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Sixth- to Ninth-generation Offspring of Nucleo-cytoplasmic Hybrids Consisting of *Rana ornativentris* Cytoplasm and *R. japonica* Nuclei

By

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(With 1 Text-figure)

INTRODUCTION

In two previous papers (1963a, 1972), the authors reported that the low reproductive capacities of three male nucleo-cytoplasmic hybrids of brown frogs consisting of *Rana ornativentris* cytoplasm and *R. japonica* nuclei were not remarkably improved even in their fifth-generation offspring, when five consecutive generations were produced from them by repeating matings with females of the nuclear species, *Rana japonica*. Moreover, sex-reversal occurred in most of the genetic females of each generation, when they were reared until the sexually mature stage.

Similar phenomena in sex and reproductive capacity were found in the offspring of nucleo-cytoplasmic hybrids between two Japanese pond frogs, *Rana nigromaculata* and *R. brevipoda*, too (KAWAMURA and NISHIOKA, 1963b; NISHIOKA 1972).

In order to elucidate how many generations are required for perfect improvement in reproductive capacity as well as in sex ratio, four more generations were produced from male fifth-generation offspring of the above three male nucleo-cytoplasmic hybrids consisting of *Rana ornativentris* cytoplasm and *R. japonica* nuclei by repeating matings with female *Rana japonica*.

MATERIALS AND METHODS

From male fifth-generation offspring of three male nucleo-cytoplasmic hybrids, Nos. 61OT2, 61AT8 and 61AT12, consisting of *Rana ornativentris* cytoplasm and *R. japonica* nuclei, sixth- to ninth-generation offspring were produced. The nucleo-cytoplasmic hybrids and their first- to fifth-generation offspring have already been reported previously (KAWAMURA and NISHIOKA, 1963a, 1972). The symbols used for indicating the fifth-generation offspring in the previous paper (1972) are abbreviated as follows in the present paper for convenience sake. The number of males of each kind is presented on the right of each symbol.

Symbol of fifth-generation offspring	Abbreviation	No. of males
$J\times J[J[J\{J(61OT2)\}_1]_5]_1$ ·4	$J_4^5(61OT2)$	5(Nos. 1～5)
$J\times J[J[J\{J(61AT8)\}_4]_2]_1$ ·1	$J_1^5(61AT8)$	4(Nos. 1～4)
$J\times J[J[J\{J(61AT12)\}_6]_2]_3$·3	$J_3^5(61AT12)$	3(Nos. 1～3)
J×J.L66.2(Control)	$J_2^5(J\times J)$	3(Nos. 1～3)

The females used for mating with male offspring of nucleo-cytoplasmic hybrids were collected from the suburbs of Hiroshima. The ovulation of these females were accelerated by injection of frog pituitary suspension. Matings were always performed by the method of artificial insemination. The following abbreviations are used for designation of these females and the offspring produced from them by mating with the male offspring of nucleo-cytoplasmic hybrids.

J.W67～70 — Female *Rana japonica* collected from the field and used for mating experiments in 1967～1970.

$J_4^6(61OT2)$ — Sixth-generation offspring produced from a field-caught female by mating with a male fifth-generation offspring (No. 4) of the nucleo-cytoplasmic hybrid No. 61OT2.

$J_2^7(61OT2)$ — Seventh-generation offspring produced from a field-caught female by mating with a male sixth-generation offspring (No. 2) of the nucleo-cytoplasmic hybrid No. 61OT2.

$J_3^8(61OT2)$ — Eight-generation offspring produced from a field-caught female by mating with a male seventh-generation offspring (No. 3) of the nucleo-cytoplasmic hybrid No. 61OT2.

The abbreviations for designating the offspring of the other two nucleo-cytoplasmic hybrids Nos. 61AT8 and 61AT12 are made by the same way as in the case of No. 61OT2.

OBSERVATION

I. Sixth-generation offspring of three male nucleo-cytoplasmic hybrids

1. Production of sixth-generation offspring

a. Control series, J.W67, Nos. 1～3♀×$J_2^5(J\times J)$, Nos. 1～3♂

Of the fifth-generation offspring produced in 1966 from a male fourth-generation offspring (No. 2) in the control series by mating with two females from the field, 25 frogs matured in the breeding season of 1967. Of these frogs, 12 were females and 13 were males. Three (Nos. 1～3) of the latter were mated with three females (Nos. 1～3) collected from the field (Table 1). As a result of artificial insemination, 276 (85.2%) of 324 eggs in total cleaved normally. At various embryonic stages, 13 of the normally cleaved eggs died of abnormalities, and the remaining 263 (81.2%) hatched normally. At the tadpole stage, only five died of abnormalities; 258 (79.6%) tadpoles metamorphosed normally.

TABLE 1

Viability of sixth-generation offspring of three male nucleo-cytoplasmic hybrids

Parents		No. of eggs	No. of normally cleaved eggs	No. of normal neurulae	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally metamorphosed frogs
Female	Male						
J. W67, Nos. 1～3	J_2^5(J×J), No. 1	103	92 (89.3%)	90 (87.4%)	90 (87.4%)	86 (83.5%)	85 (82.5%)
	J_2^5(J×J), No. 2	95	83 (87.4%)	83 (87.4%)	83 (87.4%)	82 (86.3%)	81 (85.3%)
	J_2^5(J×J), No. 3	126	101 (80.2%)	100 (79.4%)	97 (77.0%)	95 (75.4%)	92 (73.0%)
	Total	324	276 (85.2%)	273 (84.3%)	270 (83.3%)	263 (81.2%)	258 (79.6%)
J. W67, Nos. 1～3	J_4^5(61OT2), No. 1	155	137 (88.4%)	133 (85.8%)	128 (82.6%)	126 (81.3%)	125 (80.6%)
	J_4^5(61OT2), No. 2	167	146 (87.4%)	146 (87.4%)	138 (82.6%)	19 (11.4%)	14 (8.4%)
	J_4^5(61OT2), No. 3	166	150 (90.4%)	139 (83.7%)	134 (80.7%)	123 (74.1%)	75 (45.2%)
	J_4^5(61OT2), No. 4	157	133 (84.7%)	106 (67.5%)	97 (61.8%)	97 (61.8%)	90 (57.3%)
	J_4^5(61OT2), No. 5	170	162 (95.3%)	148 (87.1%)	139 (81.8%)	125 (73.5%)	121 (71.2%)
	Total	815	728 (89.3%)	672 (82.5%)	636 (78.0%)	490 (60.1%)	425 (52.1%)
J. W67, Nos. 1～3	J_1^5(61AT8), No. 1	169	123 (72.8%)	112 (66.3%)	95 (56.2%)	91 (53.8%)	74 (43.8%)
	J_1^5(61AT8), No. 2	154	151 (98.1%)	143 (92.9%)	127 (82.5%)	125 (81.2%)	103 (66.9%)
	J_1^5(61AT8), No. 3	172	140 (81.4%)	103 (59.9%)	90 (52.3%)	69 (40.1%)	63 (36.6%)
	Total	495	414 (83.6%)	358 (72.3%)	312 (63.0%)	285 (57.6%)	240 (48.5%)
J. W67, Nos. 1～3	J_3^5(61AT12), No. 1	171	156 (91.2%)	131 (76.6%)	120 (70.2%)	117 (68.4%)	115 (67.3%)
	J_3^5(61AT12), No. 2	153	130 (85.0%)	123 (80.4%)	107 (69.9%)	55 (35.9%)	51 (33.3%)
	J_3^5(61AT12), No. 3	174	103 (59.2%)	98 (56.3%)	93 (53.4%)	90 (51.7%)	89 (51.1%)
	J_3^5(61AT12), No. 4	168	150 (89.3%)	150 (89.3%)	137 (81.5%)	130 (77.4%)	126 (75.0%)
	Total	666	539 (80.9%)	502 (75.4%)	457 (68.6%)	392 (58.9%)	381 (57.2%)

b. Experimental series

As fifth-generation offspring derived from the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12 matured in the breeding season of 1967, twelve males of them were mated with the same three females Nos. 1～3 as those used in the control series (Table 1). As a result of artificial insemination, 1681 (85.1%) of 1976 eggs in total cleaved normally. While 514 of the normally cleaved eggs died of abnormalities at various embryonic stages, the remaining 1167 (59.1%) hatched normally and became tadpoles. Of the latter, 1046 (52.9%) metamorphosed normally, while 121 died of abnormalities.

