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Reciprocal Nucleo-cytoplasmic Hybrids between *Rana esculenta* and *Rana brevipoda*

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(With 2 Text-figures and 1 Plate)

INTRODUCTION

Up to the present, diploid nucleo-cytoplasmic hybrids have been produced by several authors. The first one was a metamorphosing frog obtained by SAMBUICHI (1957) by the use of two Japanese pond frog species, that is, from an enucleated *Rana nigromaculata* egg transplanted with a blastula nucleus of *Rana brevipoda*. This frog was very similar to the nuclear species in color pattern. The single nucleo-cytoplasmic hybrid obtained by him afterwards (1961) from an egg of the reciprocal combination resembled the cytoplasmic species in some points, although it was very similar to the nuclear species. In this respect, reciprocal nucleo-cytoplasmic hybrids between *Rana nigromaculata* and *Rana brevipoda* produced by KAWAMURA and NISHIOKA (1963b) were the same as that by SAMBUICHI (1961), that is, some of them were similar to the cytoplasmic species or intermediate between the two species in some characters, although they were alike to the nuclear species in the main. In the nucleo-cytoplasmic hybrids between Japanese and Korean pond frog species, *Rana brevipoda* and *Rana plancyi chosenica*, all the metamorphosed frogs were quite similar to the nuclear species in external characters, except for the rise of the dorso-lateral folds (NISHIOKA, 1972c).

Concerning the reproductive ability, both male and female nucleo-cytoplasmic hybrids between *Rana nigromaculata* and *Rana brevipoda* were more or less fertile (KAWAMURA and NISHIOKA, 1963b). The males and females of nucleo-cytoplasmic hybrids between *Rana brevipoda* and *Rana plancyi chosenica* seemed to be partially fertile, too. As the males of reciprocal hybrids produced by crossing *Rana nigromaculata* and *Rana brevipoda* are nearly completely sterile and both males and females of reciprocal hybrids of *Rana brevipoda* and *Rana plancyi chosenica* are also sterile with a few exceptions, the nucleo-cytoplasmic hybrids seem to be somewhat superior to the ordinary hybrids in reproductive ability, although they are not good by any means.

It seems interesting to produce nucleo-cytoplasmic hybrids by the use of Japanese and European pond frog species, to examine their morphological characters and reproductive ability and to compare the results with those obtained from other nucleo-cytoplasmic hybrids. These two species are isolated from each other partially by hybrid inviability and perfectly by hybrid sterility.

MATERIAL AND METHODS

As the material for this research one male and two females were used for each of the two species. The Japanese species, *Rana brevipoda,* were collected from the suburbs of Okayama City, while the European species, *Rana esculenta,* came from Luxemburg. Eggs were obtained from females whose ovulation had been accelerated by transplantation of frog pituitaries. The removal of nuclei from unfertilized eggs was performed by PORTER's method (1939) and the transfer of blastula nuclei to enucleated eggs was done by BRIGGS and KING's method (1952, 1953). Reciprocal nucleo-cytoplasmic hybrids were produced from these two species. As a part of the control series reciprocal hybrids between *Rana brevipoda* and *R. esculenta* were produced.

The ploidy of tadpoles was determined by the tail-tip method, as described by KAWAMURA and NISHIOKA(1960). Chromosomes were counted in four or five good mitotic figures for each tadpole. Moreover, the dimensions of 100 resting nuclei of peridermal cells were measured on each tadpole. The results of these counts and measurements are shown in Table 2. In the control series the measurements of resting nuclei were performed on ten tadpoles selected at random from each series, while chromosome counts were made on all the tadpoles.

The following abbreviations are used in this paper.

B — A set of *Rana brevipoda* chromosomes
E — A set of *Rana esculenta* chromosomes
(B)— *Rana brevipoda* cytoplasm
(E)— *Rana esculenta* cytoplasm

The stages of development are shown by those of *Rana pipiens* (TAYLOR and KOLLROS, 1946) for convenience' sake.

OBSERVATION

I. Production of nucleo-cytoplasmic hybrids

The results of nuclear transfer experiments are roughly shown in Table 1.

1. Control series

In the control series eggs were obtained from the same females as utilized in the experimental series. The sperm of each species was obtained from a single male. Insemination was always performed artificially.

a. *Rana brevipoda*

One hundred and seventy-five (78.1%) of 224 eggs cleaved normally. They grew into normal tadpoles, excepting 15, of which 2, 6, 2 and 5 became abnormal and died at the gastrula, neurula, tail-bud and hatching stage, respectively. While two other tadpoles died at the early tadpole stage, the remaining 158 (70.5%) completed their metamorphosis.

b. *Rana esculenta*

TABLE 1

Synthesis of nucleo-cytoplasmic hybrids between *Rana brevipoda* and *Rana esculenta* by the nuclear transplantation method

