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Reproductive Capacity of Allotriploids between *Rana tsushimensis* from Tsushima and *Rana japonica* from Ichinoseki and Hiroshima

By

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ABSTRACT

Reciprocal allotriploid frogs, (T)TTI, (I)IIT, (T)TTH and (H)HHT, were produced from crossings between *Rana tsushimensis* from Tsushima, (T)TT, and *Rana japonica* from Ichinoseki, (I)II, and Hiroshima, (H)HH, by heat-shock treatment of fertilized eggs. Almost all the allotriploids developed normally and became males. In contrast, diploid hybrids between female *R. tsushimensis* and male *R. japonica* from Ichinoseki and the reciprocal diploid hybrids between *R. japonica* from Hiroshima and *R. tsushimensis* died of underdevelopment at the tadpole stage. However, diploid hybrids between female *R. japonica* from Ichinoseki and male *R. tsushimensis* could barely complete metamorphosis, although they died of infirmity within 1~3 months after metamorphosis.

Mature allotriploids were intermediate between the two parental species in external characters. They were more similar to the parental species giving two genomes than to the species giving one genome. A total of 65 mature male allotriploids including two kinds, (H)HHT and (T)TTH, between *R. japonica* from Hiroshima and *R. tsushimensis* and one kind, (I)IIT, between female *R. japonica* from Ichinoseki and male *R. tsushimensis* were completely sterile. On the other hand, 16 of 44 male allotriploids, (T)TTI, between female *R. tsushimensis* and male *R. japonica* from Ichinoseki were fertile. The spermatogenesis in these allotriploids was completely or partially normal. The other 28 male allotriploids, (T)TTI, were sterile. A total of 419 offspring were produced from seven of the above 16 male allotriploids by backcrossing with female *R. tsushimensis*. They were all diploids and of the *R. tsushimensis* type in external characters. Most of them (96.9%) were females. They showed the same electrophoretic patterns as those of *R. tsushimensis* at 14 loci of enzymes and blood proteins. These findings seemed to indicate that some of the male allotriploids reproduce by hybridogenesis, in which the *R. japonica* genome was eliminated during spermatogenesis.

INTRODUCTION

Generally speaking, triploid frogs are superior to diploid ones of the same species in viability (KAWAMURA, 1952a; NISHIOKA, 1971, 1983). BOGART (1972) reported that polyploids between distantly related species of *Bufo* were more viable than the diploids, and the triploids were found most commonly in the polyploids. According to ELINSON (1977), the diploid hybrids between female *Rana catesbeiana*

and male *R. clamitans* stopped their development at the embryonic stage. On the other hand, ELINSON and BRIEDIS (1981) reported that 36 allotriploids constructed of two genomes of *R. catesbeiana* and one genome of *R. clamitans* developed normally through metamorphosis. Of 36 allotriploids, 35 were males and one was a female. According to them, this predominance of males was probably due to triploidy. KAWAMURA and NISHIOKA (1975, 1977) reported that the reciprocal hybrids between *R. nigromaculata* and *R. lessonae* died at the embryonic stage, while according to NISHIOKA and OHTANI (1991) the allotriploids which consisted of two genomes of *R. nigromaculata* and one genome of *R. lessonae* were viable and became mature.

SUMIDA (1981) reported that in reciprocal hybrids between the Ichinoseki and Hiroshima populations of *Rana japonica,* males were more numerous than females and their spermatogenesis was always abnormal. Moreover, the abnormality was severer in the hybrids between females of the Hiroshima population and males of the Ichinoseki population than the reciprocal hybrids. KAWAMURA and NISHIOKA (1977) and KAWAMURA, NISHIOKA and UEDA (1981) have reported that two brown frog species, *R. japonica* from Hiroshima and *R. tsushimensis* from Tsushima Island, are isolated by hybrid inviability, that is, all the diploid hybrids between the two species died at the tadpole stage.

In the present study, the authors produced reciprocal diploid hybrids and allotriploids between *R. tsushimensis* and *R. japonica* from the Ichinoseki and Hiroshima populations in order to clarify the differences among various diploid and triploid hybrids having different constitutions in developmental and reproductive capacities.

MATERIALS AND METHODS

As materials, *Rana japonica* GÜNTHER were collected from the suburbs of Ichinoseki city, Iwate Prefecture, and the suburbs of Hiroshima city, Hiroshima Prefecture, and *Rana tsushimensis* STEJNEGER were collected from Tsushima Island, Nagasaki Prefecture. Ovulation was accelerated by bullfrog pituitaries. Eggs were always fertilized by the artificial method. Triploid tadpoles were produced from fertilized eggs by suppressing the extrusion of the second polar bodies by exposing the eggs to 35~38°C for 3 minutes, 22~25 minutes after insemination (KAWAMURA and NISHIOKA, 1981). Tadpoles were fed on boiled spinach, while frogs were reared with crickets, *Gryllus bimaculatus* DE GEER.

The chromosomes of tadpoles were observed in the tail-tips by the water-pretreatment squash method (NISHIOKA, 1972). Colchicine was not applied to the tadpoles, as it was necessary to rear them continuously after examining their chromosomes. The chromosomes of adult frogs were examined by the bone marrow method (OMURA, 1967). The ploidy of preserved frogs was determined by measuring the size of cell nuclei of uriniferous tubules. The gonads and kidneys were fixed in NAVASHIN's fluid, sectioned at 10 or 12 μ and stained with HEIDENHAIN's iron hematoxylin for histological observations.

Eleven kinds of enzymes extracted from skeletal muscles, AAT, CK, Est, α-GDH, GPI, IDH, LDH, MDH, MPI, PGM and SOD, and three kinds of blood proteins, Ab, Hb and Prot-C, were analyzed by starch-gel electrophoresis. The details of electrophoretic method have been described by NISHIOKA, OHTANI and SUMIDA (1980). The enzymes were detected by specific staining procedures according to BREWER (1970) and HARRIS and HOPKINSON (1976). The blood proteins were stained with amido black 10B.

The following abbreviations are used in this report.

T ---------- A set of *Rana tsushimensis* chromosomes
I ---------- A set of *Rana japonica* chromosomes from Ichinoseki
H ---------- A set of *Rana japonica* chromosomes from Hiroshima
(T) ---------- *Rana tsushimensis* cytoplasm
(I) ---------- *Rana japonica* cytoplasm from Ichinoseki
(H) ---------- *Rana japonica* cytoplasm from Hiroshima
(T)TT -------- Diploid *Rana tsushimensis*
(I)II ---------- Diploid *Rana japonica* from Ichinoseki
(H)HH ------- Diploid *Rana japonica* from Hiroshima
(T)TI --------- Diploid hybrid between a female *Rana tsushimensis* and a male *R. japonica* from Ichinoseki
(I)IT --------- Diploid hybrid between a female *Rana japonica* from Ichinoseki and a male *R. tsushimensis*
(T)TH ------- Diploid hybrid between a female *Rana tsushimensis* and a male *R. japonica* from Hiroshima
(H)HT ------- Diploid hybrid between a female *Rana japonica* from Hiroshima and a male *R. tsushimensis*
(T)TTI ------ Allotriploid composed of two *Rana tsushimensis* genomes and one *R. japonica* genome from Ichinoseki
(I)IIT -------- Allotriploid composed of two *Rana japonica* genomes from Ichinoseki and one *R. tsushimensis* genome
(T)TTH ----- Allotriploid composed of two *Rana tsushimensis* genomes and one *R. japonica* genome from Hiroshima
(H)HHT----- Allotriploid composed of two *Rana japonica* genomes from Hiroshima and one *R. tsushimensis* genome

OBSERVATION

I. Developmental capacity

1. *Rana tsushimensis* and *R. japonica* from Ichinoseki

A. Control matings

a. *Rana tsushimensis*, (T)TT♀ × (T)TT♂

In 1979, 1980, 1983 and 1984, 15 females of *R. tsushimensis*, (T)TT♀, Nos. 1~15 were mated with seven males of *R. tsushimensis*, (T)TT♂, Nos. 1~7. In 15 control

TABLE 1

Developmental capacity and ploidy of hybrids between female *Rana tsushimensis* and

Year	Parents		Heat shock	No. of eggs	No. of normally cleaved eggs (%)	No. of normal tail-bud embryos (%)
	Female	Male				
1979	(T)TT, No. 1	(T)TT, No. 1	None	62	54 (87.1)	46 (74.2)
	(T)TT, No. 2	(T)TT, No. 1	None	80	71 (88.8)	61 (76.3)
	(T)TT, No. 3	(T)TT, No. 2	None	60	48 (80.0)	40 (66.7)
1980	(T)TT, No. 4	(T)TT, No. 3	None	126	115 (91.3)	114 (90.5)
	(T)TT, No. 5	(T)TT, No. 4	None	94	86 (91.5)	86 (91.5)
1983	(T)TT, No. 6	(T)TT, No. 5	None	40	39 (97.5)	37 (92.5)
	(T)TT, No. 7	(T)TT, No. 5	None	56	54 (96.4)	53 (94.6)
	(T)TT, No. 8	(T)TT, No. 6	None	54	49 (90.7)	48 (88.9)
	(T)TT, No. 9	(T)TT, No. 6	None	53	51 (96.2)	51 (96.2)
	(T)TT, No. 10	(T)TT, No. 6	None	51	49 (96.1)	49 (96.1)
1984	(T)TT, No. 11	(T)TT, No. 7	None	129	121 (93.8)	121 (93.8)
	(T)TT, No. 12	(T)TT, No. 7	None	91	86 (94.5)	85 (93.4)
	(T)TT, No. 13	(T)TT, No. 7	None	59	55 (93.2)	53 (89.8)
	(T)TT, No. 14	(T)TT, No. 7	None	56	48 (85.7)	45 (80.4)
	(T)TT, No. 15	(T)TT, No. 7	None	65	64 (98.5)	64 (98.5)
Total				1076	990 (92.0)	953 (88.6)
1979	(T)TT, No. 1	(I)II, No. 1	None	77	62 (80.5)	32 (41.6)
	(T)TT, No. 2	(I)II, No. 1	None	181	157 (86.7)	109 (60.2)
	(T)TT, No. 3	(I)II, No. 2	None	103	79 (76.7)	66 (64.1)
1980	(T)TT, No. 4	(I)II, No. 3	None	36	32 (88.9)	31 (86.1)
	(T)TT, No. 5	(I)II, No. 4	None	116	96 (82.8)	96 (82.8)
1983	(T)TT, No. 6	(I)II, No. 5	None	67	62 (92.5)	60 (89.6)
	(T)TT, No. 7	(I)II, No. 5	None	160	142 (88.8)	139 (86.9)
	(T)TT, No. 8	(I)II, No. 6	None	105	88 (83.8)	88 (83.8)
	(T)TT, No. 9	(I)II, No. 6	None	219	196 (89.5)	194 (88.6)
	(T)TT, No. 10	(I)II, No. 6	None	189	178 (94.2)	173 (91.5)
1984	(T)TT, No. 11	(I)II, No. 7	None	66	56 (84.8)	54 (81.8)
	(T)TT, No. 12	(I)II, No. 7	None	75	72 (96.0)	69 (92.0)
	(T)TT, No. 13	(I)II, No. 8	None	120	116 (96.7)	109 (90.8)
	(T)TT, No. 14	(I)II, No. 8	None	120	92 (76.7)	89 (74.2)
	(T)TT, No. 15	(I)II, No. 8	None	102	93 (91.2)	91 (89.2)
Total				1736	1521 (87.6)	1400 (80.6)
1984	(T)TT, No. 11	(I)II, No. 7	Treated	139	106 (76.3)	100 (71.9)
	(T)TT, No. 12	(I)II, No. 7	at 36°C	202	183 (90.6)	173 (85.6)
	(T)TT, No. 13	(I)II, No. 8		113	104 (92.0)	89 (78.8)
	(T)TT, No. 14	(I)II, No. 8		141	104 (73.8)	87 (61.7)
	(T)TT, No. 15	(I)II, No. 8		134	124 (92.5)	112 (83.6)
Total				729	621 (85.2)	561 (77.0)
1984	(T)TT, No. 13	(I)II, No. 8	Treated	107	80 (74.8)	62 (57.9)
	(T)TT, No. 14	(I)II, No. 8	at 37°C	133	77 (57.9)	61 (45.9)
	(T)TT, No. 15	(I)II, No. 8		119	100 (84.0)	82 (68.9)
Total				359	257 (71.6)	205 (57.1)
1984	(T)TT, No. 13	(I)II, No. 8	Treated	189	123 (65.1)	86 (45.5)
	(T)TT, No. 14	(I)II, No. 8	at 38°C	168	97 (57.7)	71 (42.3)
	(T)TT, No. 15	(I)II, No. 8		166	142 (85.5)	134 (80.7)
Total				523	362 (69.2)	291 (55.6)

*, These were confirmed to be triploid by the observation of (T)TT, *R. tsushimensis*

male *R. japonica* from Ichinoseki and the controls

No. of normally hatched tadpoles (%)	No. of normally feeding tadpoles (%)	No. of 30-day-old tadpoles		No. of analyzed tadpoles					No. of metamorphosed frogs (%)
		Normal (%)	Underdeveloped (%)	Normal		Underdeveloped			
				2n	3n	2n	3n	2n–4n	
46 (74.2)	44 (71.0)	40 (64.5)	0	—	—	0	0	0	38 (61.3)
57 (71.3)	56 (70.0)	53 (66.3)	0	—	—	0	0	0	49 (61.3)
39 (65.0)	36 (60.0)	35 (58.3)	0	—	—	0	0	0	34 (56.7)
114 (90.5)	113 (89.7)	110 (87.3)	0	—	—	0	0	0	108 (85.7)
84 (89.4)	83 (88.3)	82 (87.2)	0	—	—	0	0	0	80 (85.1)
37 (92.5)	37 (92.5)	36 (90.0)	0	—	—	0	0	0	35 (87.5)
53 (94.6)	52 (92.9)	50 (89.3)	0	—	—	0	0	0	50 (89.3)
46 (85.2)	46 (85.2)	44 (81.5)	0	—	—	0	0	0	42 (77.8)
51 (96.2)	51 (96.2)	50 (94.3)	0	—	—	0	0	0	50 (94.3)
49 (96.1)	48 (94.1)	46 (90.2)	0	—	—	0	0	0	44 (86.3)
120 (93.0)	120 (93.0)	119 (92.2)	0	20	0	0	0	0	117 (90.7)
85 (93.4)	85 (93.4)	85 (93.4)	0	20	0	0	0	0	85 (93.4)
53 (89.8)	53 (89.8)	53 (89.8)	0	20	0	0	0	0	51 (86.4)
45 (80.4)	45 (80.4)	43 (76.8)	1 (1.8)	20	0	—	—	—	42 (75.0)
64 (98.5)	64 (98.5)	62 (95.4)	0	20	0	0	0	0	61 (93.8)
943 (87.6)	933 (86.7)	908 (84.4)	1 (0.1)	100	0	0	0	0	886 (82.3)
32 (41.6)	26 (33.8)	0 (0)	23 (29.9)	0	0	—	—	—	0 (0)
24 (13.3)	24 (13.3)	8 (4.4)	15 (8.3)	0	4*	—	—	—	7 (3.9)
22 (21.4)	22 (21.4)	10 (9.7)	10 (9.7)	—	6*	—	—	—	9 (8.7)
2 (5.6)	2 (5.6)	1 (2.8)	1 (2.8)	0	1*	—	—	—	1 (2.8)
29 (25.0)	29 (25.0)	0 (0)	28 (24.1)	0	0	—	—	—	0 (0)
15 (22.4)	15 (22.4)	1 (1.5)	14 (20.9)	0	1	—	—	—	1 (1.5)
72 (45.0)	67 (41.9)	1 (0.6)	65 (40.6)	0	1	—	—	—	1 (0.6)
66 (62.9)	66 (62.9)	0 (0)	60 (57.1)	0	0	—	—	—	0 (0)
161 (73.5)	152 (69.4)	2 (0.9)	148 (67.6)	0	2	—	—	—	2 (0.9)
13 (6.9)	13 (6.9)	1 (0.5)	12 (6.3)	0	1	—	—	—	1 (0.5)
45 (68.2)	45 (68.2)	0 (0)	44 (66.7)	0	0	20	0	0	0 (0)
67 (89.3)	66 (88.0)	0 (0)	65 (86.7)	0	0	20	0	0	0 (0)
55 (45.8)	12 (10.0)	0 (0)	12 (10.0)	0	0	12	0	0	0 (0)
65 (54.2)	20 (16.7)	0 (0)	19 (15.8)	0	0	19	0	0	0 (0)
37 (36.3)	9 (8.8)	0 (0)	9 (8.8)	0	0	9	0	0	0 (0)
705 (40.6)	568 (32.7)	24 (1.4)	525 (30.2)	0	16	80	0	0	22 (1.3)
58 (41.7)	58 (41.7)	0 (0)	58 (41.7)	0	0	58	0	0	0 (0)
137 (67.8)	135 (66.8)	23 (11.4)	109 (54.0)	0	23	109	0	0	22 (10.9)
42 (37.2)	42 (37.2)	2 (1.8)	40 (35.4)	0	2	40	0	0	1 (0.9)
63 (44.7)	63 (44.7)	17 (12.1)	46 (32.6)	0	17	46	0	0	15 (10.6)
33 (24.6)	27 (20.1)	21 (15.7)	6 (4.5)	0	21	6	0	0	18 (13.4)
333 (45.7)	325 (44.6)	63 (8.6)	259 (35.5)	0	63	259	0	0	56 (7.7)
28 (26.2)	28 (26.2)	10 (9.3)	17 (15.9)	0	10	17	0	0	8 (7.5)
54 (40.6)	54 (40.6)	25 (18.8)	28 (21.1)	0	25	27	0	1	18 (13.5)
30 (25.2)	28 (23.5)	18 (15.1)	10 (8.4)	0	18	10	0	0	14 (11.8)
112 (31.2)	110 (30.6)	53 (14.8)	55 (15.3)	0	53	54	0	1	40 (11.1)
49 (25.9)	45 (23.8)	37 (19.6)	8 (4.2)	0	37	7	0	1	12 (6.3)
48 (28.6)	47 (28.0)	44 (26.2)	0	0	44	0	0	0	41 (24.4)
22 (13.3)	22 (13.3)	22 (13.3)	0	0	22	0	0	0	14 (8.4)
119 (22.8)	114 (21.8)	103 (19.7)	8 (1.5)	0	103	7	0	1	67 (12.8)

kidney cell nuclei after metamorphosis.

(I)II, *R. japonica* from Ichinoseki

matings, 80.0~98.5% of the respective numbers of eggs, 990 (92.0%) of 1076 eggs in total, cleaved normally. A small number of normally cleaved eggs died of various abnormalities at embryonic stages and 65.0~98.5%, 943 (87.6%) in total, hatched normally. During the tadpole stage, some individuals died of underdevelopment or edema and eventually 56.7~94.3%, 886 (82.3%) in total, metamorphosed normally (Table 1).

The chromosomes were observed by the squash method in the tail-tips of 100 normal 30-day-old tadpoles derived from five control matings, (T)TT♀, Nos. 11~15×(T)TT♂, No. 7 in 1984. All the tadpoles were normal diploids (Table 1; Fig. 1).

