (5.4%)	1 (0.4%)		41 (15.7%)	15 (5.7%)			97 (37.2%)
3rd gen.	777	315 (40.5%)	39 (5.0%)	9 (1.2%)		148 (19.0%)	43 (5.5%)			223 (28.7%)
4th gen.	723	342 (47.3%)	48 (6.6%)	6 (0.8%)	22 (3.0%)	82 (11.3%)	77 (10.7%)	36 (5.0%)	1 (0.1%)	109 (15.1%)

produced by 15 control matings, there were 314(92.4%) constructed solely of normal diploid cells, while 25(7.4%) consisted of a mixture of normal diploid and abnormal cells and one (0.3%), of more than two kinds of abnormal diploid cells. Among 482 edematous embryos obtained by 22 experimental matings, on the other hand, there were no individuals which were constructed of normal diploid cells only. In this point, there were no differences between the first and the second type of edematous embryos, as well as among the second-, third- and fourth-generation offspring of the nucleo-cytoplasmic hybrids. No distinct differences were also observed among the offspring produced by different processes or from different nucleo-cytoplasmic hybrids. Of 117 edematous tail-bud embryos of second-, third- and fourth-generation offspring produced by five experimental matings, 38(32.5%) consisted of a mixture of normal diploid and abnormal cells and the others did solely of abnormal cells. Among the latter, there were embryos constructed solely of a kind of abnormal diploid, hypo- or hyperdiploid, a mixture of more than two kinds of abnormal diploid, a mixture of abnormal diploid and hypo- or hyperdiploid, or a mixture of triploid and hypo- or hypertriploid cells. Of these embryos, those constructed of a mixture of abnormal dipliod and hypo- or hyperdiploid cells were distinctly abundant, that is, 56(47.9%) in number.

Ascitic embryos at the hatching stage were very similar to the above edematous embryos of the first type in chromosome complements, as presented in Table 39. Of 365 embryos of second-, third- and fourth-generation offspring produced by 18 experimental matings, 58(15.9%) consisted of a mixture of normal diploid and abnormal cells. While all the others were solely constructed of abnormal cells, 240(65.8%) were those of a mixture of abnormal diploid and hypo- or hyperdiploid cells.

Nearly all the abnormally shaped embryos of the third type appearing about the hatching stage had also various kinds of aberrant chromosome complements, which were similar to those of the embryos of the first and second type. Only one (0.9%) of 114 embryos of third- and fourth-generation offspring produced by seven experimental matings consisted of normal diploid cells and 42(36.8%), of a mixture of normal diploid and abnormal cells. The others consisted solely of abnormal cells, such as hypo- or hyperdiploid, triploid or tetraploid, or a mixture of more than two kinds of abnormal diploid, a mixture of abnormal diploid and hypo- or hyperdiploid, a mixture of triploid and hypo- or hypertriploid, or a mixture of tetraploid and hypo- or hypertetraploid cells. Among these embryos, those constructed of a mixture of abnormal diploid and hypo- or hyperdiploid cells were most abundantly found, that is, 33(28.9%) in number.

It was remarkable that the second-, third- and fourth-generation offspring which were externally normal at the III～VI tadpole stages were not similar in chromosome complements to normal tadpoles obtained by control matings. Of 261 second-generation offspring produced from first-generation offspring of two nucleo-cytoplasmic hybrids Nos. 61OT2 and 61AT8 by 12 experimental matings, 93(35.6%) consisted of normal diploid cells and 97(37.2%), of a mixture of normal diploid and abnormal cells. The remaining 71(27.2%) tadpoles were solely constructed of abnormal cells, such as a kind of abnormal diploid, hypodiploid, a mixture of more than two kinds of abnormal diploid, or a mixture of abnormal diploid and hypo- or hyperdiploid cells. In the third generations derived from the three nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12, 315(40.5%) of 777 normally shaped tadpoles obtained by 43 matings consisted of normal diploid cells, 223(28.7%), of a mixture of normal diploid and abnormal cells and 239(30.8%), of abnormal cells only. Among the latter, there were those constructed of a kind of abnormal diploid, hypo- or hyperdiploid, a mixture of more than two kinds of abnormal diploid, or a mixture of abnormal diploid and hypo- or hyperdiploid cells. In the fourth generations derived from the three nucleo-cytoplasmic hybrids, 342(47.3%) of 723 normally shaped tadpoles obtained by 29 matings consisted of normal diploid cells, 109(15.1%), of a mixture of normal diploid and abnormal cells and 272(37.6%), of abnormal cells. Among the latter tadpoles there were those constructed of a kind of abnormal diploid, hypo- or hyperdiploid, or triploid or tetraploid, a mixture of more than two kinds of abnormal diploid, a mixture of normal diploid and hypo- or hyperdiploid, a mixture of abnormal triploid and hypo- or hypertriploid, or a mixture of abnormal tetraploid and hypo- or hypertetraploid cells.