Series / Results	Control series				Experimental series	
	brevipoda ♀ × *brevipoda* ♂ (B)BB	*brevipoda* ♀ × *esculenta* ♂ (B)BE	*esculenta* ♀ × *brevipoda* ♂ (E)EB	*esculenta* ♀ × *esculenta* ♂ (E)EE	Enucleated egg, (E) + Blastula nucleus, BB (E)BB	Enucleated egg, (B) + Blastula nucleus, EE (B)EE
No. of females	2	2	2	2	2	2
No. of treated eggs	224	250	167	213	117	121
No. of normally cleaved eggs	175 (78.1%)	164 (65.6%)	121 (72.5%)	212 (99.5%)	22 (18.8%)	34 (28.1%)
No. of late gastrulae	173 (77.2%)	161 (64.4%)	100 (59.9%)	207 (97.2%)	9 (7.7%)	17 (14.0%)
No. of neurulae	167 (74.6%)	152 (60.8%)	54 (32.3%)	202 (94.8%)	8 (6.8%)	12 (9.9%)
No. of tail-bud embryos	165 (73.7%)	113 (45.2%)	32 (19.2%)	193 (90.6%)	8 (6.8%)	9 (7.4%)
No. of hatched tadpoles	160 (71.4%)	86 (34.4%)	25 (15.0%)	185 (86.9%)	5 (4.3%)	4 (3.3%)
No. of analysed tadpoles	160 (71.4%)	65 (26.0%)	19 (11.4%)	172 (80.8%)	3 (2.6%)	1 (0.8%)
Diploid	160 (71.4%)	65 (26.0%)	19 (11.4%)	172 (80.8%)	2 (1.7%)	1 (0.8%)
Triploid	0	0	0	0	1 (0.9%)	0

As the result of artificial insemination, 212(99.5%) eggs cleaved normally. While 5, 5, 9, 8 and 13 of them became abnormal and died at the gastrula, neurula, tail-bud, hatching and hatched tadpole stage, respectively, the remaining 172(80.8%) developed normally into tadpoles at later stages. Just before and during their metamorphosis 19 tadpoles died and the other 153(71.8%) completed the metamorphosis.

c. Hybrids, *brevipoda* ♀ × *esculenta* ♂

Normally cleaved eggs were 164(65.6%) in number. Three, 9, 39 and 27 of them became edematous and died at the gastrula, neurula, tail-bud and hatching stage, respectively. Twenty-one of 86 normally hatched tadpoles died of edema or some other abnormalities before taking food. At the late tadpole stage 32 and 16 of the remaining 65 died of under-development and edema, respectively. Only 17 tadpoles completed their metamorphosis.

d. Hybrids, *esculenta* ♀ × *brevipoda* ♂

Although there were 121(72.5%) normally cleaved eggs, 21, 46, 22 and 7 of them died of various abnormalities at the gastrula, neurula, tail-bud and hatching stage, respectively. Six others became abnormal tadpoles and died before taking food. Six and 4 of the remaining 19 tadpoles died of under-development and edema, respectively. Only 9 tadpoles eventually completed their metamorphosis.

2. Experimental series

a. Combination of *esculenta* cytoplasm and *brevipoda* nuclei

Of 117 enucleated *esculenta* eggs transplanted with *brevipoda* nuclei 22(18.8%) cleaved normally, while 86 did abnormally. Six and 7 of the normally cleaved eggs became abnormal at the blastula and gastrula stage, respectively. The remaining 9 completed their gastrulation normally, but one became abnormal at the neurula stage. Three of the other 8 embryos became edematous at the hatching stage, while 5 grew into tadpoles. Although 2 tadpoles died of edema, the remaining 3 developed normally.

As a result of chromosome counting by the tail-tip method, it was found that 2 of the 3 tadpoles were diploid and the third was triploid (Table 2). The 2 diploid tadpoles completed their metamorphosis and attained the stage of sexual maturity.

TABLE 2

Identification of the ploidy of tadpoles produced by the nuclear transplantation method

Series	Kind	Individual no.	No. of analysed mitoses	Mean dimensions of 100 nuclei of peridermal cells (μ^2)	Ploidy
Control	*Rana brevipoda*, (B)BB	Nos. 1~10	3~5	108.34±1.7~119.05±1.6	2n
	R. brevipoda ♀ × *esculenta* ♂, (B)BE	Nos. 1~10	3~5	105.33±1.5~115.40±1.7	2n
	R. esculenta ♀ × *brevipoda* ♂, (E)EB	Nos. 1~10	3~5	103.22±1.7~112.74±1.8	2n
	Rana esculenta, (E)EE	Nos. 1~10	3~5	102.06±1.7~114.31±1.7	2n
Experimental	Enucleated *esculenta* egg, (E) + *brevipoda* nucleus, BB	No. 61-1	4	111.03±1.7	2n
		No. 61-2	5	112.33±1.7	2n
		No. 61-3	5	161.81±2.5	3n
	Enucleated *brevipoda* egg, (B) + *esculenta* nucleus, EE	No. 61-1	4	104.65±1.6	2n

b. Combination of *brevipoda* cytoplasm and *esculenta* nuclei

Thirty-four (28.1%) of 121 enucleated *brevipoda* eggs transplanted with *esculenta* nuclei cleaved normally. Seventy-six cleaved abnormally, while the others did not cleave. Of the normally cleaved eggs 6 and 11 became abnormal at the blastula and gastrula stage, respectively. Although the other 17 eggs completed their gastrulation normally, 5, 3 and 5 became abnormal and died at the neurula, tail-bud and hatching stage, respectively. Eventually, the remaining 4 grew into tadpoles. However, three of them died of under-development at the early tadpole stage, and the other died of edema at the XVII stage, 35 days after fertilization. This tadpole was diploid.