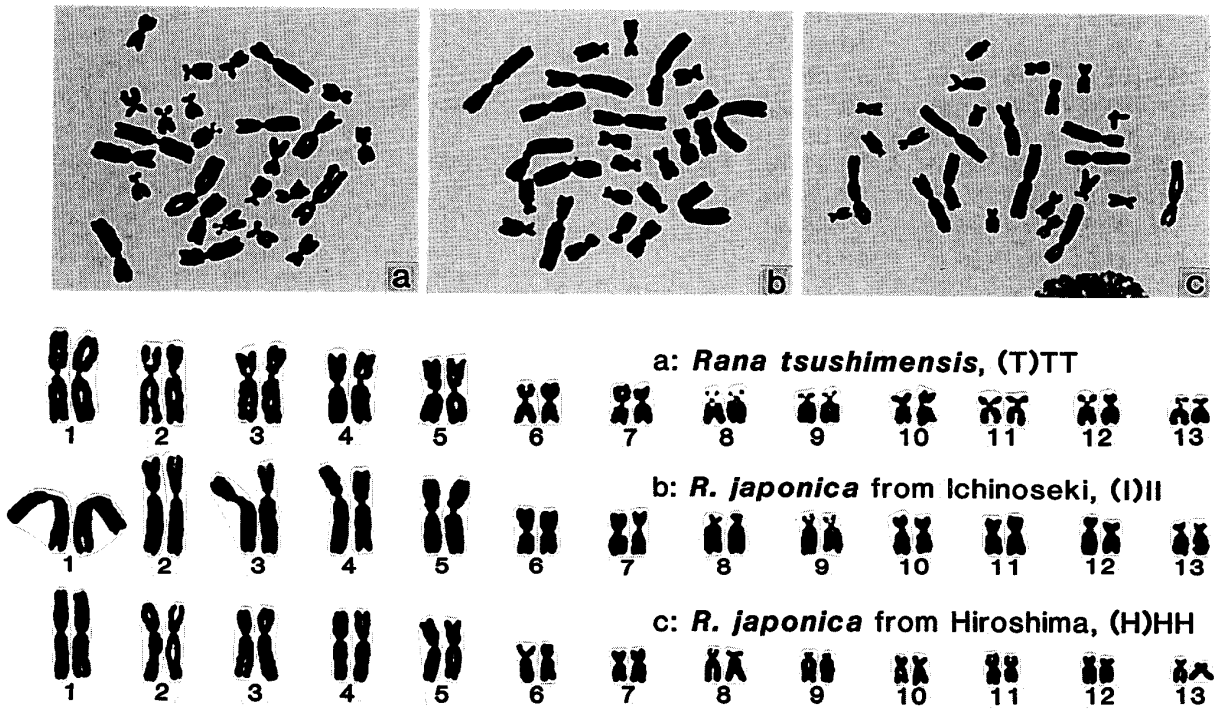

Fig. 1. Metaphase plates and karyotypes of control *Rana tsushimensis*, (T)TT, and *R. japonica* from Ichinoseki, (I)II, and Hiroshima, (H)HH. ×1200

b. *Rana japonica* from Ichinoseki, (I)II♀×(I)II♂

In 1979, 1980 and 1984, eight females of *R. japonica* from Ichinoseki, (I)II♀, Nos. 1~8 were mated with five males of *R. japonica* from Ichinoseki, (I)II♂, Nos. 1~4 and 9. In eight control matings, 74.5~92.5% of the respective numbers of eggs, 922 (85.3%) of 1081 eggs in total, cleaved normally. Some of the normally cleaved eggs died of various kinds of abnormalities at embryonic stages and 45.0~63.0%, 610 (56.4%) in total, hatched normally. During the tadpole stage, some individuals died of edema or underdevelopment and 39.1~53.3%, 477 (44.1%) in total, metamorphosed normally (Table 2).

The chromosomes were observed by the squash method in the tail-tips of 40 normal 30-day-old tadpoles derived from two control matings, (I)II♀, Nos. 7 and

8×(I)II♂, No. 9 in 1984. All the tadpoles were normal diploids (Table 2; Fig. 1).

B. Hybrid matings

a. (T)TT♀×(I)II♂

In 1979, 1980, 1983 and 1984, 15 females of *R. tsushimensis*, (T)TT♀, Nos. 1~15 were mated with eight males of *R. japonica* from Ichinoseki, (I)II♂, Nos. 1~8. In 15 hybrid matings, 76.7~96.7% of the respective numbers of eggs, 1521 (87.6%) of 1736 eggs in total, cleaved normally. Differing from the control matings, many normally cleaved eggs died of edema at the hatching stage and 5.6~89.3%, 705 (40.6%) in total, hatched normally. While most of the normally hatched tadpoles began to eat, they gradually lost their appetite and became emaciated. All the tadpoles died without attaining metamorphosis except 24 tadpoles. Thereafter, 22 of the latter completed metamorphosis (Table 1). Six frogs including four, one and one from matings Nos. 3, 4 and 10, respectively, attained sexual maturity at the age of one year (Table 4).

The chromosomes were observed by the squash method in the tail-tips of five normal 30-day-old tadpoles derived from large eggs obtained from four matings in 1983. All the tadpoles were triploids. On the other hand, the ploidy of 11 hybrid frogs derived from three matings in 1979 and 1980 was determined by measuring the size of cell nuclei of uriniferous tubules in the kidneys. The ploidy of some of these frogs was confirmed by the electrophoretic analyses of enzymes extracted from skeletal muscles and blood proteins. It was found that all these frogs were triploids (Table 1).

The chromosomes were also observed in the tail-tips of 80 underdeveloped 30-day-old tadpoles produced from five matings, (T)TT♀, Nos. 11~15×(I)II♂, Nos. 7 and 8 in 1984. All the tadpoles were normal diploids (Table 1).

b. (I)II♀×(T)TT♂

In 1979, 1980 and 1984, eight females of *R. japonica* from Ichinoseki, (I)II♀, Nos. 1~8 were mated with six males of *R. tsushimensis*, (T)TT♂, Nos. 1~4, 8 and 9. In eight hybrid matings, 72.2~89.4% of the respective numbers of eggs, 1047 (83.2%) of 1258 eggs in total, cleaved normally, 39.1~58.0%, 684 (54.4%) in total, hatched normally, and 23.8~53.0%, 558 (44.4%) in total, completed metamorphosis (Table 2). Morphological defects found at the embryonic and tadpole stages in the hybrids were nearly the same as those in the controls. Almost all the metamorphosed froglets died of infirmity within 1~3 months after metamorphosis. Only one (0.1%) frog obtained from mating No. 2 in 1979 attained sexual maturity at the age of one year (Table 4).

The chromosomes were observed by the squash method in the tail-tips of 50 normal 30-day-old tadpoles produced from two matings, (I)II♀, Nos. 7 and 8×(T)TT♂, Nos. 8 and 9 in 1984. All the tadpoles were normal diploids (Table 2). On the other hand, the ploidy of the mature hybrid frog produced from mating No. 2 in 1984 was examined by measuring the size of cell nuclei of

TABLE 2

Developmental capacity and ploidy of hybrids between female *Rana japonica*

Year	Parents		Heat shock	No. of eggs	No. of normally cleaved eggs (%)	No. of normal tail-bud embryos (%)
	Female	Male				
1979	(I)II, No. 1	(I)II, No. 1	None	165	147 (89.1)	121 (73.3)
	(I)II, No. 2	(I)II, No. 1	None	141	122 (86.5)	95 (67.4)
	(I)II, No. 3	(I)II, No. 2	None	92	85 (92.4)	60 (65.2)
1980	(I)II, No. 4	(I)II, No. 3	None	137	102 (74.5)	84 (61.3)
	(I)II, No. 5	(I)II, No. 4	None	127	106 (83.5)	86 (67.7)
	(I)II, No. 6	(I)II, No. 4	None	157	125 (79.6)	101 (64.3)
1984	(I)II, No. 7	(I)II, No. 9	None	142	124 (87.3)	106 (74.6)
	(I)II, No. 8	(I)II, No. 9	None	120	111 (92.5)	85 (70.8)
Total				1081	922 (85.3)	738 (68.3)
1979	(I)II, No. 1	(T)TT, No. 1	None	381	328 (86.1)	260 (68.2)
	(I)II, No. 2	(T)TT, No. 2	None	148	124 (83.8)	91 (61.5)
	(I)II, No. 3	(T)TT, No. 2	None	69	53 (76.8)	31 (44.9)
1980	(I)II, No. 4	(T)TT, No. 3	None	118	101 (85.6)	70 (59.3)
	(I)II, No. 5	(T)TT, No. 4	None	152	131 (86.2)	103 (67.8)
	(I)II, No. 6	(T)TT, No. 4	None	162	117 (72.2)	94 (58.0)
1984	(I)II, No. 7	(T)TT, No. 8	None	123	110 (89.4)	83 (67.5)
	(I)II, No. 8	(T)TT, No. 9	None	105	83 (79.0)	58 (55.2)
Total				1258	1047 (83.2)	790 (62.8)
1984	(I)II, No. 7	(T)TT, No. 10	Treated at 35°C	190	104 (54.7)	38 (20.0)
	(I)II, No. 8	(T)TT, No. 11		178	95 (53.4)	64 (36.0)
Total				368	199 (54.1)	102 (27.7)
1984	(I)II, No. 8	(T)TT, No. 10	Treated at 36°C	204	45 (22.1)	27 (13.2)
	(I)II, No. 8	(T)TT, No. 11		294	54 (18.4)	32 (10.9)
Total				498	99 (19.9)	59 (11.8)
1984	(I)II, No. 8	(T)TT, No. 11	Treated at 37°C	166	11 (6.6)	6 (3.6)

*, This was confirmed to be triploid by the (I)II, *R. japonica* from Ichinoseki

uriniferous tubules in the kidneys and confirmed by the electrophoretic analyses. This frog was found to be a triploid (Table 2).

C. Production of allotriploids

a. (T)TT♀×(I)II♂, with heat-shock treatment of eggs

Allotriploids, (T)TTI, were produced from eggs of five female *R. tsushimensis*, (T)TT♀, Nos. 11~15, inseminated with sperm of two male *R. japonica* from Ichinoseki, (I)II♂, Nos. 7 and 8, by heat-shock treatment for 3 minutes at 36°C,

from Ichonoseki and male *R. tsushimensis* and the controls

No. of normally hatched tadpoles (%)	No. of normally feeding tadpoles (%)	No. of 30-day-old tadpoles		No. of analyzed tadpoles				No. of metamorphosed frogs (%)
		Normal (%)	Underdeveloped (%)	Normal		Underdeveloped		
				2n	3n	2n	3n	
96 (58.2)	95 (57.6)	90 (54.5)	0	—	—	0	0	88 (53.3)
87 (61.7)	81 (57.4)	75 (53.2)	0	—	—	0	0	72 (51.1)
55 (59.8)	46 (50.0)	37 (40.2)	0	—	—	0	0	36 (39.1)
76 (55.5)	68 (49.6)	57 (41.6)	0	—	—	0	0	55 (40.1)
80 (63.0)	73 (57.5)	65 (51.2)	0	—	—	0	0	57 (44.9)
94 (59.9)	79 (50.3)	71 (45.2)	0	—	—	0	0	63 (40.1)
68 (47.9)	66 (46.5)	65 (45.8)	1 (0.7)	20	0	—	—	59 (41.5)
54 (45.0)	49 (40.8)	49 (40.8)	0	20	0	0	0	47 (39.2)
610 (56.4)	557 (51.5)	509 (47.1)	1 (0.1)	40	0	0	0	477 (44.1)
221 (58.0)	215 (56.4)	207 (54.3)	0	—	—	—	—	202 (53.0)
83 (56.1)	77 (52.0)	74 (50.0)	0	0	1*	—	—	72 (48.6)
27 (39.1)	26 (37.7)	22 (31.9)	0	—	—	—	—	19 (27.5)
61 (51.7)	53 (44.9)	49 (41.5)	0	—	—	—	—	43 (36.4)
82 (53.9)	75 (49.3)	70 (46.1)	0	—	—	—	—	69 (45.4)
85 (52.5)	78 (48.1)	72 (44.4)	0	—	—	—	—	70 (43.2)
69 (56.1)	64 (52.0)	60 (48.8)	1 (0.8)	30	0	—	—	58 (47.2)
56 (53.3)	54 (51.4)	39 (37.1)	13 (12.4)	20	0	—	—	25 (23.8)
684 (54.4)	642 (51.0)	593 (47.1)	14 (1.1)	50	1	—	—	558 (44.4)
34 (17.9)	32 (16.8)	28 (14.7)	4 (2.1)	28	0	4	0	28 (14.7)
58 (32.6)	42 (23.6)	29 (16.3)	12 (6.7)	14	15	10	2	28 (15.7)
92 (25.0)	74 (20.1)	57 (15.5)	16 (4.3)	42	15	14	2	56 (15.2)
20 (9.8)	14 (6.9)	10 (4.9)	4 (2.0)	0	10	4	0	9 (4.4)
19 (6.5)	15 (5.1)	12 (4.1)	3 (1.0)	3	9	3	0	11 (3.7)
39 (7.8)	29 (5.8)	22 (4.4)	7 (1.4)	3	19	7	0	20 (4.0)
5 (3.0)	4 (2.4)	3 (1.8)	1 (0.6)	0	3	1	0	3 (1.8)

observation of kidney cell nuclei after metamorphosis.
(T)TT, *R. tsushimensis*

37°C and 38°C, respectively, 22 minutes after insemination.

i) Treatment at 36°C

In five matings between five female *R. tsushimensis*, (T)TT♀, Nos. 11~15, and two male *R. japonica* from Ichinoseki, (I)II♂, Nos. 7 and 8, 73.8~92.5% of the respective numbers of eggs treated at 36°C for 3 minutes, 621 (85.2%) of 729 eggs in total, cleaved normally, and then 24.6~67.8%, 333 (45.7%) in total, hatched normally. Of 322 30-day-old tadpoles, 63 were normal, while 259 were underde-

veloped and died without attaining metamorphosis. Eventually, 56 of the 63 normal tadpoles metamorphosed normally (Table 1).

When the chromosomes were examined in the tail-tips of the 63 normally developed and 259 underdeveloped 30-day-old tadpoles produced from the above five matings, it was found that the 63 normally developed tadpoles were all triploids and 259 underdeveloped tadpoles were all diploids (Table 1; Fig. 2).

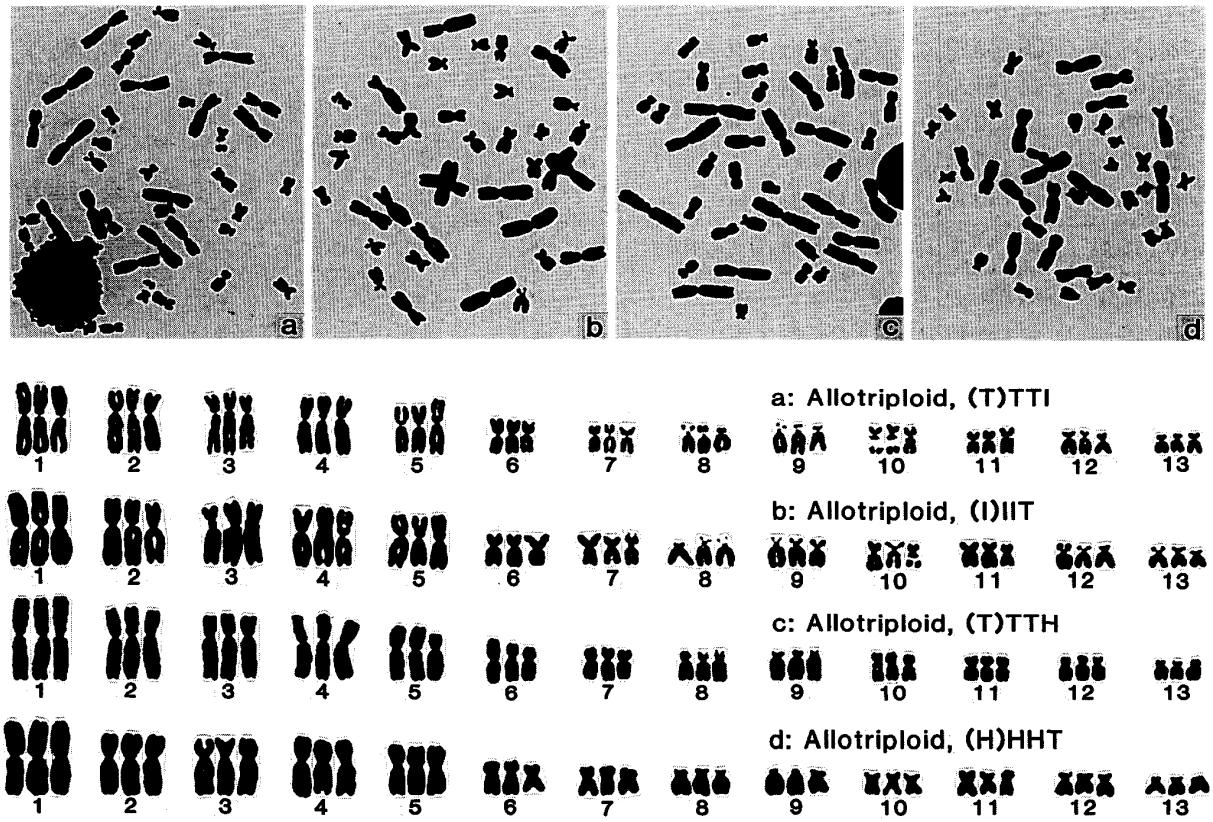

Fig. 2. Metaphase plates and karyotypes of reciprocal allotriploids between *Rana tsushimensis* and *R. japonica* from Ichinoseki, (T)TTI and (I)IIT, and Hiroshima, (T)TTH and (H)HHT. ×1200

ii) Treatment at 37°C

In three matings between three female *R. tsushimensis*, (T)TT♀, Nos. 13~15, and a male *R. japonica* from Ichinoseki, (I)II♂, No. 8, 57.9~84.0% of the respective numbers of eggs treated at 37°C for 3 minutes, 257 (71.6%) of 359 eggs in total, cleaved normally, and then 25.2~40.6%, 112 (31.2%) in total, hatched normally. Of 108 30-day-old tadpoles, 53 were normal and 55 were underdeveloped. Eventually, 40 of the 53 normal tadpoles metamorphosed normally (Table 1).

When the chromosomes were examined in the tail-tips of the 53 normal and 55 underdeveloped 30-day-old tadpoles produced from the above three matings, it was found that the 53 normal tadpoles were all triploids, while 54 of the 55 underdeveloped tadpoles were diploids and the remaining one was a 2n-4n mosaic (Table 1; Fig. 2).

iii) Treatment at 38°C

In three matings between three female *R. tsushimensis*, (T)TT♀, Nos. 13~15, and a male *R. japonica* from Ichinoseki, (I)II♂, No. 8, 57.7~85.5% of the respective numbers of eggs treated at 38°C for 3 minutes, 362 (69.2%) of 523 eggs in total, cleaved normally, and then 13.3~28.6%, 119 (22.8%) in total, hatched normally. Of 111 30-day-old tadpoles, 103 were normal and eight were underdeveloped. Eventually, 67 of the 103 normal tadpoles metamorphosed normally (Table 1).

When the chromosomes were examined in the tail-tips of the 103 normal and eight underdeveloped 30-day-old tadpoles produced from the above three matings, it was found that the 103 normal tadpoles were all triploids, while seven of the eight underdeveloped tadpoles were diploids and the other was a 2n-4n mosaic (Table 1; Fig. 2).

b. (I)II♀×(T)TT♂, with heat-shock treatment of eggs

i) Treatment at 35°C

In two matings between two female *R. japonica* from Ichinoseki, (I)II♀, Nos. 7 and 8, and two male *R. tsushimensis*, (T)TT♂, Nos. 10 and 11, 54.7 and 53.4% of the respective numbers of eggs treated at 35°C for 3 minutes, 199 (54.1%) of 368 eggs in total, cleaved normally, while 147 (39.9%) cleaved abnormally. Thereafter, 92 (25.0%) hatched normally, 57 (15.5%) became normal 30-day-old tadpoles and 56 (15.2%) metamorphosed normally (Table 2).