i) J.W67♀, Nos. 1～3×J_4^5(61OT2)♂Nos. 1～5

Seventeen fifth-generation offspring produced from a male fourth-generation offspring, $J[J[J\{J(61OT2)\}_1]_5]_1$, No. 4, by mating in 1966 with two females, J.W66, Nos. 3 and 4, from the field attained sexual maturity. Of these frogs, one was a female and 16 were males. Five (Nos. 1～5) of the latter were mated with the three normal females Nos. 1～3 from the field to produce sixth-generation offspring. As a result of insemination, 728 (89.3%) of 815 eggs in total

cleaved normally. By the hatching stage, 238 of the normally cleaved eggs died of abnormalities, while the other 490 (60.1%) hatched normally and became tadpoles. During the tadpole stage, 65 individuals died of abnormalities; 425 (52.1%) metamorphosed normally.

ii) J.W67♀, Nos. 1～3×J_1^5(61AT8)♂, Nos. 1～3

Fifteen fifth-generation offspring produced from a male fourth-generation offspring, $J[J[J\{J(61AT8)\}_4]_2]_1$, No. 1, by mating in 1966 with two normal females, J.W66, Nos. 1 and 2, from the field attained sexual maturity in the breeding season of 1967. Of these fifth-generation offspring, two were females and 13 were males. Three (Nos. 1～3) of the latter were mated with the three normal females Nos. 1～3 from the field to produce sixth-generation offspring. As a result, 414 (83.6%) of 495 eggs in total cleaved normally. Of these normally cleaved eggs, 129 died of abnormalities at various embryonic stages, while the other 285 (57.6%) hatched normally. During the tadpole stage, 45 individuals died of abnormalities and 240 (48.5%) metamorphosed normally.

iii) J.W67♀, Nos. 1～3×J_3^5(61AT12)♂, Nos. 1～4

Sixteen fifth-generation offspring produced from a male fourth-generation offspring, $J[J[J\{J(61AT12)\}_6]_2]_3$, No. 3, by mating in 1966 with two normal females, J.W66, Nos. 5 and 6, attained sexual maturity in 1967. Two of them were females and 14 were males. Four (Nos. 1～4) of the latter were mated with the three normal females Nos. 1～3 from the field to produce sixth-generation offspring. As a result of insemination, 539 (80.9%) of 666 eggs in total cleaved normally. Of the normally cleaved eggs, 392 (58.9%) hatched normally, while the other 147 died of abnormalities at various embryonic stages. During the tadpole stage, 381 (57.2%) individuals grew and metamorphosed normally.

2. Sex of sixth-generation offspring

a. Control series

Of the 258 normally metamorphosed frogs, 108 died or were killed within one month after metamorphosis. The sex of all these young frogs was examined. As a result, it was found that 52 were females, 3 were hermaphrodites and 53 were males. When the hermaphrodites were counted as males, 51.9% were males. Of the remaining 150 frogs, 107 attained sexual maturity in the next year; 53 of them were females and 54 (50.5%) were males (Table 2).

b. Experimental series

Of a total of 1046 sixth-generation offspring produced from 12 male fifth-generation offspring which had been derived from the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12, 486 died or were killed within one month after metamorphosis. As a result of sex examination of 480 of them, it was found that 196 were females, 28 were hermaphrodites and 256 were males. When the hermaphrodites were counted as males, 59.2% were males. Of the remaining 560 frogs, 301 attained sexual maturity in the next year. Of these

TABLE 2

Sex of sixth-generation offspring of three male nucleo-cytoplasmic hybrids

Parents		Age (days) at the time of climbing out (Mean)	No. of metamorphosed frogs	Sex of young frogs shortly after metamorphosis				Sex of mature frogs		
Female	Male			Total	♀	♀♂	♂ (%)	Total	♀	♂ (%)
J. W67, Nos. 1～3	J_2^5(J×J), No. 1	82～90 (85.2)	85	35	17	0	18 (51.4)	39	20	19 (48.7)
	J_2^5(J×J), No. 2	82～91 (84.9)	81	31	15	2	14 (51.6)	33	16	17 (51.5)
	J_2^5(J×J), No. 3	81～89 (84.5)	92	42	20	1	21 (52.4)	35	17	18 (51.4)
	Total	81～91 (84.9)	258	108	52	3	53 (51.9)	107	53	54 (50.5)
J. W67, Nos. 1～3	J_4^5(61OT2), No. 1	81～97 (86.3)	125	75	32	6	37 (57.3)	23	3	20 (87.0)
	J_4^5(61OT2), No. 2	83～89 (85.0)	14	—	—	—	—	7	2	5 (71.4)
	J_4^5(61OT2), No. 3	83～93 (84.5)	75	25	10	2	13 (60.0)	7	1	6 (85.7)
	J_4^5(61OT2), No. 4	85～95 (87.1)	90	40	18	3	19 (55.0)	31	7	24 (77.4)
	J_4^5(61OT2), No. 5	82～99 (86.7)	121	71	29	4	38 (59.2)	31	8	23 (74.2)
	Total	81～99 (85.9)	425	211	89	15	107 (57.8)	99	21	78 (78.8)
J. W67, Nos. 1～3	J_1^5(61AT8), No. 1	83～95 (85.2)	74	24	10	3	11 (58.3)	43	14	29 (67.4)
	J_1^5(61AT8), No. 2	83～94 (85.1)	103	53	20	2	31 (62.3)	32	12	20 (62.5)
	J_1^5(61AT8), No. 3	82～94 (84.7)	63	13	5	1	7 (61.5)	25	8	17 (68.0)
	Total	82～95 (85.0)	240	90	35	6	49 (61.1)	100	34	66 (66.0)
J. W67, Nos. 1～3	J_3^5(61AT12), No. 1	83～95 (86.9)	115	65	27	3	35 (58.5)	29	12	17 (58.6)
	J_3^5(61AT12), No. 2	82～90 (84.4)	51	—	—	—	—	17	3	14 (82.4)
	J_3^5(61AT12), No. 3	84～90 (85.9)	89	39	14	2	23 (64.1)	26	6	20 (76.9)
	J_3^5(61AT12), No. 4	81～96 (85.1)	126	75	31	2	42 (58.7)	30	4	26 (86.7)
	Total	81～96 (85.6)	381	179	72	7	100 (59.8)	102	25	77 (75.5)

frogs, 80 were females and 221 (73.4%) were males.

i) J.W67♀, Nos. 1～3×J_4^5(61OT2)♂, Nos. 1～5

Of the 425 normally metamorphosed sixth-generation offspring derived from the male nucleo-cytoplasmic hybrid No. 61OT2, 215 died or were killed within one month after metamorphosis. The sex of 211 of them was examined. As a result, 89 were females, 15 were hermaphrodites and 107 were males. When the hermaphrodites were counted as males, 57.8% were males. Of the remaining 210 frogs, 99 attained sexual maturity; 21 of them were females and 78 (78.8%) were males.

ii) J.W67♀, Nos. 1～3×J_1^5(61AT8)♂, Nos. 1～3

Of the 240 normally metamorphosed sixth-generation offspring derived from the male nucleo-cytoplasmic hybrid No. 61AT8, 90 died or were killed within one month after metamorphosis. As a result of examining their sex, it was found

that 35 were females, 6 were hermaphrodites and 49 were males. When the hermaphrodites were counted as males, 61.1% were males. One hundred of the remaining 150 frogs attained sexual maturity; 34 of them were females and 66(66.0%) were males.

iii) J.W67♀, Nos. 1～3×J_3^5(61AT12)♂, Nos. 1～4

Of the 381 normally metamorphosed sixth-generation offspring derived from the male nucleo-cytoplasmic hybrid No. 61AT12, 181 died or were killed within one month after metamorphosis. As a result of examining the sex of 179 of them, it was found that 72 were females, 7 were hermaphrodites and 100 were males. When the hermaphrodites were counted as males, 59.8% were males. Of the remaining 200 frogs, 102 attained sexual maturity; 25 were females and 77 (75.5%) were males.