When the rates of normally shaped tadpoles consisting of normal diploid cells were compared with one another among the second-, third- and fourth-generation offspring, they seemed to increase slightly with the progress of generations. In total, 750(42.6%) of 1761 tadpoles consisted of only normal diploid cells, 429 (24.4%), of a mixture of normal diploid and abnormal cells and 582(33.0%), of only abnormal cells. The fact that the majority of the normally shaped tadpoles at the III～VI stages consisted of only abnormal cells or a mixture of normal diploid and abnormal cells seemed to correspond to the existence of a considerable number of those which became abnormal immediately before or during their metamorphosis.

c. Sex

Concerning the sex of second-, third- and fourth-generation offspring, it was especially remarkable that 96.4, 94.7 and 85.9% of matured frogs were males, respectively, although there were no large differencies in number between males and females at the stage shortly after metamorphosis, as presented in Table 40.

TABLE 40
Total numbers of males and females in each of three generations derived from nucleo-cytoplasmic hybrids

Series	Generation of offspring	Young frogs shortly after metamorphosis				Matured frogs		
		Total	♀	⚥	♂	Total	♀	♂
Cont.	Second	464	232 (50.0%)	2 (0.4%)	230 (49.6%)	18	10 (55.6%)	8 (44.4%)
	Third	241	122 (50.6%)	2 (0.8%)	117 (48.5%)	57	27 (47.4%)	30 (52.6%)
	Fourth	302	145 (48.0%)	0	157 (52.0%)	20	9 (45.0%)	11 (55.0%)
		1007	499 (49.6%)	4 (0.4%)	504 (50.0%)	95	46 (48.4%)	49 (51.6%)
Exp.	Second	295	126 (42.7%)	28 (9.5%)	141 (47.8%)	56	2 (3.6%)	54 (96.4%)
	Third	892	437 (49.0%)	84 (9.4%)	371 (41.6%)	114	6 (5.3%)	108 (94.7%)
	Fourth	2334	956 (41.0%)	133 (5.7%)	1245 (53.3%)	128	18 (14.1%)	110 (85.9%)
		3521	1519 (43.1%)	245 (7.0%)	1757 (49.9%)	298	26 (8.7%)	272 (91.3%)

Among the control frogs, there were always nearly equal number of males and females at the stage of sexual maturity as well as shortly after metamorphosis. An overwhelming majority of males among matured frogs was always observed among the offspring of each generation originated from each nucleo-cytoplasmic hybrid. It has been reported by KAWAMURA and NISHIOKA (1963c) that the first-generation offspring of the male nucleo-cytoplasmic hybrid No. 61AT12 were all males, and that among those of No. 61OT2, there was a preponderance of males above females in number. As already stated in this paper, it seems certain that many of the matured second-, third- and fourth-generation offspring were sex-reversed genetic females, because at the time shortly after metamorphosis there was no large unbalance in sex ratio and moreover, there were comparatively numerous hermaphrodites, differing from the control young frogs. During the period from the completion of metamorphosis to sexual maturity, there was usually only a small mortality in each generation.

It has been reported that *Rana* frequently give rise to sex-reversal from a female to a male by various causes, such as overripeness of eggs (KUSCHAKEWITSCH, 1910; HERTWIG, R. 1921; WITSCHI, 1924), rearing of tadpoles under high temperatures (WITSCHI, 1929), and an effect of androgens (WITSCHI, 1948; KAWAMURA and YOKOTA, 1959). Interspecific hybrids of brown frogs always become sterile males (DÜRKEN, 1935, '38; KAWAMURA, 1943, '50; KAWAMURA and KOBAYASHI, 1959, '60). Excepting the case of androgens, the sex-reversal was always accompanied with some abnormalities in anatomical, cytological or physiological characters. Polyploid frogs also became males in *Rana japonica*, although they were not always perfectly sterile. All the young triploid frogs obtained from cold-treated (KAWAMURA and TOKUNAGA, 1952) or heat-shocked fertilized eggs (KAWAMURA, NISHIOKA and MYOREI, 1963; KAWAMURA and NISHIOKA, 1967b) were males. While there were a few females among young tetraploid frogs of six consecutive generations derived from tetraploid males by mating with diploid females, all the matured tetraploids were males, which were fertile to some extent. It was very probable that sex-reversal of genetic females into functional males always occurred under physiological effects of the tetraploidy (KAWAMURA and NISHIOKA, 1967b). Sex-reversal often occurred in frogs developed parthenogenetically (PARMENTER, 1925; KAWAMURA, 1939, '49; MORIWAKI, 1957, '60, '63). MORIWAKI (1957) has reported that there were nine females and seven males among 16 matured parthenogenetic frogs of *Rana japonica*. In the F_2 and F_3 generations of these parthenogenetic frogs, many males appeared, in spite of their XX constitution (MORIWAKI, 1960, '63). Especially, the males were more or less numerous in the F_2 and F_3 generations, in which there were numerous tadpoles with anatomical, histological and chromosomal abnormalities.

d. Causes for various abnormalities

In the present research, the sex-reversal found in the offspring of nucleo-cytoplasmic hybrids were always accompanied with the appearance of numerous embryos and tadpoles which were more or less abnormal in anatomical and cytological characters as well as viability. There is no doubt about that the causes for such sex-reversal, anatomical abnormalities and chromosomal aberrations should be sought in the chromosomes of gametes produced by their parents, even if there is any causation among the three matters themselves. In fact, there were many males whose testes were abnormal in inner structure among the parents used for producing their next-generation offspring by experimental matings, as presented in Tables 2, 5, 17, 18, 33 and 34.