The chromosomes were examined in the tail-tips of the 57 normal and 16 underdeveloped 30-day-old tadpoles produced from the above two matings. It was found that 42 of the 57 normal tadpoles were diploids and the other 15 were triploids. While 14 of the 16 underdeveloped tadpoles were diploids, the other two were triploids (Table 2; Fig. 2).

ii) Treatment at 36°C

In two matings between a female *R. japonica* from Ichinoseki, (I)II♀, No. 8, and two male *R. tsushimensis*, (T)TT♂, Nos. 10 and 11, 22.1% and 18.4% of the respective numbers of eggs treated at 36°C for 3 minutes, 99 (19.9%) of 498 eggs in total, cleaved normally, while 320 (64.3%) cleaved abnormally. Thereafter, 39 (7.8%) hatched normally, 22 (4.4%) became normal 30-day-old tadpoles and 20 (4.0%) metamorphosed normally (Table 2).

The chromosomes were examined in the tail-tips of the 22 normal and seven underdeveloped 30-day-old tadpoles produced from the above two matings. It was found that 19 of the 22 normal tadpoles were triploids, while the other three were diploids. The seven underdeveloped tadpoles were all diploids (Table 2; Fig. 2).

iii) Treatment at 37°C

In a mating between a female *R. japonica* from Ichinoseki, (I)II♀, No. 8, and a male *R. tsushimensis*, (T)TT♂, No. 11, 11(6.6%) of 166 eggs cleaved normally,

TABLE 3

Developmental capacity and ploidy of reciprocal hybrids between

Year	Parents		Heat shock	No. of eggs	No. of normally cleaved eggs (%)	No. of normal tail-bud embryos (%)
	Female	Male				
1984	(T)TT, No. 16	(T)TT, No. 7	None	72	66 (91.7)	65 (90.3)
	(T)TT, No. 17	(T)TT, No. 7	None	53	50 (94.3)	49 (92.5)
	(T)TT, No. 18	(T)TT, No. 7	None	58	54 (93.1)	53 (91.4)
Total				183	170 (92.9)	167 (91.3)
1984	(T)TT, No. 16	(H)HH, No. 1	None	58	57 (98.3)	55 (94.8)
	(T)TT, No. 17	(H)HH, No. 1	None	70	66 (94.3)	47 (67.1)
	(T)TT, No. 18	(H)HH, No. 2	None	88	78 (88.6)	67 (76.1)
Total				216	201 (93.1)	169 (78.2)
1984	(T)TT, No. 16	(H)HH, No. 1	Treated	155	74 (47.7)	65 (41.9)
	(T)TT, No. 17	(H)HH, No. 1	at 37.5°C	172	57 (33.1)	30 (17.4)
	(T)TT, No. 18	(H)HH, No. 2		160	56 (35.0)	38 (23.8)
Total				487	187 (38.4)	133 (27.3)
1984	(T)TT, No. 16	(H)HH, No. 1	Treated	208	88 (42.3)	76 (36.5)
	(T)TT, No. 17	(H)HH, No. 1	at 38.0°C	183	53 (29.0)	38 (20.8)
	(T)TT, No. 18	(H)HH, No. 2		242	166 (68.6)	121 (50.0)
Total				633	307 (48.5)	235 (37.1)
1984	(H)HH, No. 1	(H)HH, No. 3	None	58	53 (91.4)	52 (89.7)
	(H)HH, No. 2	(H)HH, No. 3	None	38	37 (97.4)	36 (94.7)
	(H)HH, No. 3	(H)HH, No. 3	None	38	36 (94.7)	36 (94.7)
	(H)HH, No. 4	(H)HH, No. 3	None	44	43 (97.7)	43 (97.7)
Total				178	169 (94.9)	167 (93.8)
1984	(H)HH, No. 1	(T)TT, No. 8	None	69	63 (91.3)	57 (82.6)
	(H)HH, No. 2	(T)TT, No. 8	None	65	55 (84.6)	55 (84.6)
	(H)HH, No. 3	(T)TT, No. 9	None	68	66 (97.1)	1 (1.5)
	(H)HH, No. 4	(T)TT, No. 10	None	54	53 (98.1)	53 (98.1)
Total				256	237 (92.6)	166 (64.8)
1984	(H)HH, No. 1	(T)TT, No. 8	Treated	178	147 (82.6)	82 (46.1)
	(H)HH, No. 2	(T)TT, No. 8	at 37.0°C	187	99 (52.9)	70 (37.4)
	(H)HH, No. 3	(T)TT, No. 9		164	138 (84.1)	46 (28.0)
	(H)HH, No. 4	(T)TT, No. 10		121	98 (81.0)	81 (66.9)
Total				650	482 (74.2)	279 (42.9)
1984	(H)HH, No. 1	(T)TT, No. 8	Treated	324	166 (51.2)	80 (24.7)
	(H)HH, No. 2	(T)TT, No. 8	at 38.0°C	234	31 (13.2)	9 (3.8)
	(H)HH, No. 3	(T)TT, No. 9		220	88 (40.0)	11 (5.0)
	(H)HH, No. 4	(T)TT, No. 10		280	87 (31.1)	52 (18.6)
Total				1058	372 (35.2)	152 (14.4)

(T)TT, *R. tsushimensis*

while 75(45.2%) cleaved abnormally. Thereafter, 5(3.0%) hatched normally, and 3(1.8%) became normal 30-day-old tadpoles and metamorphosed normally (Table 2).

The chromosomes were examined in the tail-tips of four 30-day-old tadpoles produced from the above mating. It was found that three normal tadpoles were triploids and one underdeveloped tadpole was a diploid (Table 2; Fig. 2).

Rana tsushimensis and *R. japonica* from Hiroshima and the controls

No. of normally hatched tadpoles (%)	No. of normally feeding tadpoles (%)	No. of 30-day-old tadpoles		No. of analyzed tadpoles				No. of metamorphosed frogs (%)
		Normal (%)	Underdeveloped (%)	Normal		Underdeveloped		
				2n	3n	2n	3n	
65 (90.3)	65 (90.3)	65 (90.3)	0	20	0	0	0	65 (90.3)
49 (92.5)	49 (92.5)	49 (92.5)	0	20	0	0	0	47 (88.7)
53 (91.4)	53 (91.4)	52 (89.7)	0	20	0	0	0	49 (84.5)
167 (91.3)	167 (91.3)	166 (90.7)	0	60	0	0	0	161 (88.0)
25 (43.1)	25 (43.1)	0	23 (39.7)	0	0	23	0	0
2 (2.9)	2 (2.9)	0	2 (2.9)	0	0	2	0	0
31 (35.2)	31 (35.2)	0	30 (34.1)	0	0	30	0	0
58 (26.9)	58 (26.9)	0	55 (25.5)	0	0	55	0	0
38 (24.5)	38 (24.5)	38 (24.5)	0	0	38	0	0	38 (24.5)
0	—	—	—	—	—	—	—	—
26 (16.3)	25 (15.6)	25 (15.6)	0	0	25	0	0	25 (15.6)
64 (13.1)	63 (12.9)	63 (12.9)	0	0	63	0	0	63 (12.9)
14 (6.7)	14 (6.7)	14 (6.7)	0	0	14	0	0	14 (6.7)
8 (4.4)	7 (3.8)	7 (3.8)	0	0	7	0	0	7 (3.8)
45 (18.6)	42 (17.4)	42 (17.4)	0	0	42	0	0	42 (17.4)
67 (10.6)	63 (10.0)	63 (10.0)	0	0	63	0	0	63 (10.0)
52 (89.7)	52 (89.7)	52 (89.7)	0	20	0	0	0	51 (87.9)
35 (92.1)	35 (92.1)	34 (89.5)	1 (2.6)	20	0	—	—	34 (89.5)
36 (94.7)	36 (94.7)	36 (94.7)	0	20	0	0	0	36 (94.7)
43 (97.7)	42 (95.5)	42 (95.5)	0	20	0	0	0	41 (93.2)
166 (93.3)	165 (92.7)	164 (92.1)	1 (0.6)	80	0	0	0	162 (91.0)
57 (82.6)	57 (82.6)	0	47 (68.1)	0	0	20	0	0
51 (78.5)	50 (76.9)	0	49 (75.4)	0	0	20	0	0
1 (1.5)	1 (1.5)	0	1 (1.5)	0	0	1	0	0
49 (90.7)	49 (90.7)	0	46 (85.2)	0	0	20	0	0
158 (61.7)	157 (61.3)	0	143 (55.9)	0	0	61	0	0
69 (38.8)	66 (37.1)	12 (6.7)	49 (27.5)	0	12	47	2	12 (6.7)
61 (32.6)	54 (28.9)	48 (25.7)	4 (2.1)	0	48	4	0	47 (25.1)
25 (15.2)	25 (15.2)	7 (4.3)	18 (11.0)	0	7	18	0	7 (4.3)
69 (57.0)	66 (54.5)	7 (5.8)	14 (11.6)	0	7	12	2	7 (5.8)
224 (34.5)	211 (32.5)	74 (11.4)	85 (13.1)	0	74	81	4	73 (11.2)
51 (15.7)	34 (10.5)	11 (3.4)	20 (6.2)	0	11	20	0	11 (3.4)
7 (3.0)	7 (3.0)	7 (3.0)	0	0	7	0	0	7 (3.0)
8 (3.6)	8 (3.6)	8 (3.6)	0	0	8	0	0	8 (3.6)
32 (11.4)	29 (10.4)	10 (3.6)	19 (6.8)	0	10	6	13	10 (3.6)
98 (9.3)	78 (7.4)	36 (3.4)	39 (3.7)	0	36	26	13	36 (3.4)

(H)HH, *R. japonica* from Hiroshima

2. *Rana tsushimensis* and *R. japonica* from Hiroshima

A. Control matings

a. *Rana tsushimensis*, (T)TT♀×(T)TT♂

In 1984, three females of the *R. tsushimensis*, (T)TT♀, Nos. 16~18, were mated with a male *R. tsushimensis*, (T)TT♂, No. 7. In three control matings, 170 (92.9%) of 183 eggs in total cleaved normally, 167 (91.3%) hatched normally and 161 (88.0%) metamorphosed normally (Table 3).

When the chromosomes were examined in the tail-tips of 60 normal 30-day-old tadpoles produced from the above three matings, it was found that they were all diploids (Table 3; Fig. 1).

b. *Rana japonica* from Hiroshima, (H)HH♀×(H)HH♂

In 1984, four females of *R. japonica* from Hiroshima, (H)HH♀, Nos. 1~4, were mated with a male *R. japonica* from Hiroshima, (H)HH♂, No. 3. In four control matings, 169 (94.9%) of 178 eggs in total cleaved normally, 166 (93.3%) hatched normally and 162 (91.0%) metamorphosed normally (Table 3).

When the chromosomes were examined in the tail-tips of 80 normal 30-day-old tadpoles produced from the above four matings, it was found that they were all diploids (Table 3; Fig. 1).

B. Hybrid matings

a. (T)TT♀×(H)HH♂

In 1984, three female *R. tsushimensis*, (T)TT♀, Nos. 16~18, were mated with two male *R. japonica* from Hiroshima, (H)HH♂, Nos. 1 and 2. In three hybrid matings, 201 (93.1%) of 216 eggs in total cleaved normally. Differing from the control matings, 143 of the normally cleaved eggs died of various abnormalities at embryonic stages and 58 (26.9%) hatched normally. While all the normally hatched tadpoles began to eat, they gradually lost their appetite and became emaciated. All the tadpoles died without attaining metamorphosis (Table 3).

When the chromosomes were examined in the tail-tips of 55 underdeveloped 30-day-old tadpoles produced from the above three matings, it was found that they were all normal diploids (Table 3).

b. (H)HH♀×(T)TT♂

In 1984, four female *R. japonica* from Hiroshima, (H)HH♀, Nos. 1~4, were mated with three male *R. tsushimensis*, (T)TT♂, Nos. 8~10. In four hybrid matings, 237 (92.6%) of 256 eggs in total cleaved normally. However, 79 of the normally cleaved eggs died of various abnormalities at embryonic stages, while 158 (61.7%) in total hatched normally. Although 157 (61.3%) attained the feeding tadpole stage, all of them were emaciated and gradually died without attaining metamorphosis (Table 3).

When the chromosomes were examined in the tail-tips of 61 underdeveloped 30-day-old tadpoles produced from the above four matings, it was found that they were all normal diploids (Table 3).

C. Production of allotriploids

a. (T)TT♀×(H)HH♂, with heat-shock treatment of eggs

i) Treatment at 37.5°C

In three matings between three female *R. tsushimensis*, (T)TT♀, Nos. 16~18, and two male *R. japonica* from Hiroshima, (H)HH♂, Nos. 1 and 2, 187 (38.4%) of 487 eggs treated at 37.5°C for 3 minutes cleaved normally. However, 123 of the

normally cleaved eggs died of various abnormalities at embryonic stages, while 64 (13.1%) in total hatched normally and eventually, 63 (12.9%) metamorphosed normally (Table 3).

When the chromosomes were examined in the tail-tips of the 63 normally developed 30-day-old tadpoles produced from the above three matings, it was found that they were all triploids (Table 3; Fig. 2).

ii) Treatment at 38°C

In three matings between three female *R. tsushimensis*, (T)TT♀, Nos. 16~18, and two male *R. japonica* from Hiroshima, (H)HH♂, Nos. 1 and 2, 307 (48.5%) of 633 eggs treated at 38°C for 3 minutes cleaved normally. However, 240 of the normally cleaved eggs died of various abnormalities at embryonic stages, while 67 (10.6%) in total hatched normally and eventually 63 (10.0%) metamorphosed normally (Table 3).

When the chromosomes were examined in the tail-tips of the 63 normally developed 30-day-old tadpoles produced from the above three matings, it was found that they were all triploids (Table 3; Fig. 2).

b. (H)HH♀×(T)TT♂, with heat-shock treatment of eggs

i) Treatment at 37°C

In four matings between four female *R. japonica* from Hiroshima, (H)HH♀, Nos. 1~4, and three male *R. tsushimensis*, (T)TT♂, Nos. 8~10, 482 (74.2%) of 650 eggs treated at 37°C for 3 minutes cleaved normally. However, 258 of the normally cleaved eggs died of various abnormalities at embryonic stages, while 224 (34.5%) in total hatched normally. Of 159 30-day-old tadpoles, 74 (11.4%) were normal and 85 (13.1%) were underdeveloped. Eventually, 73 (11.2%) of the normal tadpoles metamorphosed normally, and the 85 underdeveloped tadpoles died without attaining metamorphosis (Table 3).

When the chromosomes were examined in the tail-tips of the 74 normal and 85 underdeveloped 30-day-old tadpoles produced from the above four matings, it was found that the 74 normal tadpoles were all triploids. While four of the 85 underdeveloped tadpoles were triploids, the other 81 were diploids (Table 3; Fig. 2).

ii) Treatment at 38°C

In four matings between four female *R. japonica*, (H)HH♀, Nos. 1~4, and three male *R. tsushimensis*, (T)TT♂, Nos. 8~10, 372 (35.2%) of 1058 eggs treated at 38°C for 3 minutes cleaved normally. However, 274 of the normally cleaved eggs died of various abnormalities at embryonic stages, while 98 (9.3%) hatched normally. Of 75 30-day-old tadpoles, 36 (3.4%) became normally metamorphosed frogs, while 39 underdeveloped tadpoles died without attaining metamorphosis (Table 3).

When the chromosomes were examined in the tail-tips of 36 normal and 39 underdeveloped 30-day-old tadpoles produced from the above four matings, it was

found that the 36 normal tadpoles were all triploids, while 26 of the 39 underdeveloped tadpoles were diploids and the other 13 were triploids (Table 3; Fig. 2).

II. Sex of diploid and triploid hybrids

1. *Rana tsushimensis* and *R. japonica* from Ichinoseki

A. Control matings

a. *Rana tsushimensis*, (T)TT♀×(T)TT♂

Of 609 metamorphosed frogs produced from the 11 control matings in 1979, 1980, 1983 and 1984, the sex of 560 was examined at the juvenile or mature stage. At the juvenile stage, 172 of 363 frogs were females with normal ovaries, 17 were hermaphrodites with gonads transforming from ovaries into testes, and the remaining 174 were males with normal testes. Of 197 mature frogs, 101 were females and the other 96 were males. When the hermaphrodites were counted as males, 273 and 287 (51.3%) of the juvenile and mature frogs were females and males, respectively (Table 4).

b. *Rana japonica* from Ichinoseki, (I)II♀×(I)II♂

Of 477 metamorphosed frogs produced from the control matings in 1979, 1980 and 1984, the sex of 442 was examined at the juvenile or mature stage. At the juvenile stage, 88 of 193 frogs were females with normal ovaries, one was a hermaphrodite with gonads transforming from ovaries into testes, and the remaining 104 were males with normal testes. Of 249 mature frogs, 114 were females and the other 135 were males. When the hermaphrodite was counted as a male, 202 and 240 (54.3%) of the juvenile and mature frogs were females and males, respectively (Table 4).

B. Diploid hybrids and allotriploids obtained without heat-shock treatment

a. (T)TT♀×(I)II♂

Of 22 normally metamorphosed allotriploids, (T)TTI, produced from crosses between female *R. tsushimensis* and male *R. japonica* from Ichinoseki in 1979, 1980 and 1983, the sex of 19 was examined at the juvenile or mature stage. It was found that the 19 allotriploids including 13 juvenile and six mature frogs were all males (Table 4).

b. (I)II♀×(T)TT♂

Of 558 metamorphosed frogs produced from crosses between female *R. japonica* from Ichinoseki and male *R. tsushimensis* in 1979, 1980, and 1984, 557 were diploid hybrids, (I)IT, which died of infirmity within 1~3 months after metamorphosis, while the remainder was an allotriploid. It was found that 438 of the 557 diploid hybrids were males, six were hermaphrodites and the other 113 could not be determined owing to post-mortem changes. The only allotriploid, (I)IIT, was a mature male. When the hermaphrodites were counted as males, the 444 diploid

TABLE 4

Sex of hybrids and allotriploids between *Rana tsushimensis* and *R. japonica* from Ichinoseki and the controls