II. Life histories

The life histories of the individuals after the beginning of metamorphosis in the

experimental and control series are summarily shown in Table 3.

1. Control series

In the four control series there were 337 metamorphosed frogs in all. They were quite normal in growth and development, except a few which died of some unknown cause.

TABLE 3
Life histories of diploid nucleo-cytoplasmic hybrids and the control individuals

Kind and individual no.	Age at the time of landing (days)	No. of metamorphosed frogs	Body length immediately after metamorphosis (mm.)	Body length of one-year-old frogs (mm.)	Age at the time of death or killed (months)	Body length at the time of death or killed (mm.)
(B)BB	43～52 (47.7)	158	18.3±0.2	34.6±1.9	40.5	51.0±2.3
(B)BE	39～46 (42.4)	17	16.5±0.5	35.0±1.9	40.5	55.0±2.5
(E)EB	40～47 (43.7)	9	17.2±0.6	36.2±1.8	40.5	56.2±1.6
(E)EE	36～46 (41.1)	153	16.2±0.2	35.2±1.9	40.5	53.5±2.2
(E)BB No. 61-1	52	1	17.5	30.7	12.5	30.7
(E)BB No. 61-2	53	1	15.3	22.0	40.5	49.0

a. *Rana brevipoda*

There were 158 normally metamorphosed frogs; 141 of them were killed and preserved within three months after metamorphosis. Six, 4 and 7 frogs were afterwards killed at the ages of 1, 2, and 3 1/3 years, respectively.

b. *Rana esculenta*

Of 153 normally metamorphosed frogs, 138 were killed within three months after metamorphosis. Six, 4 and 5 frogs were killed at the ages of 1, 2, and 3 1/3 years, respectively.

c. Hybrids, *brevipoda* ♀ × *esculenta* ♂

Seventeen tadpoles normally completed their metamorphosis. Seven of them were killed immediately after metamorphosis. Five and 5 frogs were killed at the ages of 1 and 3 1/3 years, respectively.

d. Hybrids, *esculenta* ♀ × *brevipoda* ♂

Nine tadpoles normally completed their metamorphosis. Three of them were killed immediately after metamorphosis. One frog died at the age of about one year. The remaining 5 were killed at the age of 3 1/3 years.

2. Experimental series

As shown in Table 1, there were only four tadpoles whose ploidy was analysed:

three in the series combining (E) and BB, and one in that of (B) and EE. It was found that one of the former series was triploid, while the other three were diploid (Table 2).

Of the diploid nucleo-cytoplasmic hybrids, two, Nos. (E)BB61–1 and (E)BB61–2, completed their metamorphosis, while the single triploid and one of the three diploids died in the tadpole stage.

The two diploid frogs did not remarkably differ from the controls in age at the time of landing from water as well as in body length at the stage immediately after metamorphosis. However, they were rather bad in growth afterwards. At the age of about one year, Nos. (E) BB61–1 and (E) BB61–2 were 30.7 mm. and 22.0 mm. in body length, respectively, in contrast with the control frogs which were 30.6~44.5 mm.

III. External characters and reproductive ability

1. Control series

a. *Rana brevipoda*

The ground color of the upper sides was brown or greyish brown in all the frogs. On the back of the body there were a small number of large roundish black spots and no median stripe. Among 158 frogs preserved during the period from the completion of metamorphosis to the age of 3 1/3 years, there were 76 females and 82 males. Their gonads were all quite normal. At the age of one year most of the males were sexually matured; their testes were filled with bundles of spermatozoa.

b. *Rana esculenta*

Differing from *Rana brevipoda*, the back of the body was green and there was a pale median stripe and several small black spots. Among 153 frogs preserved during the period from the completion of metamorphosis to the age of 3 1/3 years there were 78 females and 75 males. The ovaries and testes of all these frogs were quite normal. Many males were sexually matured at the age of one year. Their testes were filled with bundles of spermatozoa.

c. Hybrids, *brevipoda* ♀ × *esculenta* ♂

There was a pale median stripe on the backs of all the frogs. Of ten frogs which had been kept alive for more than one year, five were green in back color, while the backs of the other five were brown with black spots. Among 17 frogs preserved during the period from the completion of metamorphosis to the age of 3 1/3 years there were nine females and eight males. The ovaries of the females were far smaller than those of the control *brevipoda* and *esculenta*, and had no growing auxocytes. The testes of all the three 1-year-old and three 3 1/3-year-old hybrids were also under-developed; germ cells were few and there were no normal spermatozoa.