II. Seventh-generation offspring of three male nucleo-cytoplasmic hybrids

1. Production of seventh-generation offspring

a. Control series, J.W68♀, Nos. 1～5×J_1^6(J×J)♂, Nos. 1～4

Of the sixth-generation offspring produced in 1967 from a male fifth-generation offspring (No. 1) in the control series by mating with three normal females from the field, 39 frogs matured in the season of 1968. Of these frogs, 20 were females and 19 were males. Four of the latter were mated with five normal females Nos. 1～5 from the field to produce seventh-generation offspring in the control series (Table 3). As a result of artificial insemination, 286 (92.6%) of 309 eggs in total cleaved normally. While 19 of the normally cleaved eggs died at various embryonic stages, the remaining 267 (86.4%) hatched normally and became normal tadpoles. Of the latter, 11 died of abnormalities and 256 (82.8%) metamorphosed normally.

b. Experimental series

A total of 16 male sixth-generation offspring derived from the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12 were mated with the same five normal females Nos. 1～5 as those in the control series to produce seventh-generation offspring (Table 3). As a result of artificial insemination, 1475 (79.6%) of 1853 eggs in total cleaved normally. Of the normally cleaved eggs, 398 died of abnormalities at various embryonic stages, while 1077 (58.1%) hatched normally. During the tadpole stage, 110 individuals died of abnormalities. The remaining 967 (52.2%) metamorphosed normally.

i) J.W68♀, Nos. 1～5×J_4^6(61OT2)♂, Nos. 1～5

Of the sixth-generation offspring produced from a male fifth-generation offspring, J_3^5(61OT2), No. 4, by mating in 1967 with three normal females from the field, 31 frogs attained sexual maturity in the breeding season of 1968. Seven of them were females and 24 were males. Five of the latter were mated with the five normal females Nos. 1～5 from the field to produce seventh-generation

TABLE 3

Viability of seventh-generation offspring of three male nucleo-cytoplasmic hybrids

Parents		No. of eggs	No. of normally cleaved eggs	No. of normal neurulae	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally metamorphosed frogs
Female	Male						
J. W68, Nos. 1～5	J_1^6(J×J), No. 1	83	80 (96.4%)	80 (96.4%)	80 (96.4%)	76 (91.6%)	75 (90.4%)
	J_1^6(J×J), No. 2	55	51 (92.7%)	50 (90.9%)	50 (90.9%)	49 (89.1%)	47 (85.5%)
	J_1^6(J×J), No. 3	69	62 (89.9%)	62 (89.9%)	62 (89.9%)	60 (87.0%)	55 (79.7%)
	J_1^6(J×J), No. 4	102	93 (91.2%)	91 (89.2%)	87 (85.3%)	82 (80.4%)	79 (77.5%)
	Total	309	286 (92.6%)	283 (91.6%)	279 (90.3%)	267 (86.4%)	256 (82.8%)
J. W68, Nos. 1～5	J_4^6(61OT2), No. 1	156	150 (96.2%)	142 (91.0%)	130 (83.3%)	76 (48.7%)	52 (33.3%)
	J_4^6(61OT2), No. 2	141	123 (87.2%)	100 (70.9%)	99 (70.2%)	97 (68.8%)	97 (68.8%)
	J_4^6(61OT2), No. 3	122	115 (94.3%)	108 (88.5%)	105 (86.1%)	95 (77.9%)	81 (66.4%)
	J_4^6(61OT2), No. 4	134	90 (67.2%)	71 (53.0%)	64 (47.8%)	53 (39.6%)	50 (37.3%)
	J_4^6(61OT2), No. 5	129	113 (87.6%)	80 (62.0%)	78 (60.5%)	75 (58.1%)	62 (48.1%)
	Total	682	591 (86.7%)	501 (73.5%)	476 (69.8%)	396 (58.1%)	342 (50.1%)
J. W68, Nos. 1～5	J_2^6(61AT8), No. 1	99	95 (96.0%)	94 (94.9%)	86 (86.9%)	71 (71.7%)	64 (64.6%)
	J_2^6(61AT8), No. 2	125	86 (68.8%)	72 (57.6%)	60 (48.0%)	51 (40.8%)	40 (32.0%)
	J_2^6(61AT8), No. 3	103	54 (52.4%)	54 (52.4%)	52 (50.5%)	41 (39.8%)	40 (38.8%)
	J_2^6(61AT8), No. 4	126	112 (88.9%)	102 (81.0%)	93 (73.8%)	93 (73.8%)	92 (73.0%)
	J_2^6(61AT8), No. 5	140	79 (56.4%)	66 (47.1%)	51 (36.4%)	34 (24.3%)	31 (22.1%)
	J_2^6(61AT8), No. 6	111	97 (87.4%)	97 (87.4%)	95 (85.6%)	95 (85.6%)	85 (76.6%)
	Total	704	523 (74.3%)	485 (68.9%)	437 (62.1%)	385 (54.7%)	352 (50.0%)
J. W68, Nos. 1～5	J_1^6(61AT12), No. 1	95	92 (96.8%)	92 (96.8%)	92 (96.8%)	90 (94.7%)	90 (94.7%)
	J_1^6(61AT12), No. 2	82	51 (62.2%)	50 (61.0%)	46 (56.1%)	44 (53.7%)	43 (52.4%)
	J_1^6(61AT12), No. 3	96	73 (76.0%)	69 (71.9%)	67 (69.8%)	59 (61.5%)	42 (43.8%)
	J_1^6(61AT12), No. 4	100	65 (65.0%)	65 (65.0%)	60 (60.0%)	31 (31.0%)	27 (27.0%)
	J_1^6(61AT12), No. 5	94	80 (85.1%)	79 (84.0%)	72 (76.6%)	72 (76.6%)	71 (75.5%)
	Total	467	361 (77.3%)	355 (76.0%)	337 (72.2%)	296 (63.4%)	273 (58.5%)

offspring. As a result of insemination, 591 (86.7%) of 682 eggs in total cleaved normally. Of the normally cleaved eggs, 195 died of abnormalities at various embryonic stages, while 396 (58.1%) hatched normally. During the tadpole stage, 54 individuals died of abnormalities, and eventually 342 (50.1%) tadpoles metamorphosed normally.

ii) J.W68♀, Nos. 1～5×J_2^6(61AT8)♂, Nos. 1～6

Thirty-two sixth-generation offspring produced from a male fifth-generation offspring, J_1^5(61AT8), No. 2, by mating in 1967 with three normal females from the field attained sexual maturity in the breeding season of 1968. They consisted of 12 females and 20 males. Six of the latter were mated with the five

normal females Nos. 1～5 from the field to produce seventh-generation offspring. As a result of insemination, 523 (74.3%) of 704 eggs in total cleaved normally. Of the normally cleaved eggs, 138 died of abnormalities at various embryonic stages, while 385 (54.7%) hatched normally. During the tadpole stage, 33 individuals died of abnormalities, while the other 352 (50.0%) metamorphosed normally.

iii) J.W68♀, Nos. 1～5×J_1^6(61AT12)♂, Nos. 1～5

Twenty-nine sixth-generation offspring produced from a male fifth-generation offspring, J_3^5(61AT12), No. 1, by mating in 1967 with three normal females from the field attained sexual maturity in the breeding season of 1968. Of these mature frogs, 12 were females and 17 were males. Five of the latter were mated with the five normal females Nos. 1～5 to produce seventh-generation offspring. As a result of insemination, 361 (77.3%) of 467 eggs in total cleaved normally. Of the normally cleaved eggs, 65 died of abnormalities at various embryonic stages, while 296 (63.4%) hatched normally. During the tadpole stage, 23 individuals died of abnormalities, and eventually 273 (58.5%) metamorphosed normally.

2. Sex of seventh-generation offspring

a. Control series

Of the 256 normally metamorphosed frogs in the control series, 136 died or were killed within one month after metamorphosis. As a result of examining their sex, it was found that 64 were females, 10 were hermaphrodites and 62 were males. When the hermaphrodites were counted as males, 52.9% were males. Of the remaining 120 frogs, 96 attained sexual maturity in the next year; 47 were females and 49 (51.0%) were males (Table 4).

b. Experimental series

Of a total of 967 seventh-generation offspring produced from 16 male sixth-generation offspring which had been derived from the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12, 498 frogs died or were killed within one month after metamorphosis. Of these young frogs, 203 were females, 31 were hermaphrodites and 264 were males. When the hermaphrodites were counted as males, 59.2% were males. Of the remaining 469 frogs, 236 attained sexual maturity in the next year; 88 were females and 148 (62.7%) were males (Table 4).

i) J.W68♀, Nos. 1～5×J_4^6(61OT2)♂, Nos. 1～5

Of the 342 normally metamorphosed seventh-generation offspring derived from the male nucleo-cytoplasmic hybrid No. 61OT2, 192 died or were killed within one month after metamorphosis. As a result of examining their sex, it was found that 80 were females, 13 were hermaphrodites and 99 were males. When the hermaphrodites were counted as males, 58.3% were males. Of the remaining 150 frogs, 79 attained sexual maturity in the next year; 29 were females and 50(63.3%) were males.