Year	Parents		Heat shock	Ploidy	Constitution	Sex of frogs observed within 3 months after metamorphosis			Sex of mature frogs		Sex of all frogs examined			
	Female	Male				♀	♂	♀♂	♀	♀♂	Total	♀	♀♂	(%)
1979	(T)TT, No. 2	(T)TT, No. 1	None	2n	(T)TT	9	1	14	12	2	38	21	17	(44.7)
	(T)TT, No. 3	(T)TT, No. 2	None	2n	(T)TT	10	0	4	6	12	32	16	16	(50.0)
1980	(T)TT, No. 4	(T)TT, No. 3	None	2n	(T)TT	13	8	15	34	27	97	47	50	(51.5)
1983	(T)TT, No. 6	(T)TT, No. 5	None	2n	(T)TT	16	0	18	—	—	34	16	18	(52.9)
	(T)TT, No. 7	(T)TT, No. 5	None	2n	(T)TT	24	1	23	—	—	48	24	24	(50.0)
	(T)TT, No. 9	(T)TT, No. 6	None	2n	(T)TT	25	1	23	—	—	49	25	24	(49.0)
	(T)TT, No. 10	(T)TT, No. 6	None	2n	(T)TT	11	0	12	12	8	43	23	20	(46.5)
1984	(T)TT, No. 12	(T)TT, No. 7	None	2n	(T)TT	29	2	23	10	13	77	39	38	(49.4)
	(T)TT, No. 13	(T)TT, No. 7	None	2n	(T)TT	12	2	13	7	9	43	19	24	(55.8)
	(T)TT, No. 14	(T)TT, No. 7	None	2n	(T)TT	8	0	11	9	10	38	17	21	(55.3)
	(T)TT, No. 15	(T)TT, No. 7	None	2n	(T)TT	15	2	18	11	15	61	26	35	(57.4)
Total				2n	(T)TT	172	17	174	101	96	560	273	287	(51.3)
1979	(T)TT, No. 2	(I)II, No. 1	None	3n	(T)TTI	0	0	4	—	—	4	0	4	(100)
	(T)TT, No. 3	(I)II, No. 2	None	3n	(T)TTI	0	0	5	0	4	9	0	9	(100)
1980	(T)TT, No. 4	(I)II, No. 3	None	3n	(T)TTI	—	—	—	0	1	1	0	1	(100)
1983	(T)TT, No. 6	(I)II, No. 5	None	3n	(T)TTI	0	0	1	—	—	1	0	1	(100)
	(T)TT, No. 7	(I)II, No. 5	None	3n	(T)TTI	0	0	1	—	—	1	0	1	(100)
	(T)TT, No. 9	(I)II, No. 6	None	3n	(T)TTI	0	0	2	—	—	2	0	2	(100)
	(T)TT, No. 10	(I)II, No. 6	None	3n	(T)TTI	—	—	—	0	1	1	0	1	(100)
Total				3n	(T)TTI	0	0	13	0	6	19	0	19	(100)
1984	(T)TT, No. 12	(I)II, No. 7	Treated	3n	(T)TTI	0	1	9	0	9	19	0	19	(100)
	(T)TT, No. 13	(I)II, No. 8	Treated	3n	(T)TTI	0	4	13	0	1	18	0	18	(100)
	(T)TT, No. 14	(I)II, No. 8	Treated	3n	(T)TTI	0	4	25	0	39	68	0	68	(100)
	(T)TT, No. 15	(I)II, No. 8	Treated	3n	(T)TTI	0	4	30	0	7	41	0	41	(100)
Total				3n	(T)TTI	0	13	77	0	56	146	0	146	(100)
1979	(I)II, No. 1	(T)TT, No. 1	None	2n	(I)IT	0	1	143	—	—	144	0	144	(100)
	(I)II, No. 2	(T)TT, No. 2	None	2n	(I)IT	0	1	62	—	—	63	0	63	(100)
				3n	(I)IIT	—	—	—	0	1	1	0	1	(100)
	(I)II, No. 3	(T)TT, No. 2	None	2n	(I)IT	0	0	13	—	—	13	0	13	(100)
1980	(I)II, No. 4	(T)TT, No. 3	None	2n	(I)IT	0	0	35	—	—	35	0	35	(100)
	(I)II, No. 5	(T)TT, No. 4	None	2n	(I)IT	0	0	56	—	—	56	0	56	(100)
	(I)II, No. 6	(T)TT, No. 4	None	2n	(I)IT	0	1	61	—	—	62	0	62	(100)
1984	(I)II, No. 7	(T)TT, No. 8	None	2n	(I)IT	0	2	49	—	—	51	0	51	(100)
	(I)II, No. 8	(T)TT, No. 9	None	2n	(I)IT	0	1	19	—	—	20	0	20	(100)
Total				2n	(I)IT	0	6	438	—	—	444	0	444	(100)
				3n	(I)IIT	—	—	—	0	1	1	0	1	(100)
1984	(I)II, No. 7	(T)TT, No. 10	Treated	2n	(I)IT	0	0	22	—	—	22	0	22	(100)
	(I)II, No. 8	(T)TT, No. 10	Treated	3n	(I)IIT	0	0	1	0	8	9	0	9	(100)
	(I)II, No. 8	(T)TT, No. 11	Treated	2n	(I)IT	0	2	15	—	—	17	0	17	(100)
				3n	(I)IIT	0	0	5	0	20	25	0	25	(100)
Total				2n	(I)IT	0	2	37	—	—	39	0	39	(100)
				3n	(I)IIT	0	0	6	0	28	34	0	34	(100)
1979	(I)II, No. 1	(I)II, No. 1	None	2n	(I)II	21	0	13	22	28	84	43	41	(48.8)
	(I)II, No. 2	(I)II, No. 1	None	2n	(I)II	10	0	14	19	25	68	29	39	(57.4)
	(I)II, No. 3	(I)II, No. 2	None	2n	(I)II	4	0	5	6	15	30	10	20	(66.7)
1980	(I)II, No. 4	(I)II, No. 3	None	2n	(I)II	17	1	18	7	11	54	24	30	(55.6)
	(I)II, No. 5	(I)II, No. 4	None	2n	(I)II	12	0	9	12	18	51	24	27	(52.9)
	(I)II, No. 6	(I)II, No. 4	None	2n	(I)II	7	0	10	20	18	55	27	28	(50.9)
1984	(I)II, No. 7	(I)II, No. 9	None	2n	(I)II	9	0	22	17	9	57	26	31	(54.4)
	(I)II, No. 8	(I)II, No. 9	None	2n	(I)II	8	0	13	11	11	43	19	24	(55.8)
Total				2n	(I)II	88	1	104	114	135	442	202	240	(54.3)

(T)TT, *R. tsushimensis* (I)II, *R. japonica* from Ichinoseki

(I)IT, Diploid hybrids between female *R. japonica* from Ichinoseki and male *R. tsushimensis*

(T)TTI, Allotriploids between female *R. tsushimensis* and male *R. japonica* from Ichinoseki

(I)IIT, Allotriploids between female *R. japonica* from Ichinoseki and male *R. tsushimensis*

hybrids were all males (Table 4).

C. Diploid hybrids and allotriploids obtained by heat-shock treatment

a. (T)TT♀×(I)II♂, with heat-shock treatment of eggs

Of 163 normally metamorphosed allotriploids, (T)TTI, produced from eggs of female *R. tsushimensis* inseminated with sperm of male *R. japonica* from Ichinoseki by heat-shock treatment, the sex of 146 allotriploids was examined at the juvenile or mature stage. At the juvenile stage, 77 of 90 allotriploids were males and the other 13 were hermaphrodites. At the mature stage, 56 allotriploids were all males. When the hermaphrodites were counted as males, the 146 allotriploids in total were all males (Table 4).

b. (I)II♀×(T)TT♂, with heat-shock treatment of eggs

Of 79 metamorphosed diploid hybrids, (I)IT, and allotriploids, (I)IIT, produced from eggs of female *R. japonica* from Ichinoseki inseminated with sperm of male *R. tsushimensis* by heat-shock treatment, the sex of 39 diploid hybrids and 34 allotriploids was examined at the juvenile or mature stage. At the juvenile stage, 37 of the 39 diploid hybrids and six allotriploids were males and the other two diploid hybrids were hermaphrodites. At the mature stage, 28 allotriploids were all males. When the hermaphrodites were counted as males, the 73 diploid hybrids and allotriploids were all males (Table 4).

2. *Rana tsushimensis* and *R. japonica* from Hiroshima

A. Control matings

a. *Rana tsushimensis*, (T)TT♀×(T)TT♂

Of 161 normally metamorphosed frogs produced from the control matings in 1984, the sex of 154 was examined at the juvenile or mature stage. At the juvenile stage, 48 of 87 frogs were females with normal ovaries, three were hermaphrodites with gonads transforming from ovaries into testes, and the remaining 36 were males with normal testes. Of 67 mature frogs, 25 were females and the other 42 were males. When the hermaphrodites were counted as males, 73 were females and 81 (52.6%) were males in total (Table 5).

b. *Rana japonica* from Hiroshima, (H)HH♀×(H)HH♂

Of 162 normally metamorphosed frogs produced from the control matings in 1984, the sex of 149 was examined at the juvenile or mature stage. At the juvenile stage, 25 of 45 frogs were females with normal ovaries, one was a hermaphrodite with gonads transforming from ovaries into testes, and the remaining 19 were males with normal testes. Of 104 mature frogs, 41 were females and the other 63 were males. When the hermaphrodite was counted as a male, 66 were females and 83 (55.7%) were males in total (Table 5).

B. Allotriploids produced by heat-shock treatment

a. (T)TT♀×(H)HH♂, with heat-shock treatment of eggs

Of 126 normally metamorphosed allotriploids, (T)TTH, produced from eggs of female *R. tsushimensis* inseminated with sperm of male *R. japonica* from Hiroshima by heat-shock treatment, the sex of 123 allotriploids was examined at the juvenile or mature stage. At the juvenile stage, 49 of 50 allotriploids were males and the other was a hermaphrodite. At the mature stage, 73 allotriploids were all males. When the hermaphrodite was counted as a male, the 123 allotriploids in total were all males (Table 5).

b. (H)HH♀×(T)TT♂, with heat-shock treatment of eggs

Of 109 normally metamorphosed allotriploids, (H)HHT, produced from eggs of female *R. japonica* from Hiroshima inseminated with sperm of male *R. tsushimensis* by heat-shock treatment, the sex of 100 allotriploids was examined at the juvenile or mature stage. At the juvenile stage, 38 of 41 allotriploids were males and the other three were hermaphrodites. At the mature stage, 59 allotriploids were all males. When the hermaphrodites were counted as males, the 100 allotriploids in total were all males (Table 5).

TABLE 5

Sex of reciprocal allotriploids between *Rana tsushimensis* and *R. japonica* from Hiroshima and the controls

Year	Parents		Heat shock	Ploidy	Constitution	Sex of frogs observed within 3 months after metamorphosis			Sex of mature frogs		Sex of all frogs examined		
	Female	Male				♀	♂	♂	♀	♂	Total	♀	♂ (%)
1984	(T)TT, No. 16	(T)TT, No. 7	None	2n	(T)TT	17	1	12	13	20	63	30	33 (52.4)
	(T)TT, No. 17	(T)TT, No. 7	None	2n	(T)TT	14	2	13	7	9	45	21	24 (53.3)
	(T)TT, No. 18	(T)TT, No. 7	None	2n	(T)TT	17	0	11	5	13	46	22	24 (52.2)
Total				2n	(T)TT	48	3	36	25	42	154	73	81 (52.6)
1984	(T)TT, No. 16	(H)HH, No. 1	Treated	3n	(T)TTH	0	0	9	0	42	51	0	51 (100)
	(T)TT, No. 17	(H)HH, No. 1	Treated	3n	(T)TTH	0	0	5	—	—	5	0	5 (100)
	(T)TT, No. 18	(H)HH, No. 2	Treated	3n	(T)TTH	0	1	35	0	31	67	0	67 (100)
Total				3n	(T)TTH	0	1	49	0	73	123	0	123 (100)
1984	(H)HH,No. 1	(T)TT, No. 8	Treated	3n	(H)HHT	0	0	11	0	9	20	0	20 (100)
	(H)HH,No. 2	(T)TT, No. 8	Treated	3n	(H)HHT	0	2	10	0	41	53	0	53 (100)
	(H)HH,No. 3	(T)TT, No. 9	Treated	3n	(H)HHT	0	0	5	0	8	13	0	13 (100)
	(H)HH,No. 4	(T)TT, No. 9	Treated	3n	(H)HHT	0	1	12	0	1	14	0	14 (100)
Total				3n	(H)HHT	0	3	38	0	59	100	0	100 (100)
1984	(H)HH,No. 1	(H)HH, No. 3	None	2n	(H)HH	11	1	6	14	23	55	25	30 (54.5)
	(H)HH,No. 2	(H)HH, No. 3	None	2n	(H)HH	3	0	2	7	13	25	10	15 (60.0)
	(H)HH,No. 3	(H)HH, No. 3	None	2n	(H)HH	4	0	5	11	10	30	15	15 (50.0)
	(H)HH,No. 4	(H)HH, No. 3	None	2n	(H)HH	7	0	6	9	17	39	16	23 (59.0)
Total				2n	(H)HH	25	1	19	41	63	149	66	83 (55.7)

(T)TT, *R. tsushimensis* (H)HH, *R. japonica* from Hiroshima

(T)TTH, Allotriploids between female *R. tsushimensis* and male *R. japonica* from Hiroshima

(H)HHT, Allotriploids between female *R. japonica* from Hiroshima and male *R. tsushimensis*

III. Morphological characters of mature male allotriploids

1. Measurements

A. Male allotriploids produced from *Rana tsushimensis* and *R. japonica* from Ichinoseki

The external characters of allotriploids were compared with those of the controls. As shown in Table 6, four control male *R. tsushimensis*, (T)TT, were 28.8~32.3 mm, 30.7 mm on the average, in body length, and four control male *R. japonica* from Ichinoseki, (I)II, were 44.4~51.3 mm, 47.7 mm on the average, in body length. On the other hand, 14 male allotriploids, (T)TTI, were 29.4~37.7 mm, 33.7 mm on the average, in body length, and five male allotriploids, (I)IIT, were 37.6~38.6 mm, 38.1 mm on the average, in body length.

The ratios of hind leg length to body length (b/a) were 1.57~1.69, 1.62 on the average, in four male *R. tsushimensis*, 1.35~1.45, 1.40 on the average, in four male *R. japonica* from Ichinoseki, 1.46~1.72, 1.58 on the average, in 14 male allotriploids, (T)TTI, and 1.45~1.69, 1.53 on the average, in five male allotriploids, (I)IIT.

The shape of tympanic membranes was round in both species. Their diameters were 2.0~2.4 mm, 2.2 mm on the average, in four male *R. tsushimensis*, 3.2~4.1 mm, 3.6 mm on the average, in four male *R. japonica* from Ichinoseki, 1.8~2.9 mm, 2.4 mm on the average, in 14 male allotriploids, (T)TTI, and 2.6~3.7 mm, 3.1 mm on the average, in five male allotriploids, (I)IIT.

The left and right testes of four male *R. tsushimensis* were 2.0~3.6 mm, 2.9 mm on the average, in length and 1.6~2.2 mm, 1.9 mm on the average, in width. The testes of four male *R. japonica* from Ichinoseki were 2.4~5.2 mm, 3.8 mm on the average, in length and 2.0~4.0 mm, 3.0 mm on the average, in width. The testes of 14 male allotriploids, (T)TTI, were 0.8~3.8 mm, 2.9 mm on the average, in length and 0.6~3.1 mm, 2.1 mm on the average, in width. The testes of five male allotriploids, (I)IIT, were 3.0~3.6 mm, 3.3 mm on the average, in length and 1.2~2.2 mm, 1.8 mm on the average, in width (Table 6; Figs. 3 and 4).

B. Male allotriploids produced from *Rana tsushimensis* and *Rana japonica* from Hiroshima

As shown in Table 7, two control male *R. tsushimensis*, (T)TT, were 31.6 mm and 32.0 mm, 31.8 mm on the average, in body length and two male *R. japonica* from Hiroshima, (H)HH , were 43.4 mm and 47.6 mm, 45.5 mm on the average, in body length. On the other hand, 10 male allotriploids, (T)TTH, were 27.0~34.6 mm, 30.8 mm on the average, in body length and five male allotriploids, (H)HHT, were 33.2~37.0 mm, 35.2 mm on the average, in body length.

The ratios of hind leg length to body length (b/a) were 1.60 and 1.61, 1.61 on the average, in two male *R. tsushimensis*, 1.51 and 1.55, 1.53 on the average, in two male *R. japonica* from Hiroshima, 1.60~1.74, 1.71 on the average, in 10 male allotriploids, (T)TTH, and 1.44~1.65, 1.54 on the average, in five male allotriploids, (H)HHT.

The shape of tympanic membranes was round in both species, and their diameters were 2.0 and 2.2 mm, 2.1 mm on the average, in two male *R. tsushimensis*, 3.2 and 3.6 mm, 3.4 mm on the average, in two male *R. japonica* from Hiroshima, 1.6~2.4 mm, 2.0 mm on the average, in 10 male allotriploids, (T)TTH, and 2.4~2.8 mm, 2.5 mm on the average, in five male allotriploids,

TABLE 6

Measurements of reciprocal allotriploids between *Rana tsushimensis* and *R. japonica* from Ichinoseki and the controls

Kind	Age (months)	Sex	Body length (a) (mm)	Fore-leg length (mm)	Hind leg length (b) (mm)	Snout length (mm)	Diameter of tympanic membrane (mm)	b/a	Size of testes left (mm)	Size of testes right (mm)
(T)TT 79, No. 1*	14	♂	32.3	20.0	51.1	4.6	2.4	1.58	3.6×2.2	3.3×2.0
(T)TT 79, No. 2*	14	♂	31.4	19.4	49.3	4.2	2.2	1.57	2.9×1.7	3.4×1.9
(T)TT 84, No. 6*	13	♂	30.4	19.8	49.6	3.8	2.0	1.63	2.8×2.2	2.6×1.8
(T)TT 84, No. 7*	13	♂	28.8	20.0	48.8	4.0	2.0	1.69	2.4×1.8	2.0×1.6
Average			30.7	19.8	49.7	4.2	2.2	1.62	2.9×2.0	2.8×1.8
(T)TTI 79, No. 1*	14	♂	37.7	25.2	61.7	5.7	2.8	1.64	2.7×2.4	3.4×3.1
(T)TTI 79, No. 2*	14	♂	36.0	22.8	59.6	5.5	2.9	1.66	3.1×2.2	2.7×2.2
(T)TTI 80, No. 3*	11.5	♂	33.0	20.8	54.4	5.0	2.3	1.65	3.2×2.5	3.5×2.5
(T)TTI 83, No. 4*	12	♂	30.9	18.6	48.2	4.8	2.5	1.56	3.8×2.8	3.6×2.4
(T)TTI 84, No. 5*	13	♂	35.2	22.0	52.8	5.0	2.2	1.50	3.0×1.8	2.6×1.4
(T)TTI 84, No. 6*	13	♂	35.6	22.6	53.8	5.2	2.4	1.51	3.0×2.4	3.2×2.4
(T)TTI 84, No. 7*	13	♂	29.4	18.4	48.0	4.2	2.0	1.63	3.8×2.0	2.6×1.8
(T)TTI 84, No. 8*	13	♂	36.4	23.0	53.6	5.4	2.4	1.47	3.0×1.8	3.4×2.0
(T)TTI 84, No. 9*	13	♂	35.6	22.8	52.0	5.2	2.4	1.46	2.6×1.8	2.8×2.2
(T)TTI 84, No. 10*	13	♂	36.6	23.0	58.2	5.2	2.4	1.59	3.6×2.4	2.2×1.4
(T)TTI 84, No. 11*	13	♂	31.4	20.0	50.4	4.0	2.2	1.61	2.6×2.4	3.2×2.6
(T)TTI 84, No. 12*	13	♂	29.6	19.0	47.4	4.0	1.8	1.60	3.2×2.2	2.0×1.8
(T)TTI 79, No. 13	14	♂	32.2	20.9	55.5	5.1	2.6	1.72	1.8×1.6	2.7×2.2
(T)TTI 79, No. 14	19	♂	32.3	22.2	50.3	5.1	2.3	1.56	2.2×1.8	0.8×0.6
Average			33.7	21.5	53.3	5.0	2.4	1.58	3.0×2.2	2.8×2.0
(I)IIT 79, No. 1*	14	♂	38.3	25.3	64.8	6.2	3.7	1.69	3.0×2.1	3.4×2.2
(I)IIT 84, No. 2*	12.5	♂	37.8	23.4	56.4	5.8	3.0	1.49	3.4×1.4	3.0×1.2
(I)IIT 84, No. 3*	12.5	♂	37.6	24.6	58.2	6.2	3.0	1.55	3.0×1.6	3.0×1.4
(I)IIT 84, No. 4*	12.5	♂	38.0	23.4	55.2	6.4	2.6	1.45	3.2×2.0	3.4×2.0
(I)IIT 84, No. 5*	12.5	♂	38.6	23.8	57.2	6.0	3.0	1.48	3.4×1.8	3.6×2.0
Average			38.1	24.1	58.4	6.1	3.1	1.53	3.2×1.8	3.3×1.8
(I)II 84, No. 1*	12.5	♂	44.4	25.0	60.6	6.8	3.4	1.36	3.4×2.4	3.0×2.4
(I)II 84, No. 2*	12.5	♂	44.6	25.4	60.4	6.8	3.2	1.35	2.4×2.0	3.2×2.4
(I)II 79, No. 3	14	♂	51.3	28.2	74.3	7.7	4.1	1.45	4.7×4.0	5.2×3.4
(I)II 79, No. 4	14	♂	50.6	28.8	72.0	8.0	3.7	1.42	4.7×3.6	3.8×3.3
Average			47.7	26.9	66.8	7.3	3.6	1.40	3.8×3.0	3.8×2.9

*, These frogs were used in the backross matings to examine the reproductive capacity.