d. Hybrids, *esculenta* ♀ × *brevipoda* ♂

All the frogs had a pale dorso-median stripe. The backs of frogs which had been kept alive for more than one year were deep green with small black spots. Among nine frogs preserved during the period from the completion of metamor-

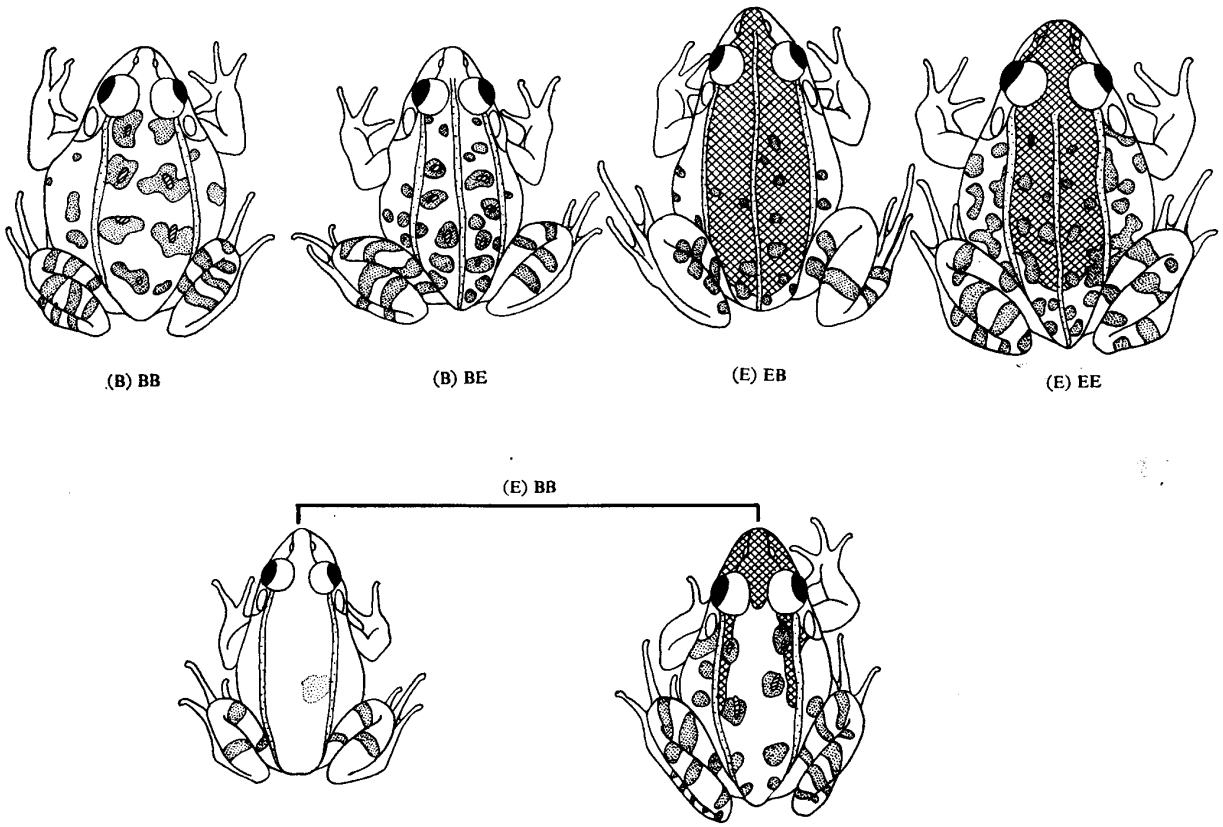

Fig. 1. Diagrammatic drawings showing the color patterns of the two diploid nucleo-cytoplasmic hybrids constructed of *Rana esculenta* cytoplasm and *Rana brevipoda* nuclei.

(B)BB, *Rana brevipoda* (E)EE, *Rana esculenta*
(B)BE, hybrid, *Rana brevipoda* ♀ × *Rana esculenta* ♂
(E)EB, hybrid, *Rana esculenta* ♀ × *Rana brevipoda* ♂
(E)BB, nucleo-cytoplasmic hybrid
Crosshatching area, green in ground color
Closely dotted area, black spot or crossbar
Diagonally hatching area, dermal protuberance

phosis to the age of 3 1/3 years there were three females and six males. The ovaries of two 3 1/3-year-old females were very degenerative; germ cells were scarcely found, and there were abundant empty cavities. The testes of one 1-year-old male were about 3.0 mm. in length and 2.7 mm. in width, and contained no germ cells. They were mostly filled with the ductules of the rete apparatus. Two of three 3 1/3-year-old males had no germ cells in their testes, while the other had a few abnormal spermatozoa and pycnotic nuclei, besides primary and secondary spermatogonia and first spermatocytes in the seminal tubules of the testes.