In these tables, the testes of the experimental and the control males were divided into five types, in accordance to the amount of normal spermatozoa in their seminal tubules, from those filled with bundles of numerous normal spermatozoa to those containing no normal spermatozoa. The number of males with testes of each type in each generation is presented in Table 41. While all the control males had testes of Type 1, the male parents of each generation derived from nucleo-cytoplasmic hybrids had mostly abnormal testes: 32 of 92 were of Type 1 and the others were of Types 2～5. Five males had no normal spermatozoa and 12 others had only a few. Such a state of affairs indicates the occurrence of abnormal spermatogeneses in their testes and seems somewhat similar to that of matured interspecific hybrids of brown frogs, although they are not always quite sterile. It is believed that the same kind of abnormalities occurred in the oogeneses of female parents, too, since the latter were nearly the same in reproductive capacity as the male parents.

TABLE 41

Type of the gonads and the number of males used as parents for experimental and the control matings in each generation derived from nucleo-cytoplasmic hybrids

Generation of male parents	Type of testes					
	1	2	3	4	5	Total
Experimental						
1st	2	3	1	0	0	6
2nd	8	4	8	9	4	33
3rd	17	2	9	3	1	32
4th	5	7	9	0	0	21
	32	16	27	12	5	92
Control						
1st	6	0	0	0	0	6
2nd	4	0	0	0	0	4
3rd	5	0	0	0	0	5
4th	9	0	0	0	0	9
	24	0	0	0	0	24

It seems quite clear that the cause for these abnormal gametogeneses is situated in the chromosome complements of the parents. In the strains which produced five generations from the three male nucleo-cytoplasmic hybrids by repeated matings with female *japonica*, such matured male offspring as quite normal in appearance at least were selected and used as parents for producing the next-generation offspring. Although the chromosome complements of the male parents were not analysed in detail, they were rightly diploid. The matter that these diploid males revealed abnormal spermatogeneses in their testes seems to indicate that the haploid set of chromosomes contributed by the sperm of their male parent had some latent or inactive damages which became active and made the spermatogenesis abnormal by unusual pairing with the other normal haploid set of chromosomes derived from their female parent. Owing to the unusual pairing, various abnormal behaviors of chromosomes, such as non-disjunction, fragmentation, translocation etc. seem to occur at the meiotic divisions. They should be the main cause for the abnormal inner structures of the testes of male parents as well as the poor reproductive capacities of the latter.

The existence of many embryos and tadpoles constructed of a kind of hypo- or hyperdiploid or abnormal diploid cells in the next generations of these male parents mated with normal diploid females seems to be attributable to the production of various kinds of spermatozoa which are functional in spite of abnormal constitution of chromosomes (Tables 10, 12, 25 and 27). However, the existence of numerous embryos and tadpoles consisting of a mixture of normal diploid and abnormal cells, a mixture of more than two kinds of abnormal diploid, or a mixture of abnormal diploid and hypo- or hyperdiploid cells, seems to indicate that more than one kind of abnormal cells are newly produced after fertilization during the development of these individuals. Moreover, the existence of numerous individuals constructed of a mixture of normal diploid and abnormal cells seems to show that the spermatozoa of their male parent had a normal haploid set of chromosomes, and some abnormalities occurred in a cell or cells some time after fertilization. The same assumption can be expressed about the next generations of female parents derived from nucleo-cytoplasmic hybrids (Tables 10 and 12). Quite the same has also been made about the offspring of nucleo-cytoplasmic hybrids between *Rana nigromaculata* and *R. brevipoda* (NISHIOKA, 1971a, b, '72a, b).

It is very probable that the chromosomes of nucleo-cytoplasmic hybrids had a lot of latent or inactive damages which had been produced by abnormal interaction with the foreign cytoplasm, as stated in the above paper by NISHIOKA (1971a, b, '72a). A part of such latent damages in the chromosomes may become active and give rise to various chromosomal aberrations at the time of meioses of germ cells as well as mitoses of somatic cells. The latent damages seem to be transmitted from generation to generation until no traces of the original chromosomes with such latent damages are contained in sperm or eggs produced by the offspring of some later generation. However, the state of affairs that there was no remarkable improvement in the development, viability and sex-differentiation of the offspring from the first to the fifth generation, in spite of repeated matings with normal diploid females, seems to make difficult the explanation that the abnormalities are brought about by a part of the original chromosomes which are reduced by half per generation. Accordingly, it seems reasonable to presume that some similar latent damages are newly induced in the homologous chromosomes probably at the time of synapsis from pre-existing latent damages. Nothing can be stated at present as to the nature of latent damages of chromosomes and how the induction of new latent damages occurs in homologous chromosomes. However, it is probable that the latent damages are intimately related to DNA double helices, as DNA is considered the permanently conserved molecule in the chromosome.