(T)TT, *R. tsushimensis* (I)II, *R. japonica* from Ichinoseki

(T)TTI, Allotriploids between female *R. tsushimensis* and male *R. japonica* from Ichinoseki

(I)IIT, Allotriploids between female *R. japonica* from Ichinoseki and male *R. tsushimensis*

(H)HHT.

The left and right testes of two male *R. tsushimensis* were 3.0~3.6 mm, 3.3 mm on the average, in length and 1.8 and 2.2 mm, 1.9 mm on the average, in width. The testes of two male *R. japonica* from Hiroshima were 3.8~5.2 mm, 4.6 mm on

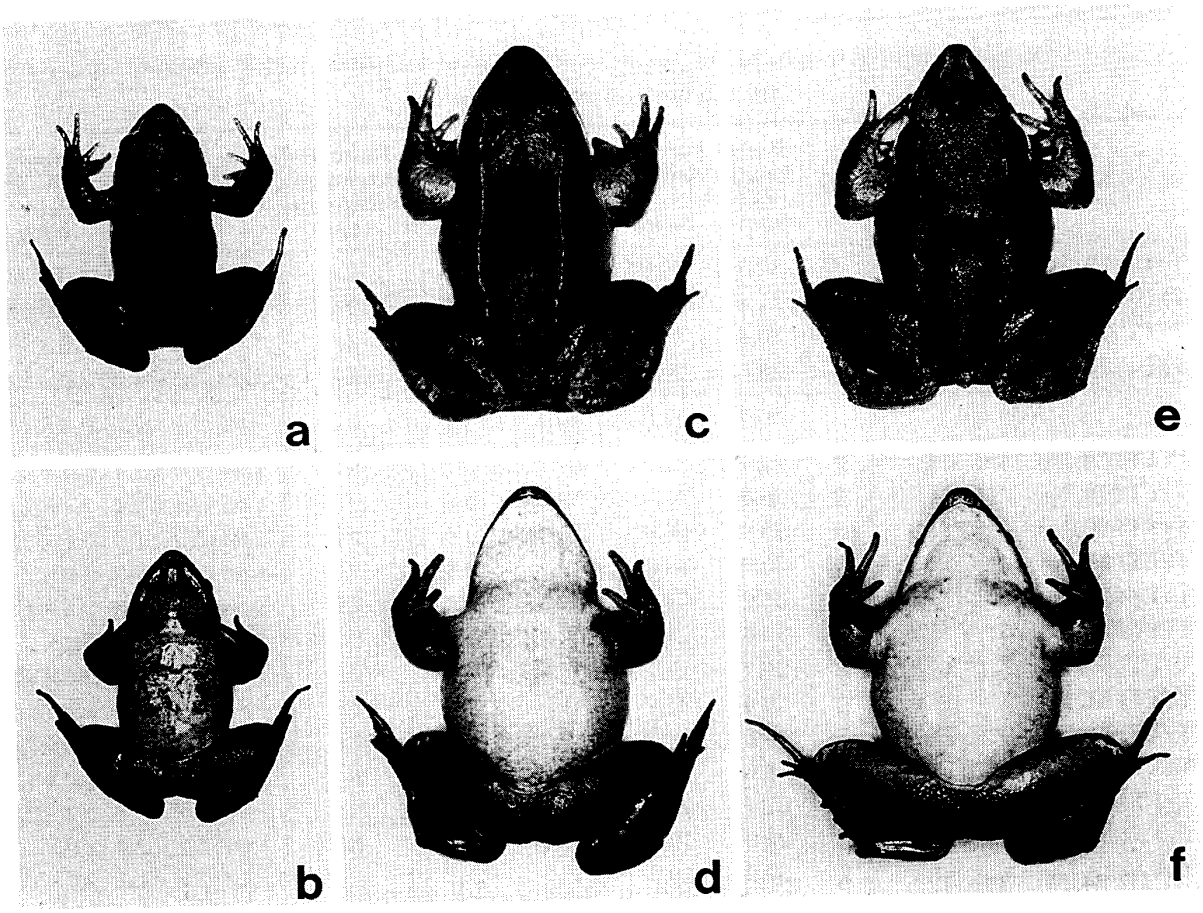

Fig. 3.

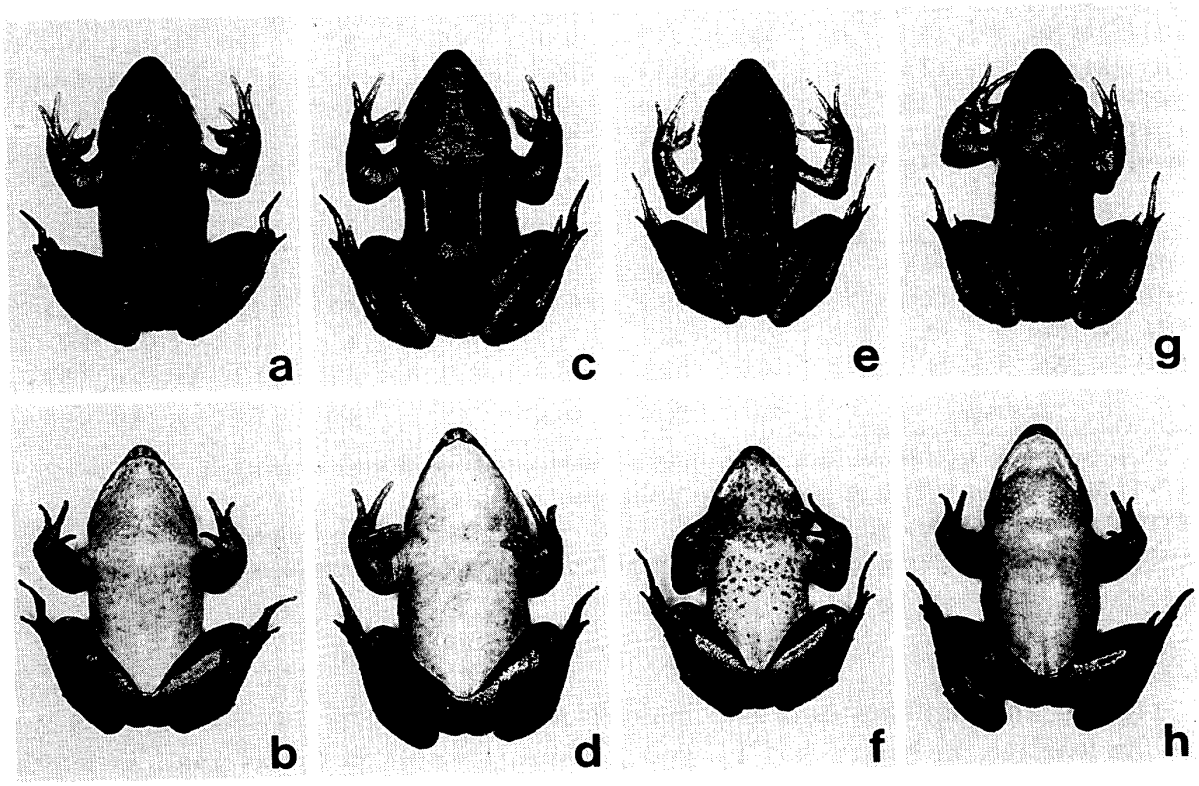

Fig. 4.

TABLE 7

Measurements of reciprocal allotriploids between *Rana tsushimensis* and *R. japonica* from Hiroshima and the controls

Kind	Age (months)	Sex	Body length (a) (mm)	Fore-leg length (mm)	Hind leg length (b) (mm)	Snout length (mm)	Diameter of tympanic membrane (mm)	b/a	Size of testes left (mm)	Size of testes right (mm)
(T)TT 84, No. 1*	12.5	♂	31.6	20.6	50.6	4.0	2.0	1.60	3.2×1.8	3.4×1.8
(T)TT 84, No. 2*	12.5	♂	32.0	20.6	51.6	4.2	2.2	1.61	3.6×2.2	3.0×1.8
Average			31.8	20.6	51.1	4.1	2.1	1.61	3.4×2.0	3.2×1.8
(T)TTH 84, No. 1*	12.5	♂	30.8	20.4	49.4	4.8	2.2	1.60	0	3.6×3.2
(T)TTH 84, No. 2*	12.5	♂	29.0	19.6	48.2	4.0	1.6	1.66	1.8×1.4	2.8×1.8
(T)TTH 84, No. 3*	12.5	♂	31.4	21.4	53.8	4.6	2.0	1.71	0	4.0×2.8
(T)TTH 84, No. 4*	12.5	♂	30.0	21.2	51.3	5.0	1.6	1.71	1.8×1.6	2.4×1.8
(T)TTH 84, No. 5*	12.5	♂	29.8	21.0	52.0	4.4	2.0	1.74	1.8×1.6	2.6×1.8
(T)TTH 84, No. 6*	12.5	♂	27.0	17.8	46.6	4.6	1.8	1.73	3.0×2.8	0
(T)TTH 84, No. 7*	12.5	♂	34.6	22.8	60.2	4.2	2.4	1.74	1.6×1.6	3.6×2.6
(T)TTH 84, No. 8*	12.5	♂	32.4	21.8	56.4	4.6	2.2	1.74	2.6×2.0	2.6×2.0
(T)TTH 84, No. 9*	12.5	♂	31.4	20.0	54.6	4.8	2.0	1.74	2.6×2.0	0.8×0.6
(T)TTH 84, No. 10*	12.5	♂	31.4	21.6	53.8	4.4	1.8	1.71	2.4×2.0	3.2×1.6
Average			30.8	20.8	52.6	4.5	2.0	1.71	2.2×1.9	2.8×2.0
(H)HHT 84, No. 1*	12.5	♂	35.8	22.2	54.8	5.2	2.4	1.53	3.8×2.2	3.6×2.2
(H)HHT 84, No. 2*	12.5	♂	34.4	20.8	49.4	4.8	2.4	1.44	3.2×2.0	3.0×2.2
(H)HHT 84, No. 3*	12.5	♂	35.8	22.8	55.6	5.4	2.4	1.55	3.6×2.2	3.0×2.0
(H)HHT 84, No. 4*	12.5	♂	33.2	22.0	50.4	4.6	2.6	1.52	2.8×1.4	2.6×1.4
(H)HHT 84, No. 5*	12.5	♂	37.0	23.6	61.2	4.8	2.8	1.65	2.8×1.4	2.6×1.4
Average			35.2	22.3	54.3	5.0	2.5	1.54	3.2×1.8	3.0×1.8
(H)HH 84, No. 1*	12.5	♂	47.6	29.0	73.8	7.0	3.6	1.55	5.2×3.0	4.2×2.8
(H)HH 84, No. 2*	12.5	♂	43.4	25.4	65.6	6.2	3.2	1.51	5.0×2.8	3.8×2.4
Average			45.5	27.2	69.7	6.6	3.4	1.53	5.1×2.9	4.0×2.6

*, These frogs were used in the backross matings to examine the reproductive capacity.

(T)TT, *R. tsushimensis* (H)HH, *R. japonica* from Hiroshima

(T)TTH, Allotriploids between female *R. tsushimensis* and male *R. japonica* from Hiroshima

(H)HHT, Allotriploids between female *R. japonica* from Hiroshima and male *R. tsushimensis*

Fig. 3. Adult male frogs of *Rana tsushimensis* and *R. japonica* from Ichinoseki and Hiroshima. ×0.87

a, b. *R. tsushimensis*, (T)TT 84♂, No. 6

c, d. *R. japonica* from Ichinoseki, (I)II 79♂

e, f. *R. japonica* from Hiroshima, (H)HH 79♂

Fig. 4. Mature reciprocal male allotriploids between *Rana tsushimensis* and *R. japonica* from Ichinoseki and Hiroshima. ×0.87

a, b. Allotriploid, (T)TTI 84♂, No. 8

c, d. Allotriploid, (I)IIT 84♂, No. 3

e, f. Allotriploid, (T)TTH 84♂, No. 8

g, h. Allotriploid, (H)HHT 84♂, No. 1

the average, in length and 2.4~3.0 mm, 2.8 mm on the average, in width. The testes of 10 male allotriploids, (T)TTH, were 0.8~4.0 mm, 2.5 mm on the average, in length and 0.6~3.2 mm, 2.0 mm on the average, in width. The testes of five male allotriploids, (H)HHT, were 2.6~3.8 mm, 3.1 mm on the average, in length and 1.4~2.2 mm, 1.8 mm on the average, in width (Table 7; Figs. 3 and 4).

2. External characters

The snout of *R. tsushimensis* was short and somewhat pointed, while that of *R. japonica* from Ichinoseki and Hiroshima was comparatively long and pointed. In both reciprocal allotriploids, the snouts were intermediate between those of the two species in length, but variable in shape. The dorso-lateral folds of *R. tsushimensis* were bent laterally at the back of the tympanic membranes and interrupted at their anterior ends, while those of *R. japonica* were nearly parallel with each other and nearly straight from the upper eyelids to the posterior ends of the body. In the allotriploids, (T)TTI and (T)TTH, the dorso-lateral folds were clear and thick lines and were conspicuously bent laterally at the back of the tympanic membranes. Both folds were interrupted at the anterior ends in almost all the allotriploids, (T)TTI and (T)TTH. In the allotriploids, (I)IIT and (H)HHT, the dorso-lateral folds were distinct and thick lines and were slightly bent laterally towards the upper margins of the tympanic membranes. The ventral surface of *R. tsushimensis* was suffused with brownish gray spots, except the posterior part of the abdomen, while that of *R. japonica* was white, grayish white, pale yellow or rarely orange, and sometimes was partly mottled with dark color. In most individuals of reciprocal allotriploids, the ventral surfaces were mottled with grayish color, except the posterior part of the abdomen(Figs. 3 and 4).

IV. Inner structures of the testes of mature male allotriploids

1. Allotriploids produced from *Rana tsushimensis* and *Rana japonica* from Ichinoseki

The inner structures of the testes of 18 control male *R. tsushimensis,* (T)TT, 10 control male *R. japonica* from Ichinoseki, (I)II , 44 male allotriploids between the two species, (T)TTI, and 15 males of the reciprocal allotriploids, (I)IIT, produced in 1979, 1980, 1983 and 1984, were examined(Figs. 5~7). They could be divided into five types. The testis of Type 1 was normal in the inner structures. All the seminiferous tubules were filled with bundles of normal spermatozoa(Fig. 5a~d). The testis of Type 2 showed two different parts of seminiferous tubules. One part was nearly normal and was filled with bundles of normal spermatozoa, while the other part was almost filled with abnormal spermatozoa and pycnotic nuclei. In each transverse section of the testis, there were one to several normal seminiferous tubules which were always less than half the number of all the seminiferous tubules(Fig. 6a, b). In the testis of Type 3, the seminiferous tubules were usually filled with abnormal spermatozoa and large and small pycnotic nuclei, while a very few normal spermatozoa were sparsely distributed in some seminiferous tubules(Fig. 6c, d). The testis of Type 4 contained a small number of abnormal

TABLE 8

Histological classification of testes of mature reciprocal allotriploids between *Rana tsushimensis* and *R. japonica* from Ichinoseki and the controls

Year	Parents		Heat shock	No. of mature male frogs	Ploidy	Constitution	No. of analyzed frogs					
	Female	Male					Total	Type of testes				
								1	2	3	4	5
1979	(T)TT, No. 3	(T)TT, No. 2	None	12	2n	(T)TT	4	4				
1980	(T)TT, No. 4	(T)TT, No. 3	None	27	2n	(T)TT	3	3				
1983	(T)TT, No. 10	(T)TT, No. 6	None	8	2n	(T)TT	3	3				
1984	(T)TT, No. 12	(T)TT, No. 7	None	13	2n	(T)TT	3	3				
	(T)TT, No. 14	(T)TT, No. 7	None	10	2n	(T)TT	5	5				
Total				70	2n	(T)TT	18	18	0	0	0	0
1979	(T)TT, No. 3	(I)II, No. 2	None	4	3n	(T)TTI	4	1	2			1
1980	(T)TT, No. 4	(I)II, No. 3	None	1	3n	(T)TTI	1		1			
1983	(T)TT, No. 10	(I)II, No. 6	None	1	3n	(T)TTI	1		1			
1984	(T)TT, No. 12	(I)II, No. 7	Treated	9	3n	(T)TTI	7		2		3	2
	(T)TT, No. 13	(I)II, No. 8	Treated	1	3n	(T)TTI	1			1		
	(T)TT, No. 14	(I)II, No. 8	Treated	39	3n	(T)TTI	28		9	9	2	8
	(T)TT, No. 15	(I)II, No. 8	Treated	7	3n	(T)TTI	2			1	1	
Total				62	3n	(T)TTI	44	1	15	11	6	11
1979	(I)II, No. 2	(T)TT, No. 2	None	1	3n	(I)IIT	1					1
1984	(I)II, No. 8	(T)TT, No. 10	Treated	8	3n	(I)IIT	2					2
	(I)II, No. 8	(T)TT, No. 11	Treated	20	3n	(I)IIT	12			1	2	9
Total				29	3n	(I)IIT	15	0	0	1	2	12
1979	(I)II, No. 2	(I)II, No. 1	None	25	2n	(I)II	4	4				
1984	(I)II, No. 8	(I)II, No. 9	None	11	2n	(I)II	6	6				
Total				36	2n	(I)II	10	10	0	0	0	0

(T)TT, *R. tsushimensis* (I)II, *R. japonica* from Ichinoseki

(T)TTI, Allotriploids between female *R. tsushimensis* and male *R. japonica* from Ichinoseki

(I)IIT, Allotriploids between female *R. japonica* from Ichinoseki and male *R. tsushimensis*

spermatozoa, which were large and small in size, in seminiferous tubules. Besides, the latter were abundantly occupied with large and small pycnotic nuclei (Fig. 7a, b). In the testis of Type 5, the seminiferous tubules contained a few large abnormal spermatozoa and a small number of large pycnotic nuclei(Fig. 7c, d). The eggs could not develop normally, when inseminated with sperm obtained from the testes of Types 3, 4 and 5.

Of the four kinds of frogs mentioned above, all the 18 males of the control *R. tsushimensis* and all the 10 males of the control *R. japonica* from Ichinoseki had testes of Type 1. Of the 44 male allotriploids, (T)TTI, one had testes of Type 1, while 15, 11, six and 11 had testes of Types 2, 3, 4 and 5, respectively. Of the 15 male allotriploids, (I)IIT, one, two and 12 had testes of Types 3, 4 and 5, respectively. There were no male allotriploids, (I)IIT, whose testes were of Types 1 and 2 (Table 8).