2. Experimental series

a. No. (E)BB61–1, 1-year-old male

This nucleo-cytoplasmic hybrid was a male with secondary sexual characters and nearly of the *brevipoda* type in appearance. On the back there were eight roundish black spots and no median stripe. The upper side of the head and the

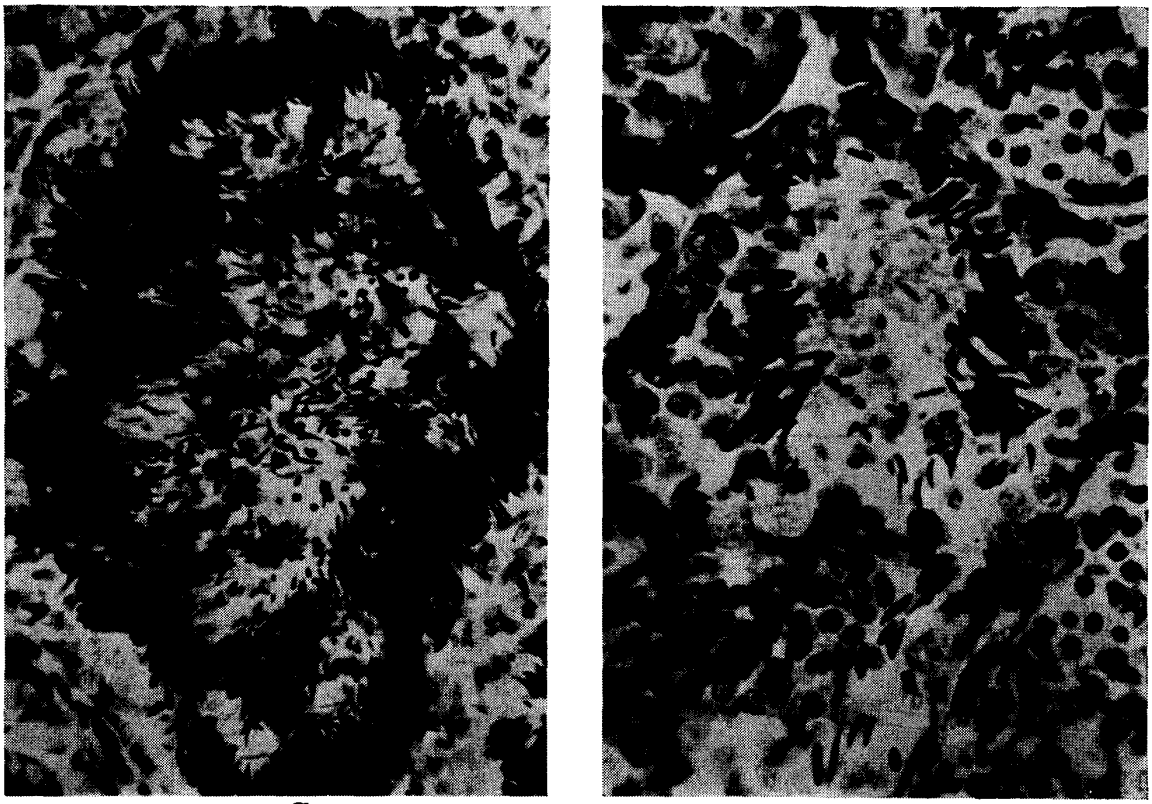

Fig. 2. Cross-sections of seminal tubules of testes. ×350.
a, *Rana brevipoda*, one year old. b, Nucleo-cytoplasmic hybrid No. (E)BB61–1, one year old.

TABLE 4
Reproductive capacity of male

Parents		No. of eggs	No. of cleaved eggs		No. of gastrulae	
Female no.	Male no.		Norm.	Abnorm.	Norm.	Abnorm.
(B)BB Nos. 1~2	(B)BB No. 1	213	176 (82.6%)	0	164 (77.0%)	12 (5.6%)
	(E)EE No. 1	205	143 (69.8%)	0	129 (62.9%)	14 (6.8%)
	(E)BB No. 61–1	216	5 (2.3%)	7 (3.2%)	5 (2.3%)	0
(E)EE No. 1	(B)BB No. 1	202	165 (81.7%)	0	126 (62.4%)	39 (19.3%)
	(E)EE No. 1	224	196 (87.5%)	0	192 (85.7%)	4 (1.8%)
	(E)BB No. 61–1	204	13 (6.4%)	5 (2.5%)	12 (5.9%)	1 (0.5%)

narrow areas along the anterior halves of the dorso-lateral folds were pale green, while the other areas of the back were greyish brown.

This male was killed at the age of one year to examine the reproductive ability by artificial insemination. The testes were quite normal in appearance; they were about 4.0 mm. and 3.7 mm. in length and width. However, they were abnormal in inner structures: normal spermatozoa were scarcely found, while large abnormal ones were rather abundant. Germ cells were large in size and seemed to be tetraploid. The kidneys were normal in size and shape; they consisted of diploid cells.