TABLE 4

Sex of seventh-generation offspring of three male nucleo-cytoplasmic hybrids

Parents		Age (days) at the time of climbing out (Mean)	No. of metamorphosed frogs	Sex of young frogs shortly after metamorphosis				Sex of mature frogs		
Female	Male			Total	♀	⚥	♂ (%)	Total	♀	♂ (%)
J. W68, Nos. 1～5	J_1^6(J×J), No. 1	84～97 (86.0)	75	45	21	4	20 (53.3)	25	12	13 (52.0)
	J_1^6(J×J), No. 2	84～95 (85.7)	47	17	8	3	6 (52.9)	26	12	14 (53.8)
	J_1^6(J×J), No. 3	83～100 (86.1)	55	25	12	2	11 (52.0)	18	9	9 (50.0)
	J_1^6(J×J), No. 4	84～99 (85.6)	79	49	23	1	25 (53.1)	27	14	13 (48.1)
	Total	83～100 (85.9)	256	136	64	10	62 (52.9)	96	47	49 (51.0)
J. W68, Nos. 1～5	J_4^6(61OT2), No. 1	83～96 (87.0)	52	22	7	3	12 (68.2)	14	5	9 (64.3)
	J_4^6(61OT2), No. 2	83～99 (87.2)	97	67	31	2	34 (53.7)	12	4	8 (66.7)
	J_4^6(61OT2), No. 3	83～97 (85.9)	81	51	22	2	27 (56.9)	17	7	10 (58.8)
	J_4^6(61OT2), No. 4	83～100 (85.3)	50	20	8	0	12 (60.0)	15	6	9 (60.0)
	J_4^6(61OT2), No. 5	84～97 (86.2)	62	32	12	6	14 (62.5)	21	7	14 (66.7)
	Total	83～100 (86.3)	342	192	80	13	99 (58.3)	79	29	50 (63.3)
J. W68, Nos. 1～5	J_2^6(61AT8), No. 1	85～103 (87.2)	64	34	13	2	19 (61.8)	12	5	7 (58.3)
	J_2^6(61AT8), No. 2	83～97 (85.1)	40	10	4	0	6 (60.0)	13	4	9 (69.2)
	J_2^6(61AT8), No. 3	84～99 (86.3)	40	10	5	1	4 (50.0)	7	2	5 (71.4)
	J_2^6(61AT8), No. 4	84～98 (86.0)	92	62	26	3	33 (58.1)	16	5	11 (68.8)
	J_2^6(61AT8), No. 5	83～92 (84.3)	31	—	—	—	—	13	4	9 (69.2)
	J_2^6(61AT8), No. 6	86～95 (85.0)	85	64	25	5	34 (60.9)	21	9	12 (57.1)
	Total	83～103 (85.7)	352	180	73	11	96 (59.4)	82	29	53 (64.6)
J. W68, Nos. 1～5	J_1^6(61AT12), No. 1	84～97 (85.9)	90	60	24	4	32 (60.0)	20	9	11 (55.0)
	J_1^6(61AT12), No. 2	84～97 (84.7)	43	13	6	0	7 (53.8)	15	6	9 (60.0)
	J_1^6(61AT12), No. 3	83～98 (85.2)	42	12	4	2	6 (66.7)	10	5	5 (50.0)
	J_1^6(61AT12), No. 4	84～90 (84.1)	27	—	—	—	—	11	3	8 (72.7)
	J_1^6(61AT12), No. 5	83～100 (86.3)	71	41	16	1	24 (61.0)	19	7	12 (63.2)
	Total	83～100 (85.2)	273	126	50	7	69 (60.3)	75	30	45 (60.0)

ii) J.W68♀, Nos. 1～5×J_2^6(61AT8)♂, Nos. 1～6

Of the 352 normally metamorphosed seventh-generation offspring derived from the nucleo-cytoplasmic hybrid No. 61AT8, 180 died or were killed within one month after metamorphosis. Of these young frogs, 73 were females, 11 were hermaphrodites and 96 were males. When the hermaphrodites were counted as males, 59.4% were males. Of the remaining 172 frogs, 82 attained sexual maturity in the next year, 29 were females and 53 (64.6%) were males.

iii) J.W68♀, Nos. 1～5×J_1^6(61AT12)♂, Nos. 1～5

Of the 273 normally metamorphosed seventh-generation offspring derived from the nucleo-cytoplasmic hybrid No. 61AT12, 126 died or were killed within one month after metamorphosis. The sex of these young frogs was examined. As a result, it was found that 50 were females, 7 were hermaphrodites and 69 were males. When the hermaphrodites were counted as males, 60.3% were males. Of the remaining 147 frogs, 75 attained sexual maturity in the next year. They consisted of 30 females and 45 (60.0%) males.

III. Eighth-generation offspring of three male nucleo-cytoplasmic hybrids

1. Production of eighth-generation offspring

a. Control series, J.W69♀, Nos. 1～3×J_1^7(J×J)♂, Nos. 1～3

Of the seventh-generation offspring produced in 1968 from a male sixth-generation offspring in the control series, 25 frogs attained sexual maturity in the breeding season of 1969. They consisted of 12 females and 13 males. Three of the latter were mated with three normal females (Nos. 1～3) collected from the field (Table 5). As a result of artificial insemination, 324 (86.6%) of 374 eggs in total cleaved normally. Thirteen of the normally cleaved eggs died of

TABLE 5

Viability of eighth-generation offspring of three male nucleo-cytoplasmic hybrids

Parents		No. of eggs	No. of normally cleaved eggs	No. of normal neurulae	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally metamorphosed frogs
Female	Male						
J. W69, Nos. 1～3	J_1^7(J×J), No. 1	125	95 (76.0%)	94 (75.2%)	94 (75.2%)	90 (72.0%)	90 (72.0%)
	J_1^7(J×J), No. 2	119	107 (89.9%)	106 (89.1%)	104 (87.4%)	104 (87.4%)	102 (85.7%)
	J_1^7(J×J), No. 3	130	122 (93.8%)	122 (93.8%)	120 (92.3%)	117 (90.0%)	116 (89.2%)
	Total	374	324 (86.6%)	322 (86.1%)	318 (85.0%)	311 (83.2%)	308 (82.4%)
J. W69, Nos. 1～3	J_2^7(61OT2), No. 1	114	107 (93.9%)	102 (89.5%)	100 (87.7%)	97 (85.1%)	96 (84.2%)
	J_2^7(61OT2), No. 2	106	89 (84.0%)	89 (84.0%)	89 (84.0%)	87 (82.1%)	87 (82.1%)
	J_2^7(61OT2), No. 3	117	96 (82.1%)	95 (81.2%)	93 (79.5%)	91 (77.8%)	87 (74.4%)
	Total	337	292 (86.6%)	286 (84.9%)	282 (83.7%)	275 (81.6%)	270 (80.1%)
J. W69, Nos. 1～3	J_6^7(61AT8), No. 1	112	95 (84.8%)	93 (83.0%)	92 (82.1%)	92 (82.1%)	90 (80.4%)
	J_6^7(61AT8), No. 2	135	122 (90.4%)	110 (81.5%)	110 (81.5%)	109 (80.7%)	106 (78.5%)
	J_6^7(61AT8), No. 3	136	120 (88.2%)	117 (86.0%)	117 (86.0%)	115 (84.6%)	110 (80.9%)
	Total	383	337 (88.0%)	320 (83.6%)	319 (83.3%)	316 (82.5%)	306 (79.9%)
J. W69, Nos. 1～3	J_1^7(61AT12), No. 1	121	115 (95.0%)	115 (95.0%)	113 (93.4%)	107 (88.4%)	102 (84.3%)
	J_1^7(61AT12), No. 2	126	105 (83.3%)	103 (81.7%)	103 (81.7%)	103 (81.7%)	100 (79.4%)
	J_1^7(61AT12), No. 3	152	126 (82.9%)	121 (79.6%)	120 (78.9%)	120 (78.9%)	115 (75.7%)
	Total	399	346 (86.7%)	339 (85.0%)	336 (84.2%)	330 (82.7%)	317 (79.4%)

abnormalities at various embryonic stages, while 311 (83.2%) hatched normally. After the hatching stage, three tadpoles died of abnormalities, while 308 (82.4%) metamorphosed normally.

b. Experimental series

Nine male seventh-generation offspring derived from the three male nucleocytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12 were mated with the same three normal females Nos. 1～3 as those in the control series to produce eighth-generation offspring (Table 5). As a result of artificial insemination, 975 (87.1%) of 1119 eggs in total cleaved normally. At the embryonic stage, 54 of the normally cleaved eggs died of abnormalities, while 921 (82.3%) hatched normally. During the tadpole stage, 28 individuals died of abnormalities, and eventually 893 (79.8%) metamorphosed normally.

i) J.W69♀, Nos. 1～3×J_2^7(61OT2)♂, Nos. 1～3

Of the seventh-generation offspring produced in 1968 from a male sixth-generation offspring, J_4^6(61OT2)♂, No. 2, by mating with five normal females from the field, 12 frogs attained sexual maturity in the breeding season of 1969. Four of them were females and eight were males. Three of the latter were mated with the three normal females Nos. 1～3 from the field to produce eighth-generation offspring. As a result, it was found that 292 (86.6%) of 337 eggs in total cleaved normally. Seventeen of the normally cleaved eggs died of abnormalities at various embryonic stages, while the remaining 275 (81.6%) hatched normally. Only five tadpoles died of abnormalities by the stage of metamorphosis, and 270 (80.1%) metamorphosed normally.