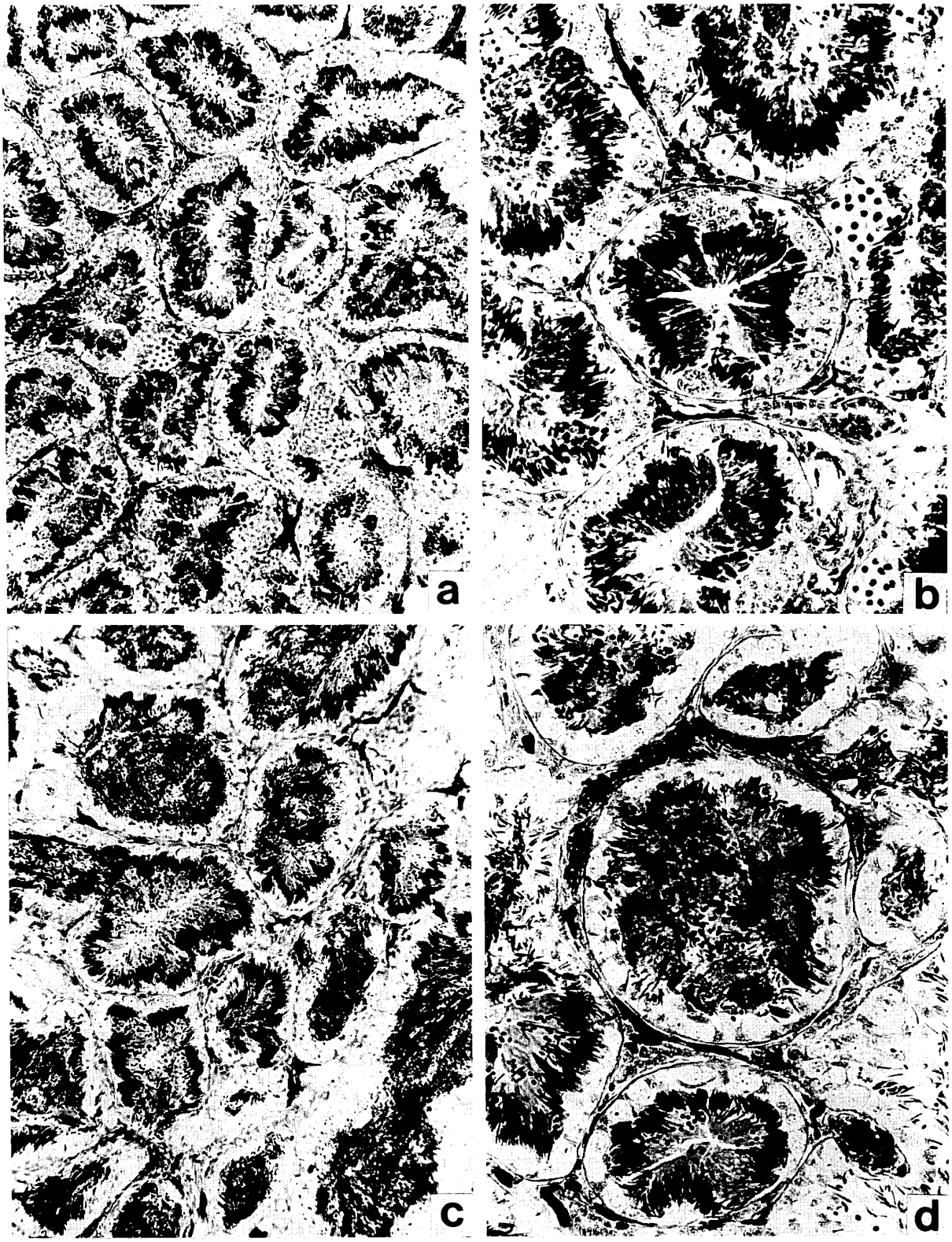

Fig. 5. Cross-sections of the testes of a control *Rana tsushimensis* and a male allotriploid between *R. tsushimensis* and *R. japonica* from Ichinoseki.

a. Control *R. tsushimensis*, (T)TT 79♂, No. 1 ×130
b. Ditto ×260
c. Type 1: Allotriploid, (T)TTI 79♂, No. 1 ×130
d. Ditto ×260

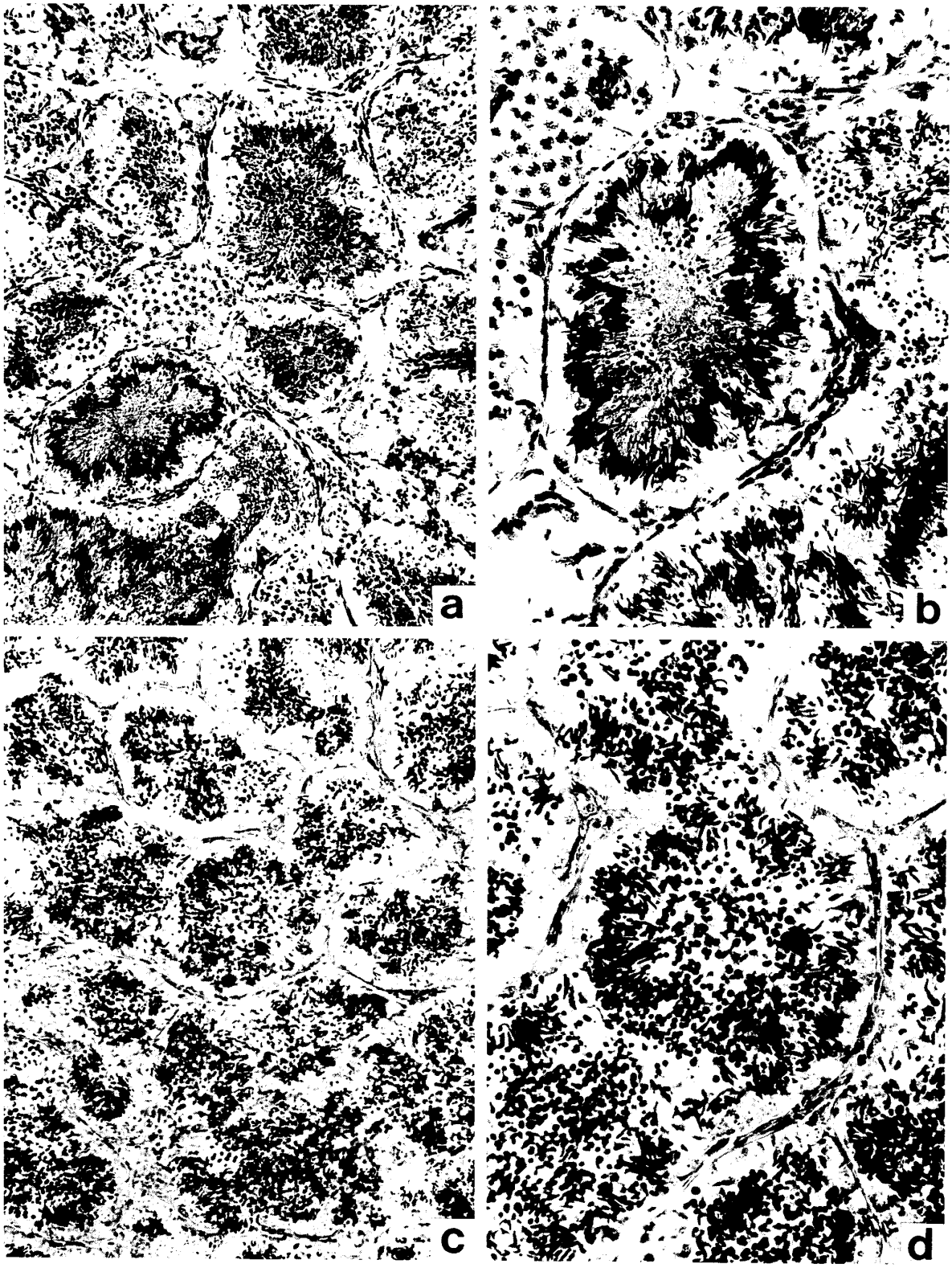

Fig. 6. Cross-sections of the testes of male allotriploids between *Rana tsushimensis* and *R. japonica* from Ichinoseki.

a. Type 2: Allotriploid, (T)TTI 84♀, No.6 ×130
b. Ditto ×260
c. Type 3: Allotriploid, (T)TTI 84♀, No. 9 ×130
d. Ditto ×260

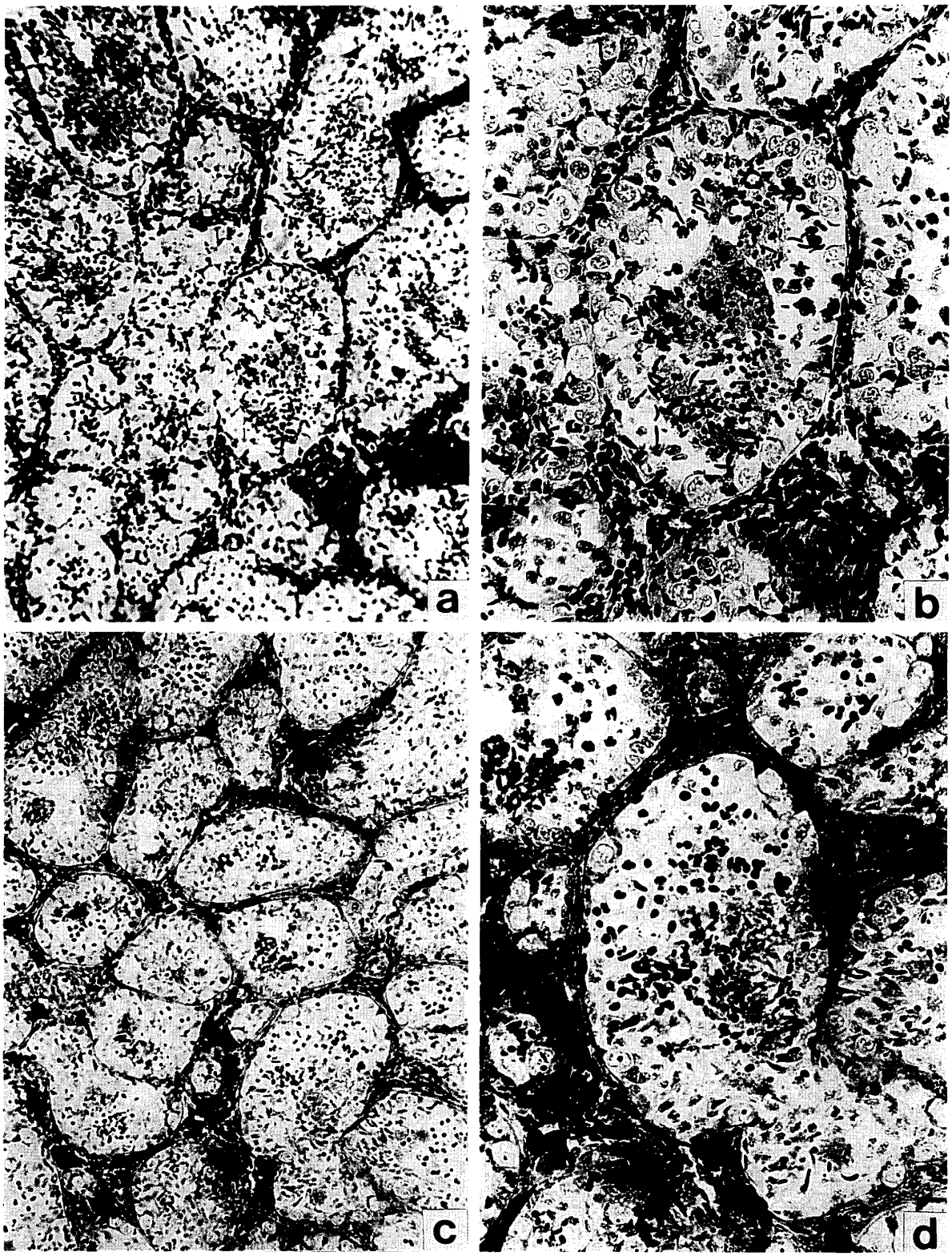

Fig. 7. Cross-sections of the testes of male allotriploids between *Rana tsushimensis* and *R. japonica* from Ichinoseki.

a. Type 4: Allotriploid, (T)TTI 84♂, No.11 ×130
b. Ditto ×260
c. Type 5: Allotriploid, (I)IIT 79♂, No. 1 ×130
d. Ditto ×260

2. Allotriploids produced from *Rana tsushimensis* and *R. japonica* from Hiroshima

The inner structures of the testes of eight control male *R. tsushimensis*, (T)TT, 10 control male *R. japonica* from Hiroshima, (H)HH, 28 male allotriploids, (T)TTH, and 22 males of the reciprocal allotriploids, (H)HHT, produced in 1984 were examined with the use of microscopic sections. It was found that the control 18 male *R. tsushimensis* and *R. japonica* all had the testes of Type 1 in the inner structures of testes. Of the 28 male allotriploids, (T)TTH , 10 had testes of Type 4 and 18 had testes of Type 5. There were no males having testes of Types 1, 2 and 3. All the 22 male allotriploids, (H)HHT, had testes of Type 5 (Table 9).

TABLE 9

Histological classification of testes of mature reciprocal allotriploids between *Rana tsushimensis* and *R. japonica* from Hiroshima and the controls

Year	Parents		Heat shock	No. of mature male frogs	Ploidy	Constitution	No. of analyzed frogs					
	Female	Male					Total	Type of testes				
								1	2	3	4	5
1984	(T)TT, No. 16	(T)TT, No. 7	None	20	2n	(T)TT	4	4				
	(T)TT, No. 18	(T)TT, No. 7	None	13	2n	(T)TT	4	4				
Total				33	2n	(T)TT	8	8	0	0	0	0
1984	(T)TT, No. 16	(H)HH, No. 1	Treated	42	3n	(T)TTH	24				7	17
	(T)TT, No. 18	(H)HH, No. 2	Treated	31	3n	(T)TTH	4				3	1
Total				73	3n	(T)TTH	28	0	0	0	10	18
1984	(H)HH, No. 1	(T)TT, No. 8	Treated	9	3n	(H)HHT	6					6
	(H)HH, No. 2	(T)TT, No. 8	Treated	41	3n	(H)HHT	13					13
	(H)HH, No. 3	(T)TT, No. 9	Treated	8	3n	(H)HHT	3					3
Total				58	3n	(H)HHT	22	0	0	0	0	22
1984	(H)HH, No. 1	(H)HH, No. 3	None	23	2n	(H)HH	4	4				
	(H)HH, No. 2	(H)HH, No. 3	None	13	2n	(H)HH	3	3				
	(H)HH, No. 3	(H)HH, No. 3	None	10	2n	(H)HH	3	3				
Total				46	2n	(H)HH	10	10	0	0	0	0

(T)TT, *R. tsushimensis* (H)HH, *R. japonica* from Hiroshima

(T)TTH, Allotriploids between female *R. tsushimensis* and male *R. japonica* from Hiroshima

(H)HHT, Allotriploids between female *R. japonica* from Hiroshima and male *R. tsushimensis*

V. Reproductive capacity of mature male allotriploids

In 1980, 1981, 1984 and 1985, one-year-old reciprocal allotriploids and the controls attained sexual maturity. After the sizes of the testes of males were measured, the left testes were fixed and used for histological observation of the inner structure, while the right testes were used to examine the reproductive capacity by backcrossing. The sizes and inner structures of the testes are shown in Tables 6~9 and Figs. 5~7.

1. Allotriploids produced from *Rana tsushimensis* and *R. japonica* from Ichinoseki

A. Controls

a. *Rana tsushimensis*

Seven mature male *R. tsushimensis*, (T)TT 79~84♂, Nos. 1~7, which were produced from control matings in 1979, 1980, 1983 and 1984 had normal testes of Type 1. In 1980, 1981, 1984 and 1985, these seven males were crossed with eight field-caught female *R. tsushimensis*, (T)TT♀, Nos. 19~26. In eight control matings, 73.6~95.7% of the respective numbers of eggs, 396 (88.2%) of 449 eggs in total, cleaved normally. After six of the normally cleaved eggs died of various abnormalities at the tail-bud and hatching stages, 71.4~95.7%, 390 (86.9%) in

TABLE 10

Reproductive capacity of mature male allotriploids between *Rana tsushimensis* and *R. japonica* from Ichinoseki and ploidy of the offspring

Year	Parents		No. of eggs	No. of normally cleaved eggs (%)	No. of normal tail-bud embryos (%)	No. of normally hatched tadpoles (%)	No. of normally feeding tadpoles (%)	No. of analyzed tadpoles		No. of matamorphosed frogs (%)
	Female	Male						2n	Others	
1980	(T)TT, No. 19	(T)TT 79, No. 1	59	54 (91.5)	52 (88.1)	52 (88.1)	52 (88.1)	20	0	51 (86.4)
	(T)TT, No. 20	(T)TT 79, No. 2	72	53 (73.6)	52 (72.2)	52 (72.2)	52 (72.2)	20	0	51 (70.8)
1981	(T)TT, No. 21	(T)TT 80, No. 3	33	31 (93.9)	31 (93.9)	30 (90.9)	30 (90.9)	20	0	30 (90.9)
	(T)TT, No. 22	(T)TT 80, No. 4	35	26 (74.3)	26 (74.3)	25 (71.4)	25 (71.4)	20	0	24 (68.6)
1984	(T)TT, No. 23	(T)TT 83, No. 5	129	121 (93.8)	121 (93.8)	120 (93.0)	120 (93.0)	20	0	118 (91.5)
1985	(T)TT, No. 24	(T)TT 84, No. 6	36	30 (83.3)	30 (83.3)	30 (83.3)	30 (83.3)	20	0	29 (80.6)
	(T)TT, No. 25	(T)TT 84, No. 6	38	36 (94.7)	36 (94.7)	36 (94.7)	36 (94.7)	20	0	36 (94.7)
	(T)TT, No. 26	(T)TT 84, No. 7	47	45 (95.7)	45 (95.7)	45 (95.7)	45 (95.7)	20	0	45 (95.7)
Total			449	396 (88.2)	393 (87.5)	390 (86.9)	390 (86.9)	160	0	384 (85.5)
1980	(T)TT, No. 19	(T)TTI 79, No. 1	75	71 (94.7)	68 (90.7)	66 (88.0)	66 (88.0)	30	0	61 (81.3)
	(T)TT, No. 20	(T)TTI 79, No. 1	74	27 (36.5)	26 (35.1)	26 (35.1)	26 (35.1)	26	0	25 (33.8)
	(T)TT, No. 19	(T)TTI 79, No. 2	47	38 (80.9)	34 (72.3)	33 (70.2)	32 (68.1)	32	0	32 (68.1)
	(T)TT, No. 20	(T)TTI 79, No. 2	45	8 (17.8)	7 (15.6)	6 (13.3)	6 (13.3)	6	0	6 (13.3)
1981	(T)TT, No. 21	(T)TTI 80, No. 3-L	103	99 (96.1)	99 (96.1)	97 (94.2)	97 (94.2)	30	0	97 (94.2)
	(T)TT, No. 22	(T)TTI 80, No. 3-R	55	8 (14.5)	8 (14.5)	8 (14.5)	8 (14.5)	8	0	8 (14.5)
1984	(T)TT, No. 23	(T)TTI 83, No. 4	48	1 (2.1)	1 (2.1)	1 (2.1)	1 (2.1)	1	0	1 (2.1)
1985	(T)TT, No. 24	(T)TTI 84, No. 5	33	3 (9.1)	3 (9.1)	3 (9.1)	3 (9.1)	3	0	3 (9.1)
	(T)TT, No. 24	(T)TTI 84, No. 6	47	19 (40.4)	18 (38.3)	18 (38.3)	18 (38.3)	18	0	17 (36.2)
	(T)TT, No. 25	(T)TTI 84, No. 7	106	80 (75.5)	78 (73.6)	75 (70.8)	72 (67.9)	30	0	71 (67.0)
	(T)TT, No. 26	(T)TTI 84, No. 7	154	117 (76.0)	115 (74.7)	113 (73.4)	112 (72.7)	30	0	111 (72.1)
Total			787	471 (59.8)	457 (58.1)	446 (56.7)	441 (56.0)	214	0	432 (54.9)
1985	(I)II, No. 9	(I)II 84, No. 1	53	48 (90.6)	43 (81.1)	40 (75.5)	38 (71.7)	—	—	36 (67.9)
	(I)II, No. 9	(I)II 84, No. 2	65	55 (84.6)	50 (76.9)	48 (73.8)	46 (70.8)	—	—	42 (64.6)
Total			118	103 (87.3)	93 (78.8)	88 (74.6)	84 (71.2)	—	—	78 (66.1)

(T)TT, *R. tsushimensis* (I)II, *R. japonica* from Ichinoseki

(T)TTI, Allotriploids between female *R. tsushimensis* and male *R. japonica* from Ichinoseki

L, Left testis R, Right testis

total, hatched normally and became normally feeding tadpoles. Eventually, 68.6~95.7%, 384 (85.5%) in total, metamorphosed normally (Table 10). The metamorphosed frogs corresponded to 92.3~100%, 97.0% on the average, of the normally cleaved eggs.

b. *Rana japonica* from Ichinoseki

Two mature male *R. japonica* from Ichinoseki, (I)II 84♂, Nos. 1 and 2, which were produced from control matings in 1984, were males having normal testes of Type 1. In 1985, these two males were crossed with one field-caught female *R. japonica* from Ichinoseki, (I)II♀, No. 9. In two control matings, 84.6% and 90.6%, 103 (87.3%) of 118 eggs in total, cleaved normally, 73.8% and 75.5%, 88 (74.6%) in total, hatched normally, 70.8% and 71.7%, 84 (71.2%) in total, became normally feeding tadpoles and 64.6% and 67.9%, 78 (66.1%) in total, metamorphosed normally (Table 10). The metamorphosed frogs corresponded to 75.0% and 76.4%, 75.7% on the average, of the normally cleaved eggs.