In order to examine the reproductive ability, this nucleo-cytoplasmic hybrid was mated with *brevipoda* and *esculenta* females by artificial insemination (Table 4). As a result, five (2.3%) of 216 *brevipoda* eggs and 13(6.4%) of 204 *esculenta* ones cleaved normally. These cleaved eggs, however, all became abnormal by the hatching stage, differing from the control eggs.

b. No. (E)BB61–2, 3 1/3-year-old frog

This frog was similar to the control *brevipoda* in main characters; there was neither median stripe nor green part on the back. However, there were no black spots, differing from any of the control frogs. The upper sides were dark purplish brown in ground color and had a large dark brown spot on the right side of the middle area.

When preserved at the age of more than three years, it was 49.0 mm. in body length. Although the Müllerian ducts were distinctly developed, the gonads were very degenerative, irregularly shaped and closely attached to the fat bodies. However, the cortical and the medullary portions were clearly distinguished from each other. In the medullary portion there were a few ill-developed seminal

nucleo-cytoplasmic hybrid

No. of neurulae		No. of tail-bud embryos		No. of hatched tadpoles		No. of metamorphosed frogs	
Norm.	Abnorm.	Norm.	Abnorm.	Norm.	Abnorm.	Norm.	Abnorm.
160 (75.1%)	4 (1.9%)	160 (75.1%)	0	152 (71.4%)	8 (3.8%)	137 (64.3%)	0
107 (52.2%)	22 (10.7%)	103 (50.2%)	4 (2.0%)	58 (28.3%)	45 (22.0%)	19 (9.3%)	0
4 (1.9%)	1 (0.5%)	3 (1.4%)	1 (0.5%)	0	3 (1.4%)	0	0
76 (37.6%)	30 (14.9%)	53 (26.2%)	43 (21.3%)	17 (8.4%)	36 (17.8%)	4 (2.0%)	0
187 (83.5%)	5 (2.2%)	187 (83.5%)	0	181 (80.8%)	6 (2.7%)	177 (79.0%)	0
12 (5.9%)	0	11 (5.4%)	1 (0.5%)	0	11 (5.4%)	0	0

tubules as well as ductules of the rete apparatus, although the tubules contained no germ cells. The cortical portions were only found in places, where they consisted of two layers, i.e. the peritoneal epithelium and a layer of oogonium-like cells. The latter had oval nuclei and were far smaller than common oogonia, although remarkably larger than follicular or rete cells. There was moderate connective tissue between the cortical and medullary portions as well as around the ductules of the rete apparatus. From the structures stated above, this nucleo-cytoplasmic hybrid was presumed to be a hermaphrodite with very degenerative gonads, transformed from ovaries.

DISCUSSION

The two nucleo-cytoplasmic hybrids produced from enucleated *Rana esculenta* eggs by transplanting a blastula nucleus of *Rana brevipoda* were not always identical with the nuclear species in appearance. In one of the two, the upper side of the head and the narrow areas along the anterior halves of the dorso-lateral folds were pale green, differing from those of the control *brevipoda*. The latter were 158 in number and all of them were brown or greyish brown in the ground color of the back. On the other hand, all the control *esculenta* were green.

As the green color of pond frogs is controlled by dominant genes, it does not usually appear in the offspring of brown parents, although rare appearance of small greenish patches on the anterior portion of the back can not be disregarded. Accordingly, it seems very probable that the pale green color of the nucleo-cytoplasmic hybrid is due to participation of the cytoplasm derived from the *esculenta* egg. However, it is also thinkable that this color is a kind of sporadic variation owing to disharmony of the nucleus with the cytoplasm. The peculiar color pattern of the other nucleo-cytoplasmic hybrid seems to show the latter possibility.

In contrast with these two, intraspecific nucleo-cytoplasmic hybrids are nearly the same in morphological characters as the nuclear subspecies or variety. This fact was found by McKINNELL (1960) between the wild type and a variety kandiyohi of *Rana pipiens*, by GURDON (1961) between *Xenopus l. laevis* and *X. l. victorianus*, and by ORTOLANI, FISCHBERG and SLATKINE (1966) between *Xenopus l. laevis* and *X. l. petersi*. This seems to show that the transplanted nucleus conducts itself as if it were in its own cytoplasm when the nucleus and cytoplasm are so intimate as they belong to the same species. The nucleo-cytoplasmic hybrids between *Pleurodeles waltlii* and *P. poireti* obtained by C.-L. GALLIEN (1970) were very similar to the nuclear species in various characters. This seems to be related to the fact that these two species are so closely related, as their reciprocal hybrids show good growth and are usually fertile.