It is an interesting problem to pursue the reproductive capacities of the offspring of the three male nucleo-cytoplasmic hybrids further than the fifth generation in oder to clarify how many generations are required for perfect improvement of their poor capacities. Although a sudden improvement seemed to occur in the eighth or ninth generation, it is premature to say positively for the present. The fate of the strains derived from the nucleo-cytoplasmic hybrids after the sixth generation will later be reported in another paper by the present writers.

SUMMARY

1. A large number of offspring of four consecutive generations from the second to the fifth, were obtained from first-generation offspring of three male nucleo-cytoplasmic hybrids constructed of *Rana ornativentris* cytoplasm and *Rana japonica* nuclei.

2. All the male and female parents used for producing their next generations were examined in terms of the structures of testes as well as the size of eggs. The testes of the males were divided into the following five types: Type 1, seminal tubules being filled with bundles of numerous normal spermatozoa, Type 2, spermatozoa in each seminal tubule being far fewer than those of Type 1, Type 3, seminal tubules being mostly filled with abnormal spermatozoa and pycnotic nuclei, besides a few small bundles of normal spermatozoa, Type 4, seminal tubules being filled with abnormal spermatozoa and pycnotic nuclei, besides a few sparsely distributed normal spermatozoa and Type 5, no normal spermatozoa being found in seminal tubules. Among 92 male first-, second-, third- and fourth-generation offspring of the three male nucleo-cytoplasmic hybrids, there were 32 males with Type 1 testes, 16 with Type 2, 27 with Type 3, 12 with Type 4 and 5 with Type 5. All the 24 control males had testes of Type 1. The eggs of 18 female first- and second-generation offspring of the three male nucleo-cytoplasmic hybrids were nearly the same in size as those of the control females.

All these male and female first-, second-, third- and fourth-generation offspring of the nucleo-cytoplasmic hybrids were remarkable inferior to the control frogs in reproductive capacity.

3. When the reproductive capacities of male offspring derived from the three male nucleo-cytoplasmic hybrids by repeated matings with females of the nuclear species, *Rana japonica*, were followed up from generation to generation by comparing the mean percentages of normal, metamorphosed frogs in the next generations with one another, it was quite clear that the low reproductive capacities of the male nucleo-cytoplasmic hybrids were not remarkably improved with the progress of generations of offspring, although the third- and fourth-generation offspring as a whole seemed slightly better than the first- and second-generation offspring.

4. The individuals of the four generations, from the second to the fifth, mostly revealed various kinds of severe abnormalities at the embryonal or tadpole

stages. These abnormal and inviable individuals were roughly divided into the following seven kinds: (1) Embryos becoming edematous at the tail-bud stage, (2) embryos becoming ascitic at the hatching stage, (3) abnormally shaped embryos at the stages from the gastrula to the hatching, (4) ill-developed tadpoles, (5) tadpoles becoming ascitic before metamorphosis, (6) metamorphosed frogs with ill-developed forelegs, (7) metamorphosed frogs with ill-developed hind legs. All or a part of the individual produced from a male or female offspring often revealed one of the seven kinds of abnormalities.

5. The chromosome complements of abnormal embryos of the former three kinds as well as normal tadpoles at the II～VI stages were examined. There were no individuals constructed merely of normal diploid cells among 596 abnormal embryos of three generations, from the second to the fourth, derived from the three male nucleo-cytoplasmic hybrids; 38(32.5%) of 117 edematous embryos at the tail-bud stage, 58(15.9%) of 365 ascitic embryos at the hatching stage and 42(36.8%) of 114 abnormally shaped embryos consisted of a mixture of normal diploid and abnormal cells. The remaining embryos were constructed of a kind of abnormal diploid, hypo- or hyperdiploid, triploid or tetraploid, a mixture of more than two kinds of abnormal diploid, a mixture of abnormal diploid and hypo- or hyperdiploid, a mixture of triploid and hypo- or hypertriploid, or a mixture of tetraploid and hypo- or hypertetraploid cells. Among the embryos with abnormal cells alone, those constructed of a mixture of abnormal diploid and hypo- or hyperdiploid cells were overwhelmingly numerous.

6. Of 340 control tadpoles at the II～VI stages, 314(92.4%) consisted purely of normal diploid cells, while 25(7.4%) consisted of a mixture of normal diploid and abnormal cells and one, of a mixture of more than two kinds of abnormal diploid cells. Differing from these control tadpoles, normally shaped tadpoles at the II～VI stages in three consecutive generations, from the second to the fourth, were mostly abnormal in their chromosome complements. Of 261 second-generation offspring, 97(37.2%) consisted of a mixture of normal diploid and abnormal cells and 71(27.2%), solely of abnormal cells, while the other 93 (35.6%) consisted purely of normal diploid cells. Among 777 third-generation offspring, there were 223(28.7%) constructed of a mixture of normal diploid and abnormal cells, and 239(30.8%), of abnormal cells, besides 315(40.5%), of normal diploid cells. Among 723 fourth-generation offspring, there were 109(15.1%) constructed of a mixture of normal diploid and abnormal cells and 272(37.6%), of abnormal cells, besides 342(47.3%), of normal diploid cells. Roughly speaking, the normal tadpoles constructed purely of normal diploid cells seemed to increase slightly in percentage with the progress of generations from the second to the fourth, in contrast with that the normally shaped tadpoles constructed of a mixture of normal diploid and abnormal cells gradually decreased.