ii) J.W69♀, Nos. 1～3×J_6^7(61AT8)♂, Nos. 1～3

Twenty-one seventh-generation offspring produced in 1968 from a male sixth-generation offspring, J_2^6(61AT8)♂, No. 6, by mating with five normal females from the field attained sexual maturity in the breeding season of 1969. They consisted of 9 females and 12 males. Three of the latter were mated with the three normal females Nos. 1～3 to produce eighth-generation offspring. As a result, 337 (88.0%) of 383 eggs in total cleaved normally. Of the normally cleaved eggs, 21 died of abnormalities at various embryonic stages, while 316 (82.5%) hatched normally. During the tadpole stage, ten individuals died of abnormalities, and 306 (79.9%) metamorphosed normally.

iii) J.W69♀, Nos. 1～3×J_1^7(61AT12)♂, Nos. 1～3

Twenty seventh-generation offspring produced in 1968 from a male sixth-generation offspring, J_1^6(61AT12)♂, No. 1, attained sexual maturity in the breeding season of 1969. They consisted of 9 females and 11 males. Three of the latter were mated with the three normal females Nos. 1～3 from the field to produce eighth-generation offspring. As a result, 346 (86.7%) of 399 eggs in total cleaved normally. Sixteen of the normally cleaved eggs died of abnormalities at various embryonic stages, while 330 (82.7%) hatched normally. During the tadpole stage, 13 individuals died of abnormalities, and 317 (79.4%) metamorphosed normally.

2. Sex of eighth-generation offspring

a. Control series

Of the 308 normally metamorphosed frogs in the control series, 218 died or were killed within one month after metamorphosis. As a result of sex examination, it was found that 108 were females and 110 (50.5%) were males. Of the remaining 90 frogs, 71 attained sexual maturity in the next year. They consisted of 35 females and 36 (50.7%) males (Table 6).

b. Experimental series

From matings between 9 male seventh-generation offspring derived from the three male nucleo-cytoplasmic hybrids Nos. 61OT2, 61AT8 and 61AT12 and the three normal females Nos. 1～3 from the field, 893 normally metamorphosed eighth-generation offspring were produced. Of these frogs, 803 died or were killed within one month after metamorphosis. As a result of sex examination, it was found that 400 were females, 7 were hermaphrodites and 396 were males. When the hermaphrodites were counted as males, 50.2% were males. Of the remaining 90 frogs, 63 attained sexual maturity in the next year. They con-

TABLE 6

Sex of eighth-generation offspring of three male nucleo-cytoplasmic hybrids

Parents		Age (days) at the time of climbing out (Mean)	No. of metamorphosed frogs	Sex of young frogs shortly after metamorphosis				Sex of mature frogs		
Female	Male			Total	♀	☿	♂ (%)	Total	♀	♂ (%)
J. W69, Nos. 1～3	J_1^7(J×J), No. 1	90～115 (97.4)	90	60	29	0	31 (51.7)	24	12	12 (50.0)
	J_1^7(J×J), No. 2	91～121 (98.5)	102	72	37	0	35 (48.6)	26	12	14 (53.8)
	J_1^7(J×J), No. 3	90～119 (97.6)	116	86	42	0	44 (51.2)	21	11	10 (47.6)
	Total	90～121 (97.8)	308	218	108	0	110 (50.5)	71	35	36 (50.7)
J. W69, Nos. 1～3	J_2^7(61OT2), No. 1	89～117 (86.3)	96	66	34	0	32 (48.5)	23	12	11 (47.8)
	J_2^7(61OT2), No. 2	89～119 (97.0)	87	57	28	2	27 (50.9)	17	8	9 (52.9)
	J_2^7(61OT2), No. 3	89～120 (98.2)	87	57	29	0	28 (49.1)	23	10	13 (56.5)
	Total	89～120 (93.8)	270	180	91	2	87 (49.4)	63	30	33 (52.4)
J. W69, Nos. 1～3	J_6^7(61AT8), No. 1	91～115 (97.7)	90	90	44	0	46 (51.1)			
	J_6^7(61AT8), No. 2	89～123 (95.3)	106	106	56	0	50 (47.2)			
	J_6^7(61AT8), No. 3	89～120 (97.2)	110	110	43	1	66 (60.9)			
	Total	89～123 (96.7)	306	306	143	1	162 (53.3)			
J. W69, Nos. 1～3	J_1^7(61AT12), No. 1	90～116 (98.0)	102	102	51	2	49 (50.0)			
	J_1^7(61AT12), No. 2	91～115 (97.7)	100	100	54	1	45 (46.0)			
	J_1^7(61AT12), No. 3	88～121 (97.5)	115	115	61	1	53 (47.0)			
	Total	88～121 (97.7)	317	317	166	4	147 (47.6)			

sisted of 30 females and 33 (52.4%) males (Table 6).

i) J.W69♀, Nos. 1～3×J_2^7(61OT2)♂, Nos. 1～3

Of the 270 eighth-generation offspring derived from the male nucleo-cytoplasmic hybrids No. 61OT2, 180 frogs died or were killed within one month after metamorphosis. Of these frogs, 91 were females, 2 were hermaphrodites and 87 were males. When the hermaphrodites were counted as males, 49.4% were males. Sixty-three of the remaining 90 frogs attained sexual maturity in the next year; 30 were females and 33 (52.4%) were males.

ii) J.W69♀, Nos. 1～3×J_6^7(61AT8)♂, Nos. 1～3

The 306 normally metamorphosed eighth-generation offspring derived from the male nucleo-cytoplasmic hybrid No. 61AT8 were all killed within one month after metamorphosis to examine their sex. As a result, 143 were females, one was a hermaphrodite and 162 were males. When the hermaphrodite was counted as a male, 53.3% were males.

iii) J.W69♀, Nos. 1～3×J_1^7(61AT12)♂, Nos. 1～3

All the 317 normally metamorphosed eighth-generation offspring derived from the male nucleo-cytoplasmic hybrid No. 61AT12 died or were killed within one month after metamorphosis. As a result of sex examination, it was found that 166 were females, 4 were hermaphrodites and 147 were males. When the hermaphrodites were counted as males, 47.6% were males.

IV. Ninth-generation offspring of a male nucleo-cytoplasmic hybrid

1. Production of ninth-generation offspring

a. Control series, J.W70♀, Nos. 1～3×J_1^8(J×J)♂, Nos. 1～3

Of the eighth-generation offspring produced in 1969 from a male offspring, J_1^7(J×J)♂, No. 1, in the control series by mating with three normal females from the field, 24 frogs attained sexual maturity in the breeding season of 1970. They consisted of 12 females and 12 males. Three of the latter were mated with three normal females Nos. 1～3 collected from the field to produce ninth-generation offspring (Table 7). As a result of insemination, 215 (92.7%) of 232 eggs in total cleaved normally. While five of the normally cleaved eggs died of abnormalities by the hatching stage, 210 (90.5%) hatched normally. During the tadpole stage, only four individuals died of abnormalities, and 206 (88.8%) completed normally their metamorphosis.

b. Experimental series

Twenty-three eighth-generation offspring produced in 1969 from a male seventh-generation offspring, J_2^7(61OT2)♂, No. 3, attained sexual maturity in the breeding season of 1970. Of these frogs, 10 were females and 13 were males. Five of the females were mated with a normal male (No. 1) collected from the field, while five of the males were mated with the same three normal females Nos. 1～3 as those of the control series to produce ninth-generation offspring (Table 7).