The two nucleo-cytoplasmic hybrids obtained in the present research were nearly completely sterile. In this respect, they are quite the same as those constructed of *Rana japonica* cytoplasm and European *Rana temporaria* nuclei (NISHIOKA, 1972b), and differ from some others reported by KAWAMURA and NISHIOKA (1963b, c), GALLIEN (1970) and NISHIOKA (1971a, b, 1972a). KAWAMURA and NISHIOKA

reported that all the three female nucleo-cytoplasmic hybrids between *Rana nigromaculata* and *Rana brevipoda* produced some viable offspring by mating with males of each species. They afterwards ascertained that the male nucleo-cytoplasmic hybrids were also fertile to some extent and produced some fertile offspring by mating with females of each species (KAWAMURA and NISHIOKA, 1972). The two male nucleo-cytoplasmic hybrids constructed of *Rana ornativentris* cytoplasm and *Rana japonica* nuclei were fertile in a certain degree and produced some fertile offspring by mating with female *Rana japonica*. Although the two female nucleo-cytoplasmic hybrids constructed of *Rana ornativentris* cytoplasm and *Rana japonica* nuclei produced more or less numerous embryos by mating with males of each species, all of these embryos did not develop beyond the hatching stage (KAWAMURA and NISHIOKA, 1963c). According to GALLIEN (1970), the single male and two female nucleo-cytoplasmic hybrids obtained by him between *Pleurodeles waltlii* and *P. poireti* were fertile and produced many larvae by mating with males or females of the nuclear species. Among the nucleo-cytoplasmic hybrids constructed of *Rana brevipoda* cytoplasm and *Rana plancyi chosenica* nuclei, there were females with nearly normal ovaries as well as some males with small bundles of normally shaped spermatozoa.

On the other hand, there are differences in the degree of sterility among various kinds of interspecific hybrids obtained by crossing two species of each combination stated above. In the reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda*, the males are nearly completely sterile, while the females are fertile to a large extent (MORIYA, 1960; KAWAMURA and NISHIOKA, 1960, 1963a, b). The reciprocal hybrids between *Pleurodeles waltlii* and *P. poireti* are usually normal in fertility, although reproductive barrier appears in the next generation (GALLIEN, 1969, 1970). All the hybrids between female *Rana japonica* and male *R. ornativentris* are perfectly sterile males; the reciprocal hybrids can not be obtained, owing to gametic isolation (KAWAMURA, 1950). The single matured male hybrid between female *Rana japonica* and male European *R. temporaria* was also a perfectly sterile male. However, the germ cells in the testes of this male degenerated much earlier than those of the *japonica* ♀ × *ornativentris* ♂ hybrids (KAWAMURA and KOBAYASHI, 1960).

The reciprocal hybrids between *Rana brevipoda* and *Rana esculenta* were all sterile; the ovaries of females were very degenerative and the testes of males contained no spermatozoa. Accordingly, there seems to be a parallelism between ordinary and nucleo-cytoplasmic hybrids, in the degree of sterility. This seems to indicate that the existence of two sets of chromosomes of one species does not always guarantee normal gametogenesis and that hybrid sterility is not only caused by the disharmony between two different sets of chromosomes, but by that between the nucleus and cytoplasm.

SUMMARY

1. Two diploid nucleo-cytoplasmic hybrids were obtained between Japanese

and European pond frogs, *Rana brevipoda* and *Rana esculenta*, by the combination of PORTER's and BRIGGS and KING's methods.

2. These nucleo-cytoplasmic hybrids developed from among 117 enucleated *esculenta* eggs transplanted with *brevipoda* nuclei and grew into one- and three-year-old frogs. Although they did not remarkably differ from the controls in development and growth at the stage immediately after metamorphosis, they were rather bad in growth afterwards. From 121 eggs of the reciprocal combination a single diploid tadpole was obtained. This died of edema at the premetamorphic stage.

The reciprocal hybrids of the two species in the control series showed inviability in a large degree at the embryonal and tadpole stage.

3. The one-year-old nucleo-cytoplasmic hybrid was a male with nearly the same appearance as that of the control *brevipoda*, except the existence of a pale green part on the back. This male had abnormal testes and produced a few inviable embryos by mating with *brevipoda* and *esculenta* females. The other nucleo-cytoplasmic hybrid was a frog with an appearance similar to the control *brevipoda* in main characters, too. It was a hermaphrodite with very degenerative gonads.

In the control series, all the males and females of reciprocal hybrids between the two species were quite sterile.

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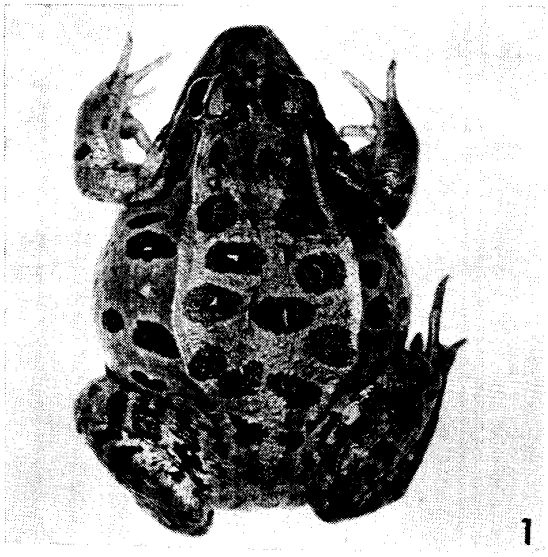
EXPLANATION OF PLATE I

Diploid nucleo-cytoplasmic hybrids and the control frogs.