7. The sex of second-, third- and fourth-generation offspring was examined shortly after metamorphosis and at the stage of sexual maturity. While there were 499(49.6%) females, four (0.4%) hermaphrodites and 504(50.0%) males among 1007 young control frogs shortly after metamorphosis, there were 1519

(43.1%) females, 245(7.0%) hermaphrodites and 1757(49.9%) males among 3521 second-, third- and fourth-generation offspring.

At the stage of sexual maturity, there were 26(8.7%) females and 272(91.3%) males among 298 second-, third- and fourth-generation offspring, while there were 46(48.4%) females and 49(51.6%) males among 95 control frogs. It was quite clear that sex-reversal occurred in most of the genetical females of each generation.

8. In order to explain the constant appearance of developmental abnormalities, chromosomal aberrations and sex-reversal in the offspring of the three male nucleo-cytoplasmic hybrids from generation to generation, in spite of repeated matings with the nuclear species, the existence of latent damages in the chromosomes of the offspring and the induction of such damages to homologous chromosomes during the time of synapsis in germ cells were presumed.

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LITERATURE

BRIGGS, R. and T. J. KING 1952. Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. Proc. Nat. Acad. Sci. **38**: 455–463.

——— 1953. Factors affecting the transplantability of nuclei of frog embryonic cells. J. Exp. Zool. **122**: 485–506.

DÜRKEN, B. 1935. Über Artbastarde *Rana arvalis* NILS. ♀ × *Rana fusca* Rös. ♂. Z. f. ind. Abst.- u. Vererbgl. **68**: 486–516.

——— 1938. Über die Keindrüsen und die Chromosomen der Artbastarde *Rana arvalis* NILS. ♀ × *Rana fusca* Rös. ♂. Z. f. ind. Abst.- u. Vererbgl. **74**: 331–353.

GALLEIN, C. L. 1969. Développement et caractères des hybrides expérimentaux entre deux espèces d'amphibiens urodèles: *Pleurodeles waltlii* MICHAH. et *Pleurodeles poireti* GERVAIS. C. R. Acad. Sc. Paris **268**: 2126–2129.

——— 1970. Recherches sur la greffe nucléaire interspécifique dans le Genre *Pleurodeles* (Amphibiens-Urodèles). Ann. Embr. Morph. **3**: 145–192.

GURDON, J. B. 1961. The transplantation of nuclei between two subspecies of *Xenopus laevis*. Heredity **16**: 305–315.

——— 1962. The transplantation of nuclei between two species of *Xenopus*. Devel. Biol. **5**: 68–83.

HENNEN, S. 1963. Chromosomal and embryological analyses of nuclear changes occurring in embryos derived from transfers of nuclei between *Rana pipiens* and *Rana sylvatica*. Devel. Biol. **6**: 133–183.

——— 1964. The transfer of diploid nuclei from *Rana pipiens* blastulae into non-enucleated eggs of *Rana palustris*. Amer. Zoologist **4**: 288.

——— 1965. Nucleocytoplasmic hybrids between *Rana pipiens* and *Rana palustris*. I. Analysis of the developmental properties of the nuclei by means of nuclear transplantation. Devel. Biol. **11**: 243–367.

——— 1967. Nuclear transplantation studies of nucleo-cytoplasmic interactions in amphibian hybrids. "The Control of Nuclear Activity" ed. by L. GOLDSTEIN. Prentice-Hall, Englewood Cliffs, New Jersey.

HERTWIG, R. 1921. Über den Einfluss der Überreife der Eier auf das Geschlechtsverhältnis von Fröschen und Schmetterlingen. Sitzungsber. bagr. Akad. Wiss. 1921, 263–294.

KAWAMURA, T. 1939. Artificial parthenogenesis in the frog. II. The sex of parthenogenetic frogs. Jour. Sci. Hiroshima Univ., Ser. B, Div. 1, **7**: 39–86.

——— 1943. Studies on hybridization in amphibians. I. The species hybrid of *Rana japonica* GÜNTHER ♀ × *Rana temporaria* L. ♂. (In Japanese with English résumé) Zool. Mag. (Tokyo) **55**: 315–330.

——— 1949. Further observations on diploid and triploid parthenogenetic frogs of *Rana nigromaculata*. Jour. Sci. Hiroshima Univ., Ser. B, Div. 1, **11**: 1–5.