TABLE 7

Viability of ninth-generation offspring of a male nucleo-cytoplasmic hybrid

Parents		No. of eggs	No. of normally cleaved eggs	No. of normal neurulae	No. of normal tail-bud embryos	No. of normally hatched tadpoles	No. of normally metamorphosed frogs
Female	Male						
J. W70, Nos. 1～3	J_1^8(J×J), No. 1	65	62 (95.4%)	62 (95.4%)	61 (93.8%)	61 (93.8%)	60 (92.3%)
	J_1^8(J×J), No. 2	79	70 (88.6%)	67 (84.8%)	67 (84.8%)	67 (84.8%)	67 (84.8%)
	J_1^8(J×J), No. 3	88	83 (94.3%)	83 (94.3%)	82 (93.2%)	82 (93.2%)	79 (89.8%)
	Total	232	215 (92.7%)	212 (91.4%)	210 (90.5%)	210 (90.5%)	206 (88.8%)
J. W70, Nos. 1～3	J_3^8(61OT2), No. 1	103	84 (81.6%)	84 (81.6%)	82 (79.6%)	82 (79.6%)	82 (79.6%)
	J_3^8(61OT2), No. 2	99	79 (79.8%)	79 (79.8%)	75 (75.8%)	72 (72.7%)	70 (70.7%)
	J_3^8(61OT2), No. 3	127	108 (85.0%)	102 (80.3%)	102 (80.3%)	90 (70.9%)	90 (70.9%)
	J_3^8(61OT2), No. 4	122	112 (91.8%)	112 (91.8%)	110 (90.2%)	103 (84.4%)	100 (82.0%)
	J_3^8(61OT2), No. 5	134	119 (88.8%)	117 (87.3%)	117 (87.3%)	117 (87.3%)	114 (85.1%)
	Total	585	502 (85.8%)	494 (84.4%)	486 (83.1%)	464 (79.3%)	456 (77.9%)
J_3^8(61OT2), No. 1	J. W70, No. 1	136	115 (84.6%)	112 (82.4%)	111 (81.6%)	102 (75.0%)	102 (75.0%)
J_3^8(61OT2), No. 2		175	156 (89.1%)	152 (86.9%)	150 (85.7%)	146 (83.4%)	145 (82.9%)
J_3^8(61OT2), No. 3		194	170 (87.6%)	167 (86.1%)	167 (86.1%)	154 (79.4%)	152 (78.4%)
J_3^8(61OT2), No. 4		201	164 (81.6%)	153 (76.1%)	150 (74.6%)	149 (74.1%)	149 (74.1%)
J_3^8(61OT2), No. 5		179	155 (86.6%)	153 (85.5%)	153 (85.5%)	140 (78.2%)	134 (74.9%)
	Total	885	760 (85.9%)	737 (83.3%)	731 (82.6%)	691 (78.1%)	682 (77.1%)

i) J.W70♀, Nos. 1～3×J_3^8(61OT2)♂, Nos. 1～5

Ninth-generation offspring were produced from the five male eighth-generation offspring by mating with the three normal females Nos. 1～3 from the field. As a result of insemination, 502 (85.8%) of 585 eggs in total cleaved normally. Of the normally cleaved eggs, 38 died of abnormalities at various embryonic stages, while 464 (79.3%) hatched normally. During the tadpole stage, only eight individuals died of abnormalities and 456 (77.9%) metamorphosed normally.

ii) J_3^8(61OT2)♀, Nos. 1～5×J.W70♂, No. 1

Ninth-generation offspring were produced from the five female eighth-generation offspring by mating with the single normal male No. 1 from the field. As a result of insemination, 760 (85.9%) of 885 eggs in total cleaved normally. Of the normally cleaved eggs, 69 died of abnormalities at various embryonic stages, while the other 691 (78.1%) hatched normally. Nine tadpoles died of abnormalities by the stage of metamorphosis, and the other 682 (77.1%) metamorphosed normally.

2. Sex of ninth-generation offspring

a. Control series

Of the 206 normally metamorphosed ninth-generation offspring in the con-

trol series, eight died within one month after metamorphosis, and 168 were killed just one month after metamorphosis to examine their sex. Of these frogs, 85 were females and 83 (49.4%) were males. Twenty-one of the remaining 30 frogs attained sexual maturity in the next year. They consisted of 10 females and 11 males (Table 8).

b. Experimental series

i) J.W70♀, Nos. 1～3×J_3^8(61OT2)♂, Nos. 1～5

Of the 456 normally metamorphosed ninth-generation offspring produced from the five males which had been derived from the male nucleo-cytoplasmic hybrid No. 61OT2, 19 died within one month after metamorphosis, and 387 were killed just one month after metamorphosis to examine their sex. As a result, it was found that 194 were females and 193 (49.9%) were males. Thirty-five of the remaining 50 frogs attained sexual maturity in the next year; 17 were females and 18 (51.4%) were males (Table 8).

ii) J_3^8(61OT2)♀, Nos. 1～5×J.W70♂, No. 1

Of the 682 normally metamorphosed ninth-generation offspring produced from

TABLE 8

Sex of ninth-generation offspring of a male nucleo-cytoplasmic hybrid

Parents		Age (days) at the time of climbing out (Mean)	No. of meta-mor-phosed frogs	Sex of young frogs shortly after metamorphosis				Sex of mature frogs		
Female	Male			Total	♀	⚥	♂ (%)	Total	♀	♂ (%)
J. W70, Nos. 1～3	J_1^8(J×J), No. 1	88～119 (96.7)	60	30	15	0	15 (50.0)	21	10	11 (52.4%)
	J_1^8(J×J), No. 2	91～107 (97.3)	67	65	34	0	31 (47.7)			
	J_1^8(J×J), No. 3	90～121 (97.1)	79	73	36	0	37 (50.7)			
	Total	88～121 (97.0)	206	168	85	0	83 (49.4)			
J. W70, Nos. 1～3	J_3^8(61OT2), No. 1	90～114 (95.9)	82	80	41	0	39 (48.8)			
	J_3^8(61OT2), No. 2	90～112 (96.6)	70	67	32	0	35 (52.2)			
	J_3^8(61OT2), No. 3	90～110 (97.2)	90	82	42	0	40 (48.8)			
	J_3^8(61OT2), No. 4	86～117 (95.8)	100	94	46	0	48 (51.1)			
	J_3^8(61OT2), No. 5	88～119 (97.2)	114	64	33	0	31 (48.4)	35	17	18 (51.4%)
	Total	86～119 (96.5)	456	387	194	0	193 (49.9)			
J_3^8(61OT2), No. 1	J. W70, No. 1	91～120 (97.0)	102	97	51	0	46 (47.4)			
J_3^8(61OT2), No. 2		90～115 (97.5)	145	114	55	0	59 (51.8)			
J_3^8(61OT2), No. 3		89～115 (96.1)	152	135	69	0	66 (48.9)			
J_3^8(61OT2), No. 4		89～112 (97.3)	149	127	62	0	65 (51.2)			
J_3^8(61OT2), No. 5		89～114 (97.2)	134	126	60	0	66 (52.4)			
	Total	89～120 (97.0)	682	599	297	0	302 (50.4)			

the five females which had been derived from the male nucleo-cytoplasmic hybrid No. 61OT2, 83 frogs died within one month after metamorphosis. All the other frogs were killed to examine their sex. As a result, it was found that 297 were females and 302 (50.4%) were males.

DISCUSSION

1. Reproductive capacity of the descendants

In 1963, the authors reported that three male nucleo-cytoplasmic hybrids constructed of *Rana ornativentris* cytoplasm and *Rana japonica* nuclei produced a small number of viable first-generation offspring by mating with normal females of the nuclear species, and that the low reproductive capacities of the nucleo-cytoplasmic hybrids were not improved in the next generation, in spite of the matings with the nuclear species. Differing from the male nucleo-cytoplasmic hybrids, two female ones produced only inviable offspring which died by the hatching stage. The reproductive capacities of the first- to fourth-generation offspring derived from the three male nucleo-cytoplasmic hybrids were reported by the authors in 1972. Male first- to fourth- and female first- and second-generation offspring were mated with normal frogs of the nuclear species. As a result, the offspring of the nucleo-cytoplasmic hybrids in each generation were remarkably inferior to the control frogs in reproductive capacity, although the third- and fourth-generation offspring as a whole seemed slightly better than the first- and second-generation offspring.

In the present study, the reproductive capacities of male fifth-, sixth- and seventh-generation offspring of the three male nucleo-cytoplasmic hybrids and male eighth-generation offspring of one of the latter were examined. The change of reproductive capacity in the male offspring from the first to the eighth generation is shown in Fig. 1. As shown in this figure, the reproductive capacity was remarkably improved in the fifth- and sixth-generation offspring; a normal reproductive capacity was almost completely recovered in the seventh-generation offspring. By the sperm of male fifth- and sixth-generation offspring in each of the three series derived from the three nucleo-cytoplasmic hybrids, Nos. 61OT2, 61AT8 and 61AT12, about half the number of eggs of normal females from the field developed into normally metamorphosed frogs. About 80 percent of eggs of normal females became normally metamorphosed frogs by the sperm of male seventh-generation offspring in each of the three series. Male eighth-generation offspring in the series No. 61OT2 also made 78 percent of the eggs of normal females to develop into normal frogs. These high percentages of eggs which developed normally in the eighth and ninth generations derived from three male nucleo-cytoplasmic hybrids show a rapid recovery of the seventh-generation offspring in reproductive capacity, although they were still slightly inferior to those in the control series.

