Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. 5: 325-358 (1981)

Developmental Capacity and Sex of Gynogenetic Diploids in *Rana japonica* and *Rana tsushimensis*

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(With 4 Text-figures)

INTRODUCTION

Although diploid frogs homozygous for every gene can be obtained by artificial parthenogenesis, it is very difficult to produce them abundantly (KAWAMURA, 1939). They are usually raised from less than 0.05% of pricked eggs. MORIWAKI (1957) refrigerated eggs immediately after pricking with a needle in *Rana japonica* with aim of making the egg diploid by union of the second polar body nucleus with the female pronucleus. In this case, the percentage of parthenogenetic frogs did not remarkably increase, while 16 mature frogs were produced.

On the other hand, it has been found by SIMON (1930) and DALCQ (1930) that gynogenetic haploids are easily produced by inseminating with UV-irradiated sperm in European brown frogs. SELMAN (1958) produced gynogenetic haploids by UV-irradiating sperm in three European *Triturus* species. POGANY (1971, 1973, 1976) has reported on the HERTWIG effect brought about by UV-irradiated sperm in *Rana pipiens*. Gynogenetic haploids have been abundantly produced since 1967 in our laboratory by inseminating with UV-irradiated sperm in several Japanese anuran species. A few gynogenetic haploids produced by this method in *Rana rugosa* attained sexual maturity (KASHIWAGI, 1980). Two articles on the effects of UV rays on the sperm of *Rana japonica* are included in the present volume (NISHIOKA, OKUMOTO and KONDO, 1981; NISHIOKA and TANAKA, 1981).

Gynogenetic diploids were first produced by VOLPE and DASGUPTA (1962) from *Rana pipiens* eggs by heat-shock after insemination with sperm of the spade-foot toad, *Scaphiopus holbrooki*. Since 1964 to the present, gynogenetic diploids have been abundantly produced in our laboratory from eggs of several anuran species by refrigeration or heat-shock after insemination with UV-irradiated sperm (KAWAMURA and NISHIOKA, 1977). Many color mutants as well as natural color strains were obtained by application of this method in *Rana nigromaculata* (NISHIOKA, 1977) and *Hyla arborea japonica* (NISHIOKA and UEDA, 1977). Sex-determining mechanism was also elucidated by this method in several Japanese anuran species (KAWAMURA and NISHIOKA, 1977).

Diploid gynogenesis in laboratory frogs is a mode of development which is simple in principle and easy in conduct. However, the present authors con-

sidered it to be of value to resolve the following questions: How many diploid frogs are gynogenetically raised from anuran eggs by heat-shock after insemination with UV-irradiated sperm of its own or foreign species? What is the sex ratio of juvenile and mature gynogenetic diploid frogs? Is there any difference in producing gynogenetic diploids between insemination with UV-irradiated sperm of its own species and that with UV-irradiated sperm of a foreign species? The results of experiments conducted to resolve these problems by using two brown frog species, *Rana japonica* and *Rana tsushimensis*, will be presented here.

MATERIALS AND METHODS

The Japanese brown frog, *Rana japonica* GÜNTHER, was collected from the suburbs of Hiroshima, while the Tsushima brown frog, *Rana tsushimensis* STEJNEGER, was from the Island of Tsushima, Nagasaki Prefecture (Fig. 1). The specimens of *Rana tsushimensis* were utilized after reared for about two years in our laboratory. Experiments were performed in February and March of 1968. Ovulation was induced by celiac injection of frog pituitary suspension. A preliminary examination for fertilization capacity of eggs was made 24 hours after commencement of ovulation. The females in which more than 90% of the eggs examined were normally fertilized were used as materials of this study within 24 hours after the test. Sperm suspension was made in Cl-free tap water. A vial with 0.5 ml of sperm suspension was placed 20 cm from the source of ultraviolet rays (U-shaped mercury-vapor lamp, GUL-5J type, Toshiba Electric Company, Tokyo) and exposed for 90 seconds at 24 erg/mm^2/sec. The ultraviolet rays were 2537 Å in main wave length. Eggs were inseminated with sperm whose nuclei were inactivated by UV-irradiation. Ten minutes later, they were put in PETRI dishes, 18 cm in diameter, containing Cl-free tap water for 10~15 minutes. Eggs of *Rana japonica* and *Rana tsushimensis* were then exposed to 37°C and 36°C, respectively, for 3 minutes in order to suppress extrusion of the second polar body. Gynogenetic diploids were produced by fusion of the female pronucleus with the second polar body nucleus retained in the eggs.