——— 1950. Studies on hybridization in amphibians. II. Interspecific hybrids in red-colored frogs. Ibid. **11**: 61–70.

KAWAMURA, T. and M. KOBAYASHI 1959. Studies on hybridization in amphibians. VI. Reciprocal hybrids between *Rana temporaria temporaria* L. and *Rana temporaria ornativentris* WERNER. Ibid. **18**: 1–15.

——— 1960. Studies on hybridization in amphibians. VII. Hybrids between Japanese and European brown frogs. Ibid. **18**: 221–238.

KAWAMURA, T. and M. NISHIOKA 1960. Amphidiploid frogs produced by artificial means. Jour. Sci. Hiroshima Univ., Ser. B, Div. 1, **18**: 195–220.

——— 1963a. Reproductive capacity of an amphidiploid male produced by nuclear transplantation in amphibians. Ibid. **21**: 1–13.

——— 1963b. Reciprocal diploid nucleo-cytoplasmic hybrids between two species of Japanese pond frogs and their offspring. Ibid. **21**: 65–84.

——— 1963c. Nucleo-cytoplasmic hybrid frogs between two species of Japanese brown frogs and their offspring. Ibid. **21**: 107–134.

——— 1964a. Viability of the offspring of nucleo-cytoplasmic hybrids. (In Japanese) Japan. Jour. Genetics **39**: 352.

——— 1964b. Chromosomal aberrations in the offspring of nucleo-cytoplasmic hybrids. (In Japanese) Ibid. **39**: 352.

——— 1965. Abnormalities in the offspring of nucleo-cytoplasmic hybrids. (In Japanese) Zool. Mag. (Tokyo) **74**: 368.

——— 1967a. Chromosomal aberrations found in the offspring of nucleo-cytoplasmic hybrids. (In Japanese) Japan. Jour. Genetics **42**: 420.

——— 1967b. On the sex and reproductive capacity of tetraploids in amphibians. Gunma Symposia on Endocrinology **4**: 23–39.

KAWAMURA, T., M. NISHIOKA, and Y. MYOREI 1963. Reproductive capacity of autotetraploid males in brown frogs. Jour. Sci. Hiroshima Univ., Ser. B, Div. 1, **21**: 15–24.

KAWAMURA, T. and C. TOKUNAGA 1952. The sex of triploid frogs, *Rana japonica* GÜNTHER. Ibid. **13**: 121–128.

KAWAMURA, T. and R. YOKOTA 1959. The offspring of sex-reversed females of *Rana japonica* GÜNTHER. Ibid. **18**: 31–38.

KUSCHAKEWITSCH, S. 1910. Die Entwicklungsgeschichte der Keimdrüse von *Rana esculenta*. Festschrift für HERTWIG **2**: 61–224.

MAKINO, S. and I. NISHIMURA 1952. Water-pretreatment squash technic. A new and simple practical method for the chromosome study of animals. Stain Technology **27**: 1–7.

MCKINNELL, R. G. 1960. Transplantation of *Rana pipiens* (kandiyohi dominant mutant) nuclei to *R. pipiens* cytoplasm. Amer. Nat. **94**: 187–188.

——— 1962. Intraspecific nuclear transplantation in frogs. J. Heredity **53**: 199–207.

MOORE, J. A. 1958. Transplantation of nuclei between *Rana pipiens* and *Rana sylvatica*. Exp. Cell Res. **14**: 532–540.

——— 1960. Serial back-transfers of nuclei in experiments involving two species of frogs. Devel. Biol. **2**: 535–550.

MORIWAKI, T. 1957. Studies on matured parthenogenetic frogs. I. The development and reproductive ability. Jour. Sci. Hiroshima Univ., Ser. B, Div. 1, **17**: 13–32.

——— 1960. Studies on matured parthenogenetic frogs. IV. On second-generation offspring of parthenogenetic frogs. Ibid. **18**: 183–193.

——— 1963. Studies on matured parthenogenetic frogs. V. On the F_3 offspring of parthenogenetic frogs. Bull. Suzugamine Women's Coll., Nat. Sci. **10**: 11–24.

NISHIOKA, M. 1971a. Nucleo-cytoplasmic hybrids in amphibians. (In Japanese) The Heredity (Japan) **25**: 18–24.

——— 1971b. Abnormal combinations of the nucleus and cytoplasm and their effects in amphibians. (In Japanese with English résumé). Symposia Cell. Biol. **22**: 189–203.

——— 1972a. Abnormalities of the offspring of nucleo-cytoplasmic hybrids between *Rana nigromaculata* and *Rana brevipoda*. Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. **1**: 1–94.

——— 1972b. Nucleo-cytoplasmic hybrids between *Rana japonica* and *Rana temporaria temporaria*. Ibid. **1**: 211–243.

——— 1972c. Reciprocal nucleo-cytoplasmic hybrids between *Rana esculenta* and *Rana brevipoda*. Ibid. **1**: 245–257.

——— 1972d. Nucleo-cytoplasmic hybrids between *Rana brevipoda* and *Rana plancyi chosenica*. Ibid. **1**: 259–275.