B. Male allotriploids

a. Allotriploids, (T)TTI

Twelve one-year-old mature male allotriploids, (T)TTI 79~84♂, Nos. 1~12, were produced from crossing experiments in 1979, 1980, 1983 and 1984. Of these allotriploids, one male, (T)TTI♂, No. 1, had normal testes of Type 1, six males, (T)TTI♂, Nos. 2~7, had abnormal testes of Type 2, two males, (T)TTI♂, Nos. 8 and 9, had abnormal testes of Type 3, two males, (T)TTI♂, Nos. 10 and 11, had abnormal testes of Type 4, and one male, (T)TTI♂, No. 12, had abnormal testes of Type 5. Sixteen matings were made between these 12 male allotriploids and the same eight field-caught females of *R. tsushimensis,* (T)TT♀, Nos. 19~26, as used in the control matings. In 11 matings between seven male allotriploids, (T)TTI♂, Nos. 1~7, having the testes of Types 1 and 2, and eight female *R. tsushimensis*, (T)TT♀, Nos. 19~26, 2.1~96.1%, 471 (59.8%) of 787 eggs in total, cleaved normally, 2.1~94.2%, 446 (56.7%) in total, hatched normally, 2.1~94.2%, 441 (56.0%) in total, became normally feeding tadpoles and 2.1~94.2%, 432 (54.9%) in total, metamorphosed normally (Table 10). The metamorphosed frogs corresponded to 75.0~100%, 91.7% on the average, of the normally cleaved eggs.

On the other hand, in five matings between the other five male allotriploids, (T)TTI♂, Nos. 8~12, with abnormal testes of Types 3~5 and two female *R. tsushimensis*, (T)TT♀, Nos. 24 and 25, 233 eggs did not cleaved normally nor abnormally.

b. Allotriploids, (I)IIT

The same three field-caught female *R. tsushimensis,* (T)TT♀, Nos. 19, 20 and 26, and one field-caught female *R. japonica* from Ichinoseki, (I)II♀, No. 9, used in the control matings were mated with five one-year-old male allotriploids, (I)IIT 79♂, No. 1 and (I)IIT 84♂, Nos. 2~5, which had abnormal testes of Types 3~5. The

results showed that none of the 612 eggs of the above four females cleaved normally.

2. Allotriploids produced from *Rana tsushimensis* and *R. japonica* from Hiroshima

A. Controls

Two mature male *R. tsushimensis*, (T)TT 84♂, Nos. 1 and 2, and two mature male *R. japonica* from Hiroshima, (H)HH 84♂, Nos. 1 and 2, which were produced from control matings in 1984, had normal testes of Type 1. In 1985, these four males were crossed with a field-caught female *R. japonica* from Hiroshima, (H)HH♀, No. 5. In four control matings, 83.9~92.5% of the respective numbers of eggs, 215 (86.7%) of 248 eggs in total, cleaved normally.

B. Male allotriploids, (T)TTH and (H)HHT

Ten one-year-old mature male allotriploids, (T)TTH 84♂, Nos. 1~10, and five one-year-old mature male allotriploids, (H)HHT 84♂, Nos. 1~5, which were produced in crossing experiments in 1984, had abnormal testes of Types 4 and 5. In 1985, these 15 allotriploids were crossed with the same field-caught female *R. japonica* from Hiroshima, (H)HH♀, No. 5, as used in the control matings. The results showed that none of the 794 eggs cleaved normally nor abnormally.

VI. Backcrosses of allotriploids, (T)TTI

1. Sex

A. Control *Rana tsushimensis*

Of 384 metamorphosed frogs produced from backcrosses between eight field-caught female *R. tsushimensis*, (T)TT♀, Nos. 19~26, and seven male *R. tsushimensis*, (T)TT 79~84♂, Nos. 1~7, the sex of 373 was examined at the juvenile or mature stage. At the juvenile stage, 87 of 173 frogs were females with normal ovaries, three were hermaphrodites with gonads transforming from ovaries into testes, and the remaining 83 were males with normal testes. At the mature stage, 110 of 200 frogs were females and the remaining 90 were males. When the juvenile hermaphrodites were counted as males, 197 frogs were females and 176 (47.2%) were males in total of the juvenile and mature ones (Table 11).

B. Backcrosses of allotriploids, (T)TTI

Of 432 metamorphosed frogs produced from backcrosses between eight field-caught female *R. tsushimensis*, (T)TT♀, Nos. 19~26, and seven male allotriploids, (T)TTI 79~84♂, Nos. 1~7, the sex of 419 was examined at the juvenile or mature stage. At the juvenile stage, 57 of 62 frogs were females with normal ovaries, four were hermaphrodites with gonads transforming from ovaries into testes, and the remaining one was a male with normal testes. At the mature stage, 349 of 357 frogs were females and the remaining eight were males. When the hermaphrodites were counted as males, 406 frogs were females and 13 (3.1%) were males in

TABLE 11
Sex of backcrosses derived from seven male allotriploids and the controls

Year	Parents		No. of metamorphosed frogs	Sex of frogs dead or killed within one month after metamorphosis			Sex of mature frogs		Sex of all frogs examined		
	Female	Male		♀	♂	⚥	♀	⚥	Total	♀	⚥ (%)
1980	(T)TT, No. 19	(T)TT, 79, No. 1	51	8		6	15	19	48	23	25 (52.1)
	(T)TT, No. 20	(T)TT, 79, No. 2	51	10		7	20	13	50	30	20 (40.0)
1981	(T)TT, No. 21	(T)TT, 80, No. 3	30	2		6	13	9	30	15	15 (50.0)
	(T)TT, No. 22	(T)TT, 80, No. 4	24	6	2	5	7	4	24	13	11 (45.8)
1984	(T)TT, No. 23	(T)TT, 83, No. 5	118	59	1	57	—	—	117	59	58 (49.6)
1985	(T)TT, No. 24	(T)TT, 84, No. 6	29	—		—	15	12	27	15	12 (44.4)
	(T)TT, No. 25	(T)TT, 84, No. 6	36	1		2	20	13	36	21	15 (41.7)
	(T)TT, No. 26	(T)TT, 84, No. 7	45	1		0	20	20	41	21	20 (48.8)
Total			384	87	3	83	110	90	373	197	176 (47.2)
1981	(T)TT, No. 19	(T)TTI, 79, No. 1	61	5	1	0	53	1	60	58	2 (3.3)
	(T)TT, No. 20	(T)TTI, 79, No. 1	25	5		0	18	0	23	23	0 (0)
	(T)TT, No. 19	(T)TTI, 79, No. 2	32	5		0	25	0	30	30	0 (0)
	(T)TT, No. 20	(T)TTI, 79, No. 2	6	4		0	2	0	6	6	0 (0)
1981	(T)TT, No. 21	(T)TTI, 80, No. 3-L	97	13	1	0	73	6	93	86	7 (7.5)
	(T)TT, No. 22	(T)TTI, 80, No. 3-R	8	1		0	7	0	8	8	0 (0)
1984	(T)TT, No. 23	(T)TTI, 83, No. 4	1	1		0	—	—	1	1	0 (0)
1985	(T)TT, No. 24	(T)TTI, 84, No. 5	3	—		—	3	0	3	3	0 (0)
	(T)TT, No. 24	(T)TTI, 84, No. 6	17	—		—	17	0	17	17	0 (0)
	(T)TT, No. 25	(T)TTI, 84, No. 7	71	15	1	1	53	1	71	68	3 (4.2)
	(T)TT, No. 26	(T)TTI, 84, No. 7	111	8	1	0	98	0	107	106	1 (0.9)
Total			432	57	4	1	349	8	419	406	13 (3.1)

(T)TT, *R. tsushimensis*
(T)TTI, Allotriploids between female *R. tsushimensis* and male *R. japonica* from Ichinoseki
L, Left testis R, Right testis

total (Table 11).

2. Chromosome number

A. Control *Rana tsushimensis*

Chromosomes were examined in the tail-tips of 160 of 390 normally developed 30-day-old tadpoles produced in 1980, 1981, 1984 and 1985 from eight control matings between eight field-caught female *R. tsushimensis*, (T)TT♀, Nos. 19~26, and seven control male *R. tsushimensis*, (T)TT 79~84⚥, Nos. 1~7, by the squash method with water pretreatment. It was found that they were all diploids (Table 10).

B. Backcrosses of male allotriploids, (T)TTI

Chromosomes were examined in the tail-tips of 214 of 441 30-day-old normally

feeding tadpoles obtained from 11 backcrosses of seven male allotriploids, (T)TTI 79~84♂, Nos. 1~7, with eight field-caught female *R. tsushimensis*, (T)TT♀, Nos. 19~26, by the squash method with water pretreatment. It was found that they were all diploids (Table 10).

These findings showed that the male allotriploids produced haploid spermatozoa by meiosis, as in the case of the control male *R. tsushimensis*.

3. External characters

The backcrosses which were obtained from male allotriploids, (T)TTI, and female *R. tsushimensis* were compared with the control *R. tsushimensis* by measuring their body lengths and observing the external characters. In 97 one-year-old female backcrosses obtained from three male allotriploids, (T)TTI♂, Nos. 1~3, by mating with two field-caught female *R. tsushimensis*, the body length was 32.8~41.0 mm, 36.7±0.62 mm on the average. In 24 one-year-old control female *R. tsushimensis*, the body length was 34.5~38.8 mm, 36.6±0.85 mm on the average. The difference in body length between the backcrosses and controls was not statistically significant by T-test. The backcrosses were also very similar to the control *R. tsushimensis* in external characters(Fig. 8).

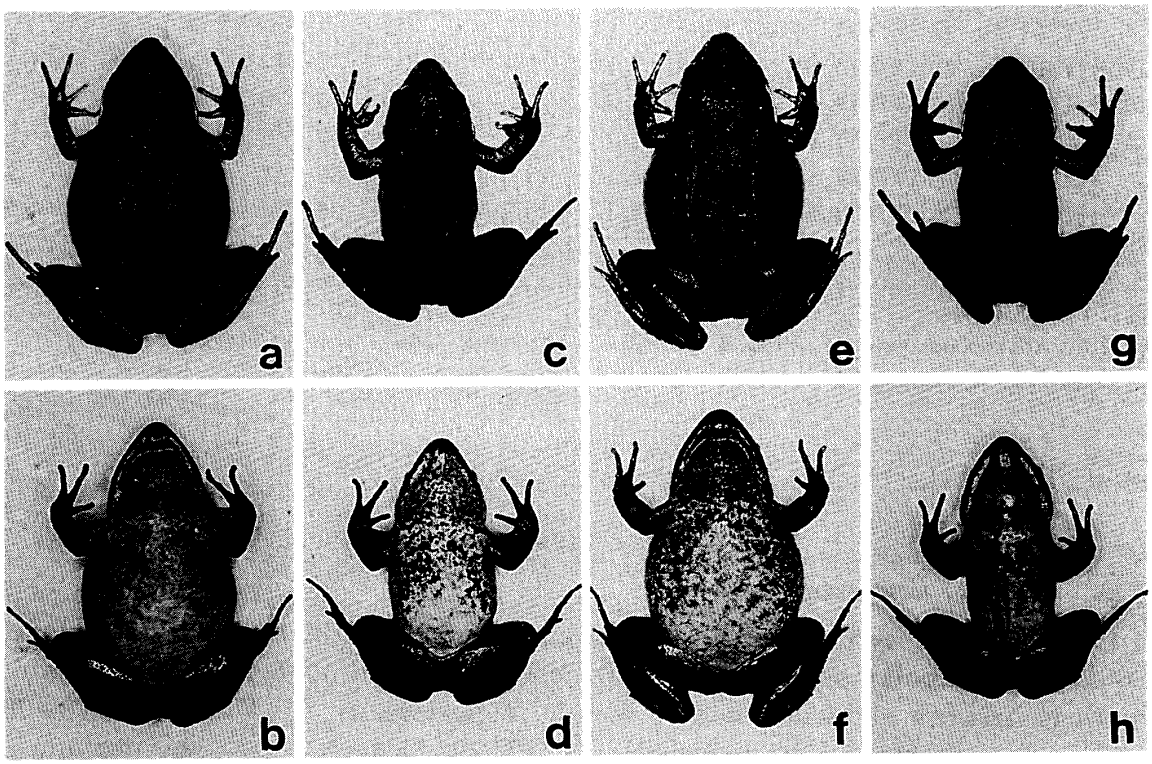

Fig. 8. Backcrosses of a male allotriploid, (T)TTI 80♂, No. 3, mated with a female *Rana tsushimensis* and the controls. ×0.87

a, b. Control female *R. tsushimensis*, (T)TT♀, No. 21×(T)TT 80♂, No. 3
c, d. Control male *R. tsushimensis*, (T)TT♀, No. 21×(T)TT 80♂, No. 3
e, f. Female backcross, (T)TT♀, No. 21×(T)TTI 80♂, No. 3
g, h. Male backcross, (T)TT♀, No. 21×(T)TTI 80♂, No. 3

4. Electrophoretic patterns

A. Controls

Electrophoretic patterns were examined at 18 loci of 11 enzymes extracted from skeletal muscles, AAT, CK, Est, α-GDH, GPI, IDH, LDH, MDH, MPI, PGM and SOD, and three blood proteins, Ab, Prot-C and Hb, in 30 *R. tsushimensis* and 35 *R. japonica* from Ichinoseki, which were collected in the field. It was found that the two species evidently differed from each other at 14 loci, AAT-B, Est-1, GPI,

TABLE 12

Electrophoretic phenotypes at 14 loci in *Rana tsushimensis*, *R. japonica* from Ichinoseki, allotriploids, backcrosses and the controls

Kind \ Locus	AAT-B	Est-1	GPI	IDH A	IDH B	LDH A	LDH B	MDH-B	MPI	PGM	SOD	Ab	P.-C	Hb
Rana tsushimensis (T)TT (30)	*ad, bb, bd dd, ee*	*aa*	*cc*	*bb*	*ab, bb*	*bb*	*aa*	*aa*	*aa*	*aa*	*aa*	*dd*	*aa*	*bb*
Rana japonica (I)II (35)	***cc***	***bb***	***bb***	***aa***	***aa***	***aa***	***bb***	***cc***	***bb***	***bb***	***bb***	***bb***	***bb***	***aa***
(T)TT ♀, No. 19 (T)TT ♀, No. 21	*dd*	*aa*	*cc*	*bb*	*ab*	*bb*	*aa*	*aa*	*aa*	*aa*	*aa*	*dd*	*aa*	*bb*
(T)TT ♀, No. 24 (T)TT ♀, No. 26	*dd*	*aa*	*cc*	*bb*	*ab*	*bb*	*aa*	*aa*	*aa*	*aa*	*aa*	*dd*	*aa*	*bb*
(T)TT 79 ♂, No. 1 (T)TT 80 ♂, No. 3	*ad*	*aa*	*cc*	*bb*	*bb*	*bb*	*aa*	*aa*	*aa*	*aa*	*aa*	*dd*	*aa*	*bb*
(T)TT 84 ♂, No. 6 (T)TT 80 ♂, No. 7	*dd*	*aa*	*cc*	*bb*	*bb*	*bb*	*aa*	*aa*	*aa*	*aa*	*aa*	*dd*	*aa*	*bb*
(T)TTI 79 ♂, No. 1	***bbc***	***aab***	***ccb***	***bba***	***aba***	***bba***	***aab***	***aac***	***aab***	***aab***	***aab***	***ddb***	***aab***	***bba***
(T)TTI 79 ♂, No. 2	***bdc***	***aab***	***ccb***	***bba***	***aba***	***bba***	***aab***	***aac***	***aab***	***aab***	***aab***	***ddb***	***aab***	***bba***
(T)TTI 80 ♂, No. 3	***eec***	***aab***	***ccb***	***bba***	***aaa***	***bba***	***aab***	***aac***	***aab***	***aab***	***aab***	***ddb***	***aab***	***bba***
(T)TTI 84 ♂, No. 6	***ddc***	***aab***	***ccb***	***bba***	***aaa***	***bba***	***aab***	***aac***	***aab***	***aab***	***aab***	***ddb***	***aab***	***bba***
(T)TTI 84 ♂, No. 7	***bdc***	***aab***	***ccb***	***bba***	***aaa***	***bba***	***aab***	***aac***	***aab***	***aab***	***aab***	***ddb***	***aab***	***bba***
(T)TT ♀, No. 19 × (T)TT 79 ♂, No. 1 (13)	*ad, dd* (8) (5)	*aa* (13)	*cc* (13)	*bb* (13)	*ab, bb* (7) (6)	*bb* (13)	*aa* (13)	*aa* (13)	*aa* (13)	*aa* (13)	*aa* (13)	*dd* (13)	*aa* (13)	*bb* (13)
(T)TT ♀, No. 19 × (T)TTI 79 ♂, No. 1 (26)	*bd* (26)	*aa* (26)	*cc* (26)	*bb* (26)	*aa, ab, bb* (8)(10)(8)	*bb* (26)	*aa* (26)	*aa* (26)	*aa* (26)	*aa* (26)	*aa* (26)	*dd* (26)	*aa* (26)	*bb* (26)
(T)TT ♀, No. 19 × (T)TTI 79 ♂, No. 2 (10)	*bd, dd* (3) (7)	*aa* (10)	*cc* (10)	*bb* (10)	*aa, ab, bb* (2)(5)(3)	*bb* (10)	*aa* (10)	*aa* (10)	*aa* (10)	*aa* (10)	*aa* (10)	*dd* (10)	*aa* (10)	*bb* (10)
(T)TT ♀, No. 21 × (T)TT 80 ♂, No. 3 (8)	*ad, dd* (4) (4)	*aa* (8)	*cc* (8)	*bb* (8)	*ab, bb* (5) (3)	*bb* (8)	*aa* (8)	*aa* (8)	*aa* (8)	*aa* (8)	*aa* (8)	*dd* (8)	*aa* (8)	*bb* (8)
(T)TT ♀, No. 21 × (T)TTI 80 ♂, No. 3 (14)	*de* (14)	*aa* (14)	*cc* (14)	*bb* (14)	*aa, ab* (10) (4)	*bb* (14)	*aa* (14)	*aa* (14)	*aa* (14)	*aa* (14)	*aa* (14)	*dd* (14)	*aa* (14)	*bb* (14)
(T)TT ♀, No. 24 × (T)TT 84 ♂, No. 6 (12)	*dd* (12)	*aa* (12)	*cc* (12)	*bb* (12)	*ab, bb* (7) (5)	*bb* (12)	*aa* (12)	*aa* (12)	*aa* (12)	*aa* (12)	*aa* (12)	*dd* (12)	*aa* (12)	*bb* (12)
(T)TT ♀, No. 24 × (T)TTI 84 ♂, No. 6 (17)	*dd* (17)	*aa* (17)	*cc* (17)	*bb* (17)	*aa, ab* (9) (8)	*bb* (17)	*aa* (17)	*aa* (17)	*aa* (17)	*aa* (17)	*aa* (17)	*dd* (17)	*aa* (17)	*bb* (17)
(T)TT ♀, No. 26 × (T)TT 84 ♂, No. 7 (15)	*dd* (15)	*aa* (15)	*cc* (15)	*bb* (15)	*ab, bb* (7) (8)	*bb* (15)	*aa* (15)	*aa* (15)	*aa* (15)	*aa* (15)	*aa* (15)	*dd* (15)	*aa* (15)	*bb* (15)
(T)TT ♀, No. 26 × (T)TTI 84 ♂, No. 7 (31)	*bd, dd* (9) (22)	*aa* (31)	*cc* (31)	*bb* (31)	*aa, ab* (13) (18)	*bb* (31)	*aa* (31)	*aa* (31)	*aa* (31)	*aa* (31)	*aa* (31)	*dd* (31)	*aa* (31)	*bb* (31)

Parentheses show the numbers of frogs analyzed.