As already stated in the authors' previous paper (1972), the males used for pro-

ducing their offspring by mating with normal females from the field were selected as the most normal in appearance and behavior from the groups in which comparatively numerous frogs were obtained in each generation of each series, that is, the best frogs were selected from generation to generation to obtain their offspring. As dominant gene mutations or visible chromosomal aberrations which occurred in germ cells of the nucleo-cytoplasmic hybrids should rapidly diminish with the progress of generations, the low reproductive capacities of the first- to sixth-generation offspring seemed to be attributable to chromosomal aberrations which newly occurred in germ cells of these offspring probably at the time of synapsis by activation of latent damages existing in their chromosomes. These latent damages in the chromosomes of germ cells were considered to have been transmitted from generation to generation since the production of the nucleo-cytoplasmic hybrids. Accordingly, the recovery of the seventh-generation offspring in reproductive capacity seems to show almost complete disappearance of chromosomes having such latent damages from their germ cells.

2. Sex of the descendants

The three male nucleo-cytoplasmic hybrids, Nos. 61OT2, 61AT8 and 61AT12, were in 14 metamorphosed nucleo-cytoplasmic hybrids obtained in 1961. Of these frogs, nine were females and five were males. The sex of the first-generation offspring produced from the three male nucleo-cytoplasmic hybrids and of the second-generation offspring from 18 male first-generation offspring by mating with normal females of the nuclear species, *Rana japonica*, has been reported by the authors (1963a). The sex of the second- and third-generation offspring produced from male and female first- and second-generation offspring as well as the sex of the fourth- and fifth-generation offspring produced from male third- and fourth-generation offspring has also been repoted by the authors (1972). In each of these five generations, an overwhelming majority of mature frogs was males.

The sex of the sixth- to ninth-generation offspring was examined in the present investigation to elucidate the recovery of a 1 : 1 sex ratio. While the sixth- to eighth-generation offspring were produced by male fifth- to seventh-generation offspring derived from the three male nucleo-cytoplasmic hybrids, Nos. 61OT2, 61AT8 and 61AT12, the ninth-generation offspring were produced by male eighth-generation offspring derived from No. 61OT2 alone, as there were no remarkable differences in sex ratio among the three series derived from the three nucleo-cytoplasmic hybrids. The sex ratio in the nine consecutive generations each of which was produced from males of the preceding generation by mating with normal females of the nuclear species is presented in Table 9, in comparison with the percentages of normally metamorphosed frogs shown in Fig. 1.

In the four generations from the second to the fifth in the three series derived from the three nucleo-cytoplasmic hybrids, Nos. 61OT2, 61AT8 and 61AT12, 55~69 percent of young frogs shortly after metamorphosis were males including a few hermaphrodites, except 48 percent in the second-generation offspring of No. 61OT2 and 39 percent in the fourth-generation offspring of No. 61AT12

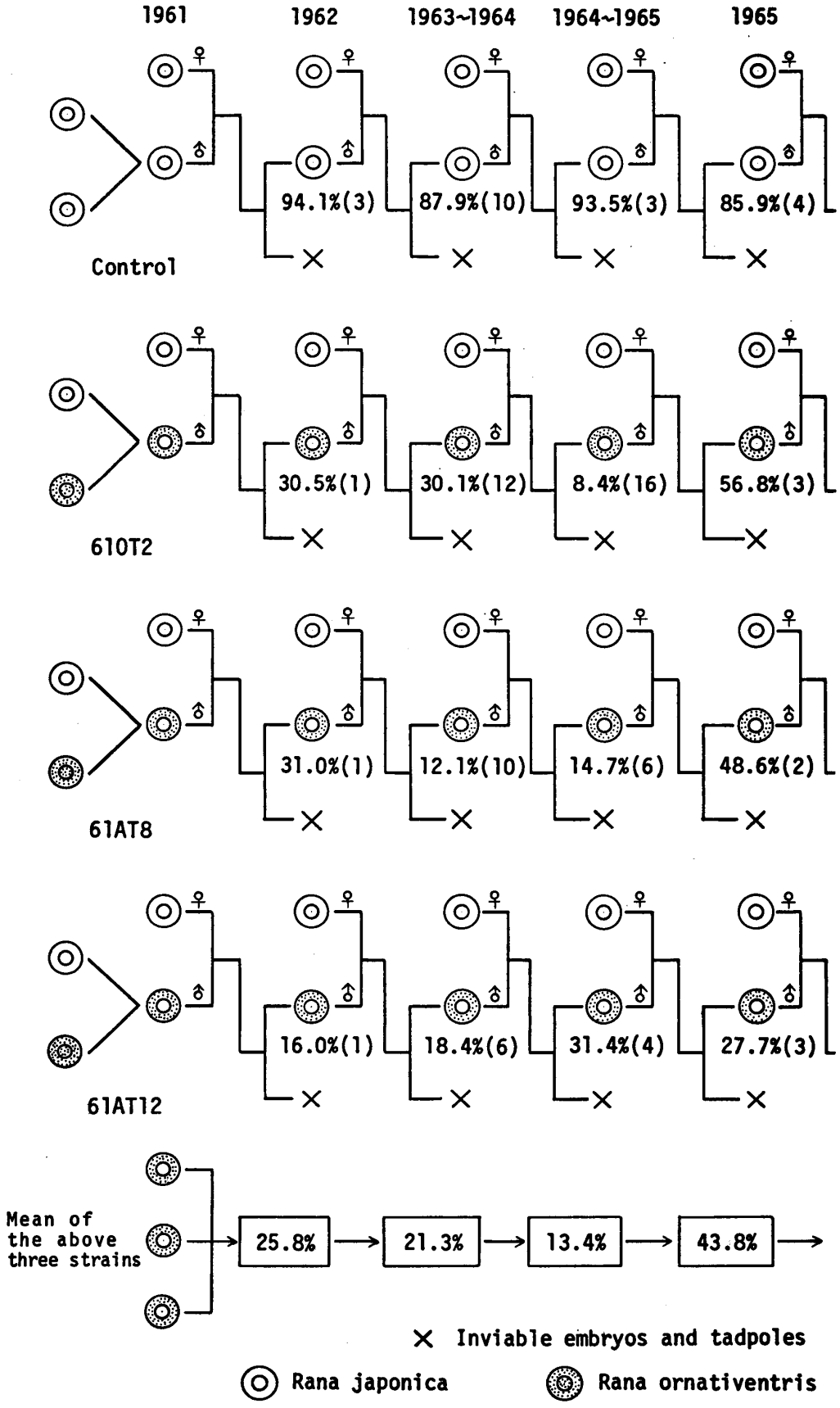
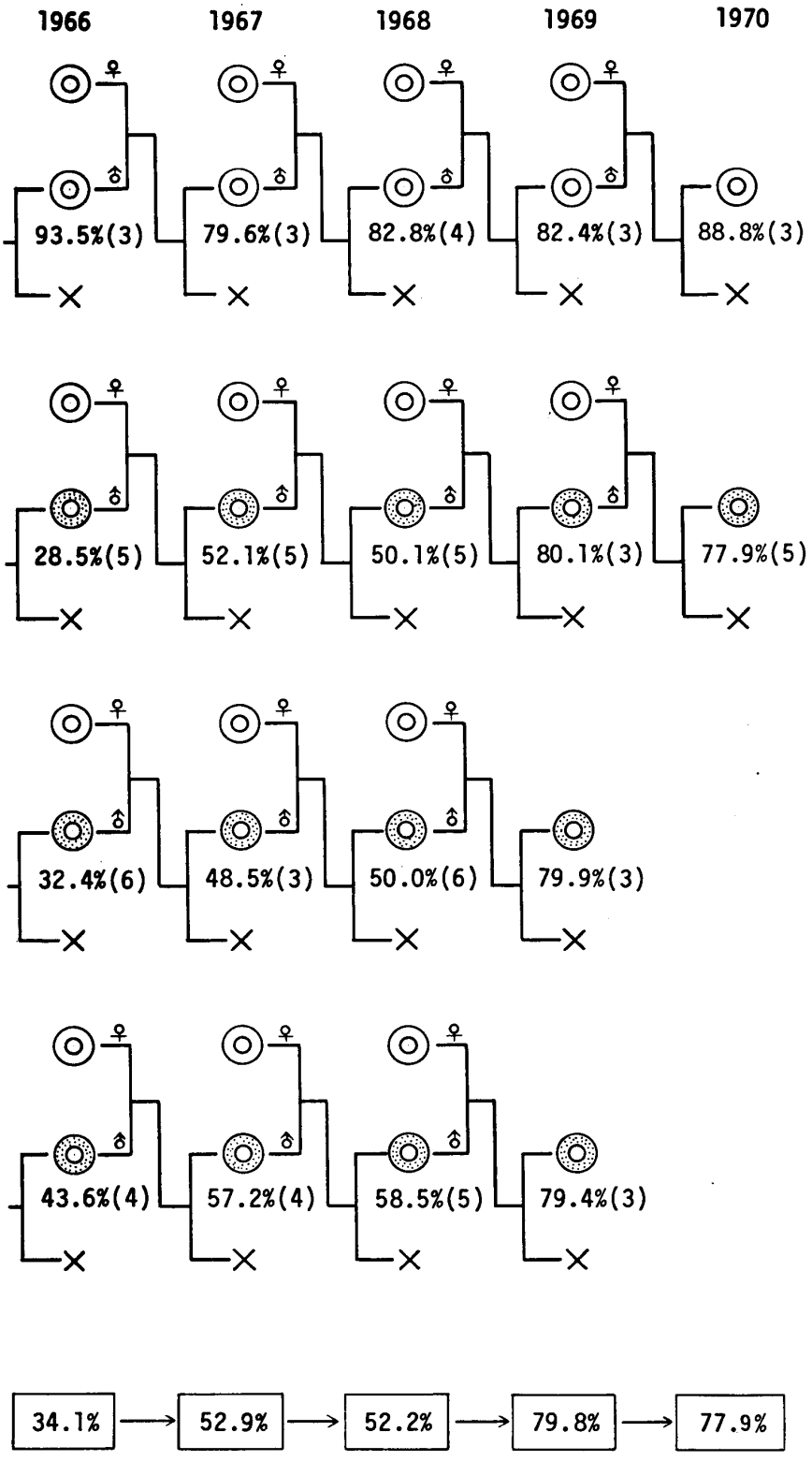