ORTOLANI, G., M. FISCHBERG, et S. SLATKINE 1966. Nuclear transplantation between two subspecies of *Xenopus laevis* (*Xenopus laevis laevis* and *Xenopus laevis petersi*). Acta Emb. Morph. Exp. **9**: 187–202.

PARMENTER, C. L. 1925. The chromosomes of parthenogenetic frogs and tadpoles. J. Gen. Physiol. **8**: 1–20.

SAMBUICHI, H. 1957. The roles of the nucleus and the cytoplasm in development. I. An intersubspecific hybrid frog, developed from a combination of *Rana nigromaculata nigromaculata* cytoplasm and a diploid nucleus of *Rana nigromaculata brevipoda*. Jour. Sci. Hiroshima Univ., Ser. B, Div. 1, **17**: 33–41.

——— 1961. The roles of the nucleus and the cytoplasm in development. III. Diploid nucleo-cytoplasmic hybrids, derived from *Rana nigromaculata brevipoda* cytoplasm and *Rana nigromaculata nigromaculata* nuclei. Ibid. **20**: 1–15.

SHUMWAY, W. 1940. Stages in the normal development of *Rana pipiens*. I. External form. Anat. Rec. **78**: 139–147.

TAYLOR, A. C. and J. J. KOLLROS 1946. Stages in the normal development of *Rana pipiens* larvae. Anat. Rec. **94**: 7–24.

WITSCHI, E. 1924. Die Beweise für die Umwandlung weiblicher Jungfrösche in männliche nach uteriner Überreife der Eier. Arch. f. Entw.-Mech. **102**: 168–183.

——— 1929. Studies on sex differentiation and sex determination in amphibians. II. Sex reversal in female tadpoles of *R. sylvatica* following the application of high temperature. J. Exp. Zool. **52**: 267–291.

——— 1948. Geschlechtsbestimmung durch Chemikalien. Arch. d. Julius KLAUS-Stiftung f. Vererbf., Sozialanthrop. u. Rassenhyg. **23**: 525–538.

EXPLANATION OF PLATES

PLATE I

Cross-sections of the gonads of young frogs killed two weeks after metamorphosis. According to such inner structures of gonads as shown in the eight figures of Plates I and II, the sex of young frogs was divided into seven categories. ×300

1. Normal ovary of a female (♀$_N$) among the fifth-generation offspring produced by mating, J.W66♀ Nos. 5∼6×J〔J[J{J(61AT12)}$_6$]$_2$〕$_3$ ♂ No. 1.
2. Under-developed ovary of a female (♀$_U$) among the fifth-generation offspring produced by mating, J.W66♀ Nos. 5∼6×J〔J[J{J(61AT12)}$_6$]$_2$〕$_3$♂ No. 1.
3. Gonad of a hermaphrodite (⚥$_1$) at the beginning of sex-reversal among the fifth-generation offspring produced by mating, J.W66♀ Nos. 5∼6× J〔J[J{J(61AT12)}$_6$]$_2$〕$_3$♂ No. 1.
4. Gonad of a hermaphrodite (⚥$_1$) at the beginning of sex-reversal among the third-generation offspring produced by mating, J.L65♀ Nos. 12∼13× {J(61OT2)}$_2$J♂ No. 1.

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PLATE II

Cross-sections of the gonads of young frogs killed two weeks after metamorphosis. ×300

5. Gonad of a hermaphrodite (⚥$_2$) at the middle stage of sex-reversal among the third-generation offspring produced by mating, J.L65♀ Nos. 12～13× $\{J(61OT2)\}_2J$ ♂ No. 1.
6. Gonad of a hermaphrodite (⚥$_3$) at the last stage of sex-reversal among the third-generation offspring produced by mating, J.L65♀ Nos. 12～13× $\{J(61OT2)\}_2J$♂ No. 1.
7. Normal testis of a male (♂$_N$) among the third-generation offspring produced by mating, J.L65♀ Nos. 12～13× $\{J(61OT2)\}_2J$♂ No. 1.
8. Rudimentary testis of a male (♂$_R$) among the third-generation offspring produced by mating, J.L65♀ Nos. 12～13× $\{J(61OT2)\}_2J$♂ No. 1.

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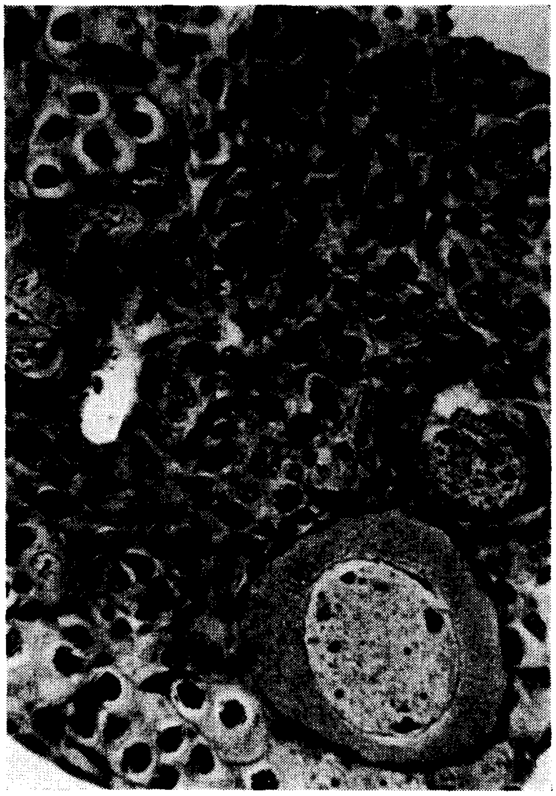