(T)TT, *R. tsushimensis* (I)II, *R. japonica* from Ichinoseki

(T)TTI, Allotriploids between female *R. tsushimensis* and male *R. japonica* from Ichinoseki

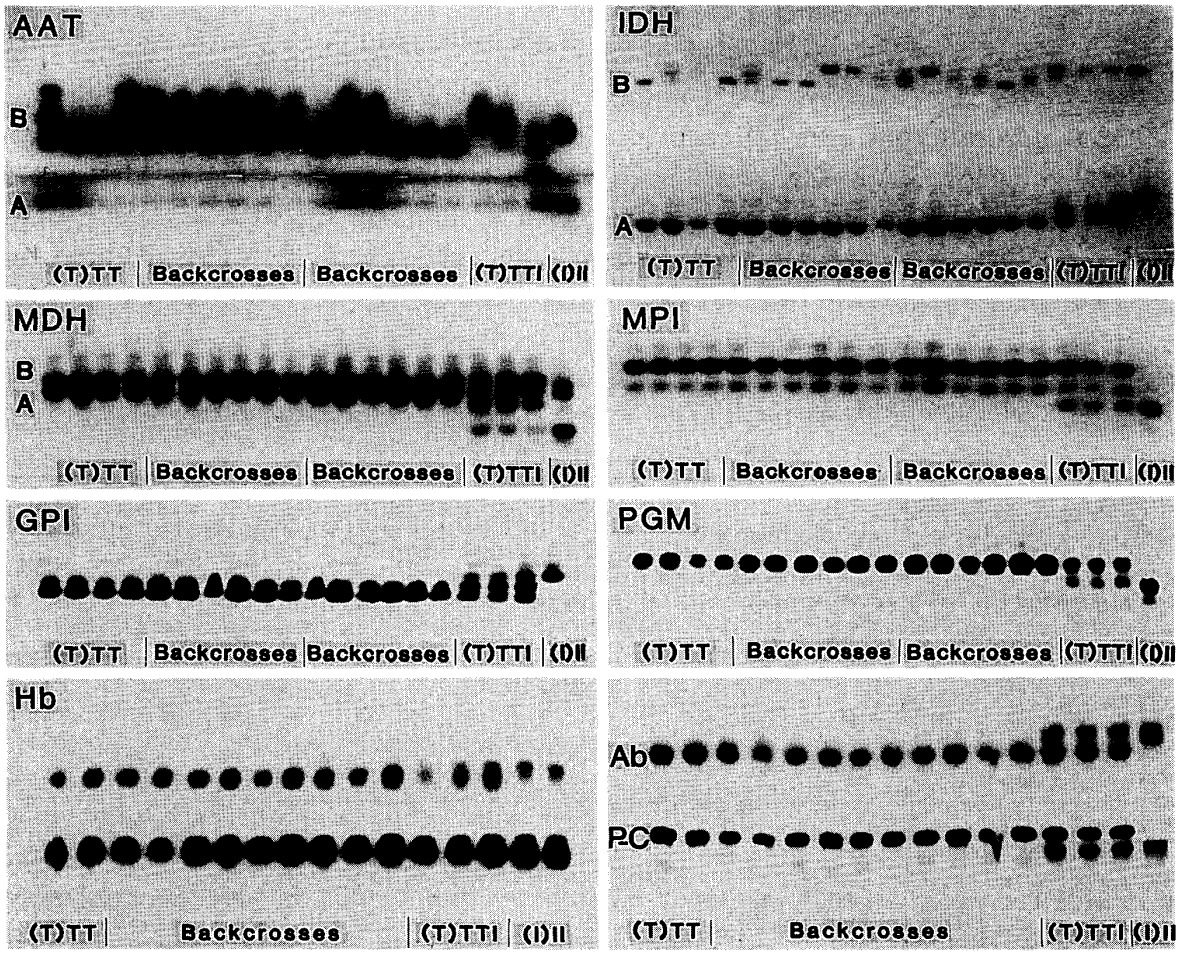

Fig. 9. Electrophoretic patterns of six enzymes and three blood proteins in backcrosses of male allotriploids, (T)TTI, and the controls, (T)TT and (I)II.

IDH-A, IDH-B, LDH-A, LDH-B, MDH-B, MPI, PGM, SOD, Ab, Prot-C and Hb (Table 12; Fig. 9).

B. Allotriploids, (T)TTI

Electrophoretic patterns at the above 14 loci were examined in five male allotriploids, (T)TTI 79♂, Nos. 1 and 2, (T)TTI 80♂, No. 3 and (T)TTI 84♂, Nos. 6 and 7. The results showed that electrophoretic patterns of these five allotriploids consisted of the sum of bands derived from the two parental species, although the bands derived from *R. tsushimensis* were more intensely stained than those derived from *R. japonica* (Table 12; Fig. 9).

C. Backcrosses of male allotriploids, (T)TTI

Electrophoretic patterns at the above 14 loci were examined in 98 two-year-old female backcrosses obtained in 1980, 1981 and 1985 from five matings between five male allotriploids, (T)TTI, and four field-caught female *R. tsushimensis*, and 48 two-year-old control female *R. tsushimensis* produced in 1980, 1981 and 1985 from four matings between female and male *R. tsushimensis*. The results showed that all

TABLE 13

Developmental capacity and sex of the offspring derived from seven male backcrosses and the controls

Year	Parents		No. of eggs	No. of normally cleaved eggs (%)	No. of metamorphosed frogs (%)	Sex						
						Juvenile frogs			Mature frogs		Total	
	Female	Male				♀	♂	♂	♀	♂	♀	♂ (%)
1982	(T)TT, No. 27	[(T)TT, No. 19× (T)TT 79, No. 1], No. 1	78	75 (96.2)	72 (92.3)	18	1	15	16	13	34	29 (46.0)
	(T)TT, No. 28	[(T)TT, No. 21× (T)TT 80, No. 3], No. 2	55	48 (87.3)	45 (81.8)	22		23	—	—	22	23 (51.1)
	(T)TT, No. 29	[(T)TT, No. 21× (T)TT 80, No. 3], No. 3	46	45 (97.8)	44 (95.7)	3		1	16	20	19	21 (52.5)
	(T)TT, No. 30	[(T)TT, No. 21× (T)TT 80, No. 3], No. 4	58	54 (93.1)	49 (84.5)	21		27	—	—	21	27 (56.3)
Total			237	222 (93.7)	210 (88.6)	64	1	66	32	33	96	100 (51.0)
1982	(T)TT, No. 27	[(T)TT, No. 19× (T)TTI 79, No. 1], No. 1	75	71 (94.7)	64 (85.3)	20	2	0	34	2	54	4 (6.9)
	(T)TT, No. 28	[(T)TT, No. 19× (T)TTI 79, No. 1], No. 1	70	62 (88.6)	57 (81.4)	20		0	34	2	54	2 (3.6)
	(T)TT, No. 28	[(T)TT, No. 21× (T)TTI 80, No. 3], No. 2	73	71 (97.3)	63 (86.3)	15	1	1	38	2	53	4 (7.0)
	(T)TT, No. 28	[(T)TT, No. 21× (T)TTI 80, No. 3], No. 3	108	101 (93.5)	94 (87.0)	84		5	—	—	84	5 (5.6)
	(T)TT, No. 29	[(T)TT, No. 21× (T)TTI 80, No. 3], No. 4	76	73 (96.1)	59 (77.6)	28	3	2	12	1	40	6 (13.0)
	(T)TT, No. 29	[(T)TT, No. 21× (T)TTI 80, No. 3], No. 5	96	87 (90.6)	83 (86.5)	67	7	5	—	—	67	12 (15.2)
	(T)TT, No. 30	[(T)TT, No. 21× (T)TTI 80, No. 3], No. 6	60	32 (53.3)	28 (46.7)	5		0	20	0	25	0 (0)
	(T)TT, No. 30	[(T)TT, No. 21× (T)TTI 80, No. 3], No. 7	74	73 (98.6)	67 (90.5)	56	5	2	—	—	56	7 (11.1)
Total			632	570 (90.2)	515 (81.5)	295	18	15	138	7	433	40 (8.5)

(T)TT, *R. tsushimensis*

(T)TTI, Allotriploids between female *R. tsushimensis* and male *R. japonica* from Ichinoseki

the backcrosses were completely the same as *R. tsushimensis* in electrophoretic patterns (Table 12; Fig. 9).

These findings showed that the *R. japonica* genome from Ichinoseki was eliminated from the germ cells of the male allotriploids and that haploid spermatozoa having a *R. tsushimensis* genome were produced by meiosis from spermatocytes having two *R. tsushimensis* genomes.

VII. Offspring of seven male backcrosses produced from male allotriploids, (T)TTI, and female Rana tsushimensis

A. Control *Rana tsushimensis*

In 1982, four control male backcrosses (Nos. 1~4) obtained in 1980 and 1981 from control matings between two control male *R. tsushimensis*, (T)TT 79♂, No. 1 and (T)TT 80 ♂, No. 3, and two field-caught female *R. tsushimensis*, (T)TT♀, Nos. 19 and 21, were mated with four field-caught female *R. tsushimensis*, (T)TT♀, Nos. 27~30. In four control matings, 87.3~97.8%, 222 (93.7%) of 237 eggs in total, cleaved normally and 81.8~95.7%, 210 (88.6%) in total, metamorphosed

normally. Of these 210 metamorphosed frogs, the sex of 196 was examined at the juvenile or mature stage. At the juvenile stage, 64 of 131 frogs were females with normal ovaries, one was a hermaphrodite and the remaining 66 were males with normal testes. At the mature stage, 32 of 65 frogs were females and the other 33 were males. When the hermaphrodite was counted as a male, 96 frogs were females and 100 (51.0%) were males in total (Table 13).

B. Seven male backcrosses from male allotriploids, (T)TTI

Seven mature male backcrosses obtained in 1980 and 1981 from two matings between two male allotriploids, (T)TTI 79♂, No. 1 and (T)TTI 80♂, No. 3 and two field-caught female *R. tsushimensis,* (T)TT♀, Nos. 19 and 21, were mated with four field-caught female *R. tsushimensis,* (T)TT♀, Nos. 27~30. In these matings, 53.3~98.6%, 570 (90.2%) of 632 eggs in total, cleaved normally, and then 51.7~94.6%, 547 (86.6%) in total, hatched normally. Eventually, 46.7~90.5%, 515 (81.5%) in total, metamorphosed normally. Of these 515 frogs, the sex of 473 was examined at the juvenile or mature stage. At the juvenile stage, 295 of 328 frogs were females with normal ovaries, 18 were hermaphrodites and the remaining 15 were males with normal testes. At the mature stage, 138 of 145 frogs were females and the remaining seven were males. When the hermaphrodites were counted as males, 433 frogs were females and 40 (8.5%) were males in total(Table 13). From these results, the seven mature male backcrosses produced in 1980 and 1981 from the two male allotriploids, (T)TTI 79♂, No. 1 and (T)TTI 80♂, No. 3, were assumed to be sex-reversed genetic females.

DISCUSSION

Triploid amphibians were first discovered by G. and P. HERTWIGS (1920). They were 18 tadpoles of *Rana esculenta* which had been reared as the control of pseudohybrids. The second discovery of triploid frogs was made by KAWAMURA (1941). More than 70 triploid tadpoles of *R. nigromaculata* which had been reared as a control series completed metamorphosis and grew normally. Some of them reached maturity and showed the secondary sexual characters. WICKBOM (1945) reported that four of eight *R. esculenta* collected on Bornhorm Island near the exit of the Baltic Sea were triploids. KAWAMURA and TOKUNAGA (1952) obtained 81 triploid frogs of *R. japonica* from cold-treated fertilized eggs. All these frogs were males. Six of them were raised to sexual maturity. KAWAMURA (1952b) produced triploid hybrids (allotriploids) from eggs of *R. japonica* fertilized with sperm of *R. ornativentris* by cold-treatment. A total of 19 allotriploids attained sexual maturity at the age older than nine months. All of them were sterile males like diploid hybrids. No normal spermatozoa were produced from them.

It has been a settled conviction that two different species are usually isolated by hybrid inviability or sterility. However, recently this conviction was partially changed by numerous investigations, since BERGER's discovery (1967). The European pond frog (green frog, water frog), *Rana esculenta,* whose name has been

believed to be the most firmly established, is now considered to be the name of the hybrid between *R. lessonae* and *R. ridibunda*. The insistence that *R. esculenta* is the hybrid between these two species was repeatedly made after BERGER by himself (1968~1973), GÜNTHER (1970, 1974, 1978), TUNNER (1970, 1972, 1973), BLANKENHORN, HEUSSER and VOGEL (1971), ENGELMANN (1972, 1973), HEYM (1974), UZZELL and BERGER (1975), VOGEL and CHEN (1976a, 1976b, 1977), EBENDAL (1977), HEPPICH (1978), KOREF-SANTIBANEZ and GÜNTHER (1980), *etc.* The phenomenon that *R. esculenta* can take part in reproduction was first assumed by TUNNER (1974). The population of pond frogs in a district of Austria consisted of female *R. esculenta* and female and male *R. lessonae*. It was found that the female *R. esculenta* produced female *R. esculenta* by mating with male *R. lessonae*. It was evident that the parental *R. esculenta* produced only eggs having the *ridibunda* genomes by hybridogenesis, and that the eggs were fertilized with spermatozoa having the *lessonae* genomes. The *lessonae* genomes of the female *R. esculenta* were eliminated from their eggs during oogenesis. The hybridogenesis of *R. esculenta* was thereafter confirmed by UZZELL and BERGER (1975), TUNNER and DOBROWSKY (1976), UZZELL, GÜNTHER and BERGER (1977), BERGER (1977), TUNNER (1978), HEPPICH (1978), UZZELL, HOTZ and BERGER (1980), TUNNER and HEPPICH (1981), HEPPICH, TUNNER and GREILHUBER (1982), BINKERT, BORNER and CHEN (1982), BERGER, UZZELL and HOTZ (1982), *etc.*

HOTZ and UZZELL (1983) reported that the diploid hybrids between *R. ridibunda* and *R. lessonae,* between *R. ridibunda* and Italian taxon of *R. lessonae,* and between *R. ridibunda* and *R. perezi* performed hybridogenesis, while the hybrids between *R. ridibunda* and Balkan taxon of *R. lessonae* did not show hybridogenesis. KAWAMURA and NISHIOKA (1986) obtained reciprocal hybrids between *R. ridibunda* from France, Turkey and Belgorod and *R. lessonae* from Luxembourg and Italy. It was found that a small number of male hybrids between female *R. lessonae* from Italy and male *R. ridibunda* from Belgorod and between female *R. ridibunda* from Belgorod and male *R. lessonae* from Luxembourg produced spermatozoa having the *ridibunda* genome derived from *R. ridibunda* by eliminating the *lessonae* genome of *R. lessonae,* and produced hybrids, (L)LR, by mating with female *R. lessonae*. It was clear that the other hybrids did not show hybridogenesis. The females of these hybrids produced two kinds of eggs in size, normal and large, which became diploid and triploid frogs, respectively. The male hybrids were all completely sterile. Thus, the hybridogenesis seemed to occur in hybrids between specific populations of *R. lessonae* and *R. ridibunda.*

It was interesting to examine the hybridogenesis in triploid *R. esculenta.* According to GÜNTHER (1975), 39.6% of all *R. esculenta* collected from 19 places were triploids in the northern and eastern parts of former East Germany. The sex ratio of triploid *R. esculenta* was nearly 1:1 (♀ : ♂) in contrast to about 1:2 (♀ : ♂) in diploid *R. esculenta.* A high frequency of triploid *R. esculenta* was also found by EBENDAL, BERGLUND and RYMAN (1981), and EBENDAL and UZZELL (1982) in Swedish *R. esculenta* complex. UZZELL and BERGER (1975), UZZELL, BERGER and GÜNTHER (1975), GÜNTHER (1975, 1979), GÜNTHER and HÄHNEL

(1976), GÜNTHER, UZZELL and BERGER (1979), EBENDAL, BERGLUND and RYMAN (1981), and EBENDAL and UZZELL (1982) confirmed that the triploid *R. esculenta* were allotriploids constructed of two *lessonae* genomes and one *ridibunda* genome in some populations or of two *ridibunda* genomes and one *lessonae* genome in the other populations.

On the production of gametes by male and female allotriploid *R. esculenta,* GÜNTHER, UZZELL and BERGER (1979) and GÜNTHER (1979) insisted that the same two genomes derived from *R. lessonae* or *R. ridibunda* in male and female allotriploids made synapsis easily and produced haploid gametes, while the remaining one genome was excluded. Thus, hybridogenesis occurs in allotriploid *R. esculenta,* as in diploid *R. esculenta,* and *R. esculenta* can be produced by crossing between hybridogenetic diploids and triploids in the populations consisting of *R. esculenta* alone.

NISHIOKA and OHTANI (1984) reported that male allotriploids, (B)BBL, consisted of two genomes of *R. brevipoda* and one genome of *R. lessonae* produced haploid spermatozoa having the *brevipoda* genome derived from *R. brevipoda* by eliminating the *lessonae* genome of *R. lessonae,* and produced diploid females by mating with diploid female *R. brevipoda.* These offspring were completely of the *R. brevipoda* type in external characters, enzymes and blood proteins. NISHIOKA and OHTANI (1985) also reported that male and female allotriploids, (L)LLB, constructed of two genomes of *R. lessonae* and one genome of *R. brevipoda* produced gametes having the *lessonae* genome derived from *R. lessonae* by eliminating the *brevipoda* genome of *R. brevipoda.* NISHIOKA and OHTANI (1991), furthermore, reported that allotriploids, (N)NNL, constructed of two genomes of *R. nigromaculata* and one genome of *R. lessonae* produced gametes having the *nigromaculata* genome derived from *R. nigromaculata* by eliminating the *lessonae* genome of *R. lessonae.*

The present study showed that male allotriploids, (T)TTI, between Japanese female *R. tsushimensis* from Tsushima Island and male *R. japonica* from Ichinoseki performed a kind of hybridogenesis, as found in the above allotriploids, (L)LLR, (R)RRL, (B)BBL, (L)LLB and (N)NNL, in which one parent was European *R. lessonae* at least. It was found that the allotriploids, (T)TTI, produced haploid spermatozoa having the *tsushimensis* genome derived from *R. tsushimensis* by eliminating the *japonica* genome of *R. japonica* from Ichinoseki. When seven male allotriploids, (T)TTI, were backcrossed with female *R. tsushimensis,* 432 offspring were obtained. When the sex was examined in 419 of these offspring, only 13 (3.1%) were diploid males, while the others were diploid females, which were all of the *R. tsushimensis* type in external characters, enzymes of skeletal muscles and blood proteins. The fact that the offspring were almost females showed that the *japonica* genome derived from *R. japonica* of Ichinoseki including the Y chromosome had been eliminated. The small number of males contained in the offspring were considered to be genetic females which were sex-reversed, as seven of the eight males of the 357 mature backcrosses produced almost females in the second-generation offspring between these males and female *R. tsushimensis.* On the other hand, it was interesting that the reciprocal allotriploids, (I)IIT, between female *R.*

japonica from Ichinoseki and male *R. tsushimensis* showed no evidence of hybridogenesis. In contrast, reciprocal allotriploids, (T)TTH and (H)HHT, between *R. tsushimensis* and *R. japonica* from Hiroshima which slightly differed morphologically, biochemically and karyologically and was isolated from *R. japonica* of Ichinoseki by incomplete hybrid sterility (SUMIDA, 1981) did not show hybridogenetic gametogenesis. In this case, the existence of the hybridogenetic gametogenesis seemed to depend on the direction of reciprocal hybridizations.

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