Fig. 1. Percentages of normally metamorphosed frogs in nine consecutive generations with females of the nuclear species. Parentheses show the number of male parents. The metamorphosed frogs obtained from all the male parents.



Nucleo-cytoplasmic hybrids and their offspring

derived from three male nucleo-cytoplasmic hybrids by repeated matings
mean value of the three strains in each generation is the percentage of the

(Table 9). At the sexually mature stage, 80~100 percent of frogs were males in all the four generations of the three experimental series, although they were small in number. These unusual sex ratios were in a striking contrast to those in the control series. In the latter, 49~52 percent of young frogs were males including a few hermaphrodites, and nearly equal numbers of males and females were always found among mature frogs. The situation in the experimental series changed in the sixth and seventh generations, where 58~61 percent of young frogs and 60~79 percent of mature frogs were males. In the sixth and seventh generations of control series, 50~53 percent of young and mature frogs were males.

In the eighth generation, 48~53 percent of young frogs in the three experimental series were males including a few hermaphrodites, while 51 percent of the control frogs were males. At the sexually mature stage, 33 (52%) of 63 offspring derived from No. 61OT2 were males, while 36 (51%) of 71 controls were males (Table 9). In the ninth generation, the sex ratio of the frogs derived from No. 61OT2 was about 1: 1 at both young and mature stages, as in the control series. Thus, a normal sex ratio 1: 1 seems to be recovered in the eighth- and ninth-generation offspring. This corresponds well to the recovery of viability found in the eighth- and ninth-generation offspring (Fig. 1).

The preponderance of males in number in the generations from the first to the

TABLE 9

Sex ratios of offspring in nine generations produced from three male nucleo-cytoplasmic

Number of female and male N-C hybrids (O)JJ produced in 1961: 9 females and 5 males (From

Sex and percentages of males in

Generation		No. 61OT2			No. 61AT8		
		♀	♂	%	♀	♂	%
First		13	38	74.5	18	21	53.8
Second	Y	152	139 (3)	47.8	88	115 (1)	56.7
	M	0	12	100.0			
Third	Y	67	87 (7)	56.5	24	36 (9)	60.0
	M	1	18	94.7	0	4	100.0
Fourth	Y	137	229 (4)	62.6	86	189 (11)	68.7
	M	6	24	80.0	1	8	88.9
Fifth	Y	55	96 (24)	63.6	97	153 (43)	61.2
	M	1	21	95.5	2	13	86.7
Sixth	Y	89	122 (15)	57.8	35	55 (6)	61.1
	M	21	78	78.8	34	66	66.0
Seventh	Y	80	112 (13)	58.3	73	107 (11)	59.4
	M	29	50	63.3	29	53	64.6
Eighth	Y	91	89 (2)	49.4	143	163 (1)	53.3
	M	30	33	52.4			
Ninth	Y	194	193 (0)	49.9			
	M	17	18	51.4			

Y, Young frogs shortly after metamorphosis.
M, Mature frogs.

seventh is principally attributable to sex-reversal of genetic females. In the authors' previous paper (1972), it was shown in detail that the sex-reversal found in the offspring of nucleo-cytoplasmic hybrids was always accompanied with anatomically and cytologically abnormal embryos and tadpoles, together with their low viability. The causes for these sex-reversal, anatomical and chromosomal abnormalities and low viability seemed eventually to exist in the chromosomes of gametes produced by their parents. The recovery of normal viability as well as normal sex ratio in the eighth- and ninth-generation offspring seems to indicate that latent damages causing abnormal gametogenesis were removed from the chromosomes of the male seventh- and eighth-generation offspring. As the males used for producing offspring by mating with normal females from the field in each generation were those which were sorted out of many brothers as the best in development and growth, their chromosomes seemed to be fewer in latent damages than those of the other males. Accordingly, it is probable that the latent damages of chromosomes derived from the interaction between *Rana ornativentris* cytoplasm and *Rana japonica* nuclei are left from generation to generation until the time of gametogenesis of the sixth-generation offspring at least.

There is a question if a sex-reversed female was used for producing offspring, since sex-reversal of genetic females into males occurred very frequently in generations from the first to the sixth (Table 9). If a sex-reversed genetic female was

hybrids (Parentheses present the number of hermaphrodites which are counted as males.)

Table 7, 1963a) Males used for producing offspring: Nos. 61OT2, 61AT8 and 61AT12

the offspring of 9 generations

No. 61AT12			Control			Remarks
♀	♂	%	♀	♂	%	
0	12	100.0	64	75	54.0	From Table 11 (1963a)
42	57	57.6	393	382 (7)	49.3	From Table 16
			10	8	44.4	(1963a), 13(1972)
50	60 (12)	54.5	143	143 (2)	50.0	From Tables 14
0	9	100.0	27	30	52.6	~16, 28 (1972)
173	109 (5)	38.7	103	111 (0)	51.9	From Tables 29
5	25	83.3	7	8	53.3	~31 (1972)
68	105 (18)	60.7	410	415 (9)	50.3	From Tables 37,
2	21	91.3	34	39	53.4	38 (1972)
72	107 (7)	59.8	52	56 (3)	51.9	From Table 2
25	77	75.5	53	54	50.5	
50	76 (7)	60.3	64	72 (10)	52.9	From Table 4
30	45	60.0	47	49	51.0	
166	151 (4)	47.6	108	110 (0)	50.5	From Table 6
			35	36	50.7	
			85	83 (0)	49.4	From Table 8
			10	11	52.4	

used on one occasion, all the descendants derived from this frog should be genetic females. However, as no sign of such a case was found in all the generations derived from the three male nucleo-cytoplasmic hybrids, all the males used for producing offspring are considered to be genetic males. The exceptional case that 39 percent of young frogs were males in the fourth generation derived from No. 61AT12 may be explained by unusually delayed differentiation of testicular structure in the gonads of some genetic males.

SUMMARY

1. Sixth- to ninth-generation offspring of three male nucleo-cytoplasmic hybrids consisting of *Rana ornativentris* cytoplasm and *Rana japonica* nuclei were produced from male offspring by mating with female *Rana japonica* collected from the field, in order to shed light on how many generations are required for improvement of reproductive capacities and for restoration of normal sex ratio of these offspring.

2. The reproductive capacity was remarkably improved in the fifth- and sixth-generation offspring and then almost completely recovered in the seventh- and eighth-generation offspring.

3. While males were overwhelmingly numerous as compared with females among mature frogs in the second, third, fourth and fifth generations derived from each male nucleo-cytoplasmic hybrid, this preponderance of males in number became remarkably lower in the sixth and seventh generations. In the six generations from the second to the seventh, males were always slightly more numerous than females among young frogs shortly after metamorphosis, with a few exceptional cases. In the eighth and ninth generations, nearly an equal number of males and females was found among both young and mature frogs.

4. The recovery of normal viability as well as normal sex ratio in the eighth- and ninth-generation offspring seems to indicate that latent damages causing abnormal gametogenesis were removed from the chromosomes of the male seventh- and eighth-generation offspring.

ACKNOWLEDGMENTS

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