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PLATE III

Cross-sections of the testes of matured male second-, third- and fourth-generation offspring. All these males were one year old. On the basis of such an amount of spermatozoa in the cross-sections of seminal tubules as found in Plates III and IV, the testes of matured males were divided into five types. ×300

9. Type 1 testis of a male second-generation offspring, $J\{J(61AT8)\}_4$ No. 2.
10. Type 2 testis of a male second-generation offspring, $J\{J(61OT2)\}_1$ No. 3.
11. Type 3 testis of a male third-generation offspring, $[J\{J(61AT12)\}_6]_2J$ No. 3.
12. Type 3 testis of a male fourth-generation offspring, $J〔J[J\{J(61AT12)\}_6]_2〕_3$ No. 2.

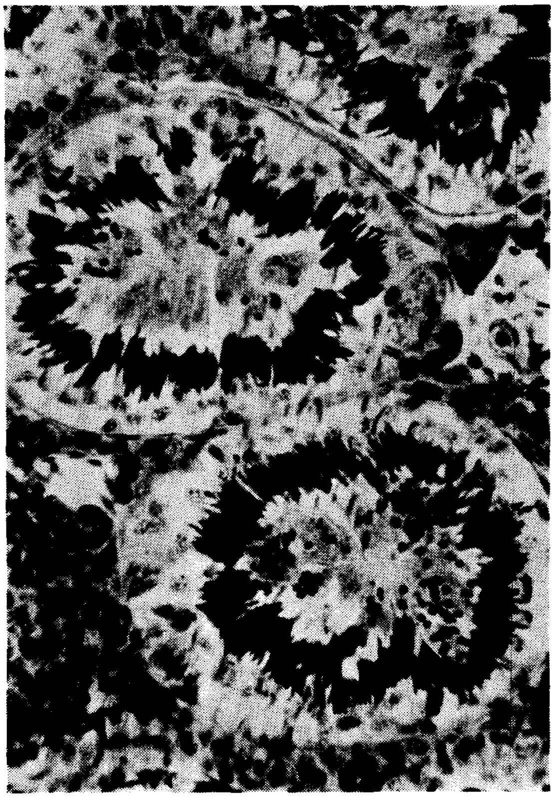

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PLATE IV

Cross-sections of the testes of matured male second- and third-generation offspring. All these males were one year old. $\times 300$

13. Type 4 testis of a male second-generation offspring, J{J(61AT8)}$_4$ No. 1.
14. Type 4 testis of a male third-generation offspring, [J{J(61AT12)}$_6$]$_2$[J{J(61AT12)}$_6$]$_2$ No. 4.
15. Type 5 testis of a male second-generation offspring, J{J(61OT2)}$_4$ No. 3.
16. Type 5 testis of a male third-generation offspring, J[J{J(61OT2)}$_1$]$_5$ No. 3.

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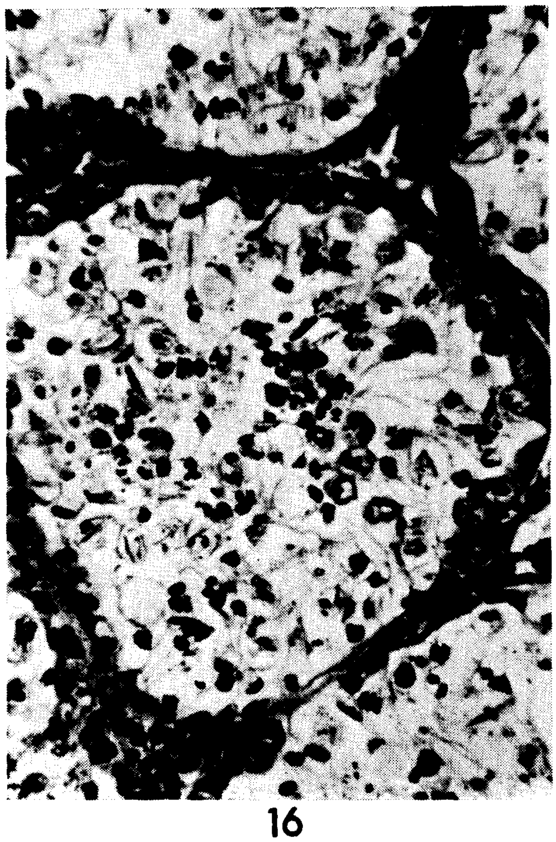

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