The eggs were reared for about 40 days after insemination under laboratory conditions. At this age, they became tadpoles having hind limb buds which began to show toe prominences. Thereafter, the tadpoles were reared outdoors in a cement tank, 95 cm in length, 65 cm in width and 20 cm in depth, until completion of metamorphosis. Most of the frogs were fixed in NAVASHIN's fluid within 1~2 months after metamorphosis. Their sex was anatomically determined from the shape, size and external structure of their gonads. When the sex could not be definitely determined by the external characters of the gonads, it was determined by observing their histological structure. In this case, the gonads were embedded in paraffin, sectioned at 12 μ and stained with HEIDENHAIN's iron-hematoxylin. The remaining frogs were reared until sexual maturity.

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

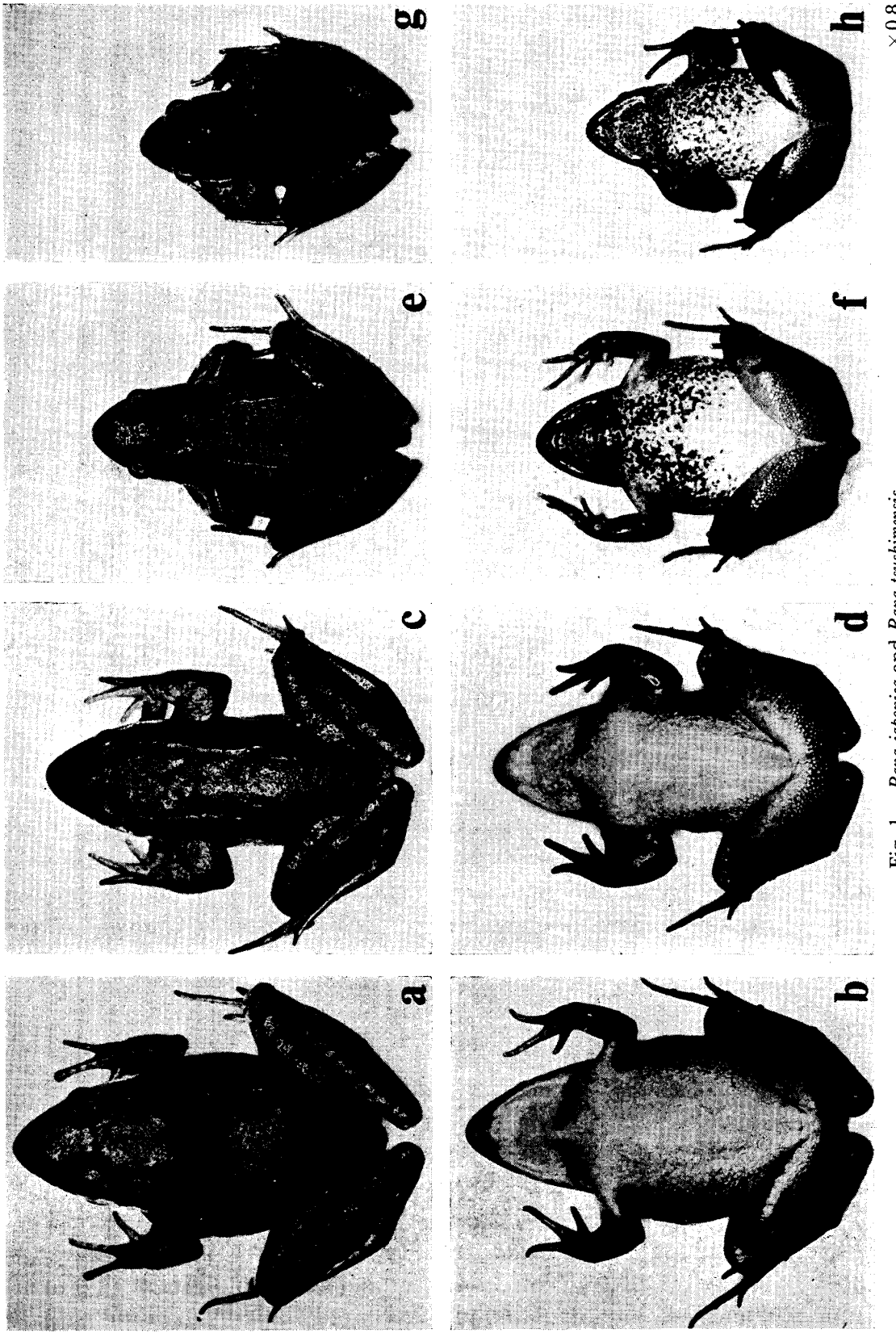

Fig. 1. *Rana japonica* and *Rana tsushimensis*. ×0.8

a, b. A female *Rana japonica* c, d. A male *Rana japonica* e, f. A female *Rana tsushimensis* g, h. A male *Rana tsushimensis*

The following abbreviations are used for designation of the brown frogs employed in this study.