1. *Rana brevipoda,* (B)BB, one year old. ×1.5.
2. *Rana esculenta,* (E)EE, one year old. ×1.5.
3. Diploid hybrid, (B)BE, one year old, produced by a cross, *Rana brevipoda* ♀ ×*Rana esculenta* ♂. ×1.5.
4. Diploid hybrid, (E)EB, one year old, produced by a cross, *Rana esculenta* ♀ ×*Rana brevipoda* ♂. ×1.5.
5. Diploid nucleo-cytoplasmic hybrid, No. (E)BB61–2, 3 1/3 year old, consisting of *Rana esculenta* cytoplasm and *Rana brevipoda* nuclei. ×1.
6. Diploid nucleo-cytoplasmic hybrid, No. (E)BB61–1, one year old, consisting of *Rana esculenta* cytoplasm and *Rana brevipoda* nuclei. ×1.5.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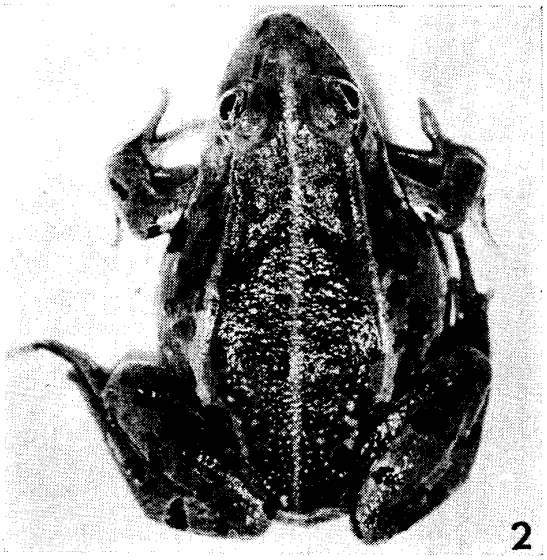
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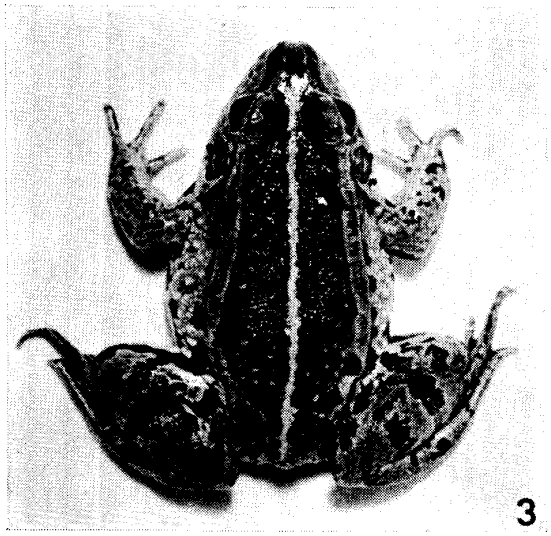
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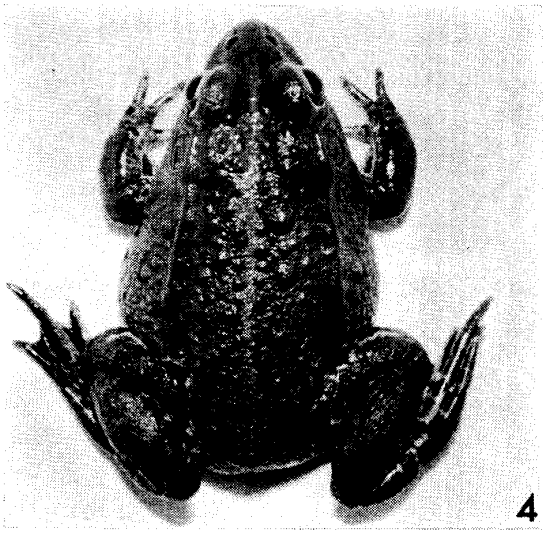
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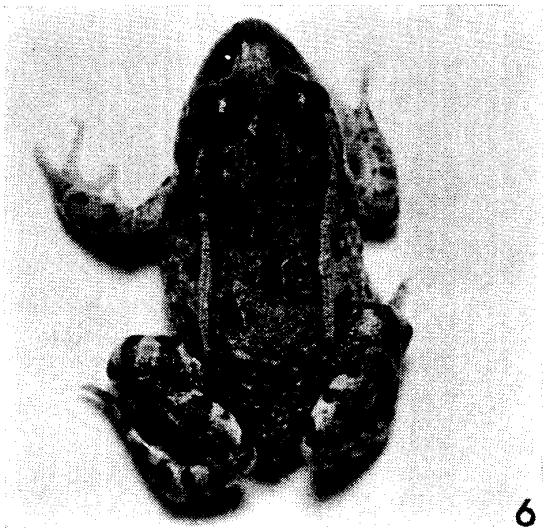
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