J..... *Rana japonica*
Ts..... *Rana tsushimensis*

OBSERVATION

I. Developmental capacity

1. Individuals produced from female *Rana japonica*

a. Control *Rana japonica*

In 12 matings between 12 females (J♀, Nos. 1～12) and 12 male (J♂, Nos. 1～12), 82.1～96.5%, average of 91.8%, of 45～234 eggs cleaved normally (Table 1). Of the normally cleaved eggs, 88.9～100%, average of 93.9%, hatched normally and became normal tadpoles. Some of the latter died of underdevelopment or edema. While about 73% of normally cleaved eggs became normally metamorphosed frogs in three matings (Nos. 2, 9 and 12), 79.9～93.6% attained this stage in the other nine matings.

b. Diploid hybrids

Eggs of 12 female *Rana japonica* (J♀, Nos. 1～12) were inseminated with sperm of 12 male *Rana tsushimensis* (Ts♂, Nos. 1～12). In the 12 crosses, 82.6～100%, average of 92.5%, of 32～115 eggs cleaved normally (Table 2). The normally cleaved eggs developed normally as those of the control *Rana japonica*; 80.4～100%, average of 92.6%, of them hatched normally. Although the tadpoles were almost normal in appearance, their external gills were somewhat retarded in development and degenerated as compared with the controls. Thereafter, they did not eat so actively as the controls did and were increasingly retarded in growth. After stage V at the age of 40 days, they were extremely retarded in growth and could not become longer than about 3 cm. All of them became emaciated and died of underdevelopment.

c. Gynogenetic haploids

Eggs of 12 female *Rana japonica* (J♀, Nos. 1～12) were inseminated with UV-irradiated sperm of 12 male *Rana japonica* (J♂, Nos. 1～12). In the 12 matings, 84.6～100%, average of 90.8%, of 40～72 eggs cleaved normally as in the control matings of *Rana japonica* (Table 3). Of the normally cleaved eggs, 83.3～100%, average of 93.7%, hatched and all became typical haploid tadpoles.

Eggs of the same 12 female *Rana japonica* were also inseminated with UV-irradiated sperm of 12 male *Rana tsushimensis* (Ts♂, Nos. 1～12). In 12 matings, 56.7～100%, average of 90.0%, of 33～91 eggs cleaved normally (Table 4). Of the normally cleaved eggs, 61.8～97.6%, average of 86.3%, hatched and all became typical haploid tadpoles.

TABLE 1

Developmental capacity of the control *Rana japonica*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos		No. of st. V tadpoles		No. of metamorphosed frogs
Female	Male			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
J, No. 1	J, No. 1	115	110	110	0	110	0	105	5	105	0	103
			95.7%	(100%)		(100%)		(95.5%)	(4.5%)	(95.5%)		(93.6%)
J, No. 2	J, No. 2	140	133	131	2	127	4	125	2	108	17	97
			95.0%	(98.5%)	(1.5%)	(95.5%)	(3.0%)	(94.0%)	(1.5%)	(81.2%)	(12.8%)	(72.9%)
J, No. 3	J, No. 3	112	103	101	2	101	0	99	2	98	1	95
			92.0%	(98.1%)	(1.9%)	(98.1%)		(96.1%)	(1.9%)	(95.1%)	(1.0%)	(92.2%)
J, No. 4	J, No. 4	136	125	120	5	120	0	120	0	118	2	112
			91.9%	(96.0%)	(4.0%)	(96.0%)		(96.0%)		(94.4%)	(1.6%)	(89.6%)
J, No. 5	J, No. 5	90	84	82	2	81	1	80	1	73	7	69
			93.3%	(97.6%)	(2.4%)	(96.4%)	(1.2%)	(95.2%)	(1.2%)	(86.9%)	(8.3%)	(82.1%)
J, No. 6	J, No. 6	131	122	122	0	119	3	112	7	109	3	105
			93.1%	(100%)		(97.5%)	(2.5%)	(91.8%)	(5.7%)	(89.3%)	(2.5%)	(86.1%)
J, No. 7	J, No. 7	120	112	112	0	111	1	106	5	105	1	98
			93.3%	(100%)		(99.1%)	(0.9%)	(94.6%)	(4.5%)	(93.8%)	(0.9%)	(87.5%)
J, No. 8	J, No. 8	123	101	101	0	101	0	101	0	99	2	94
			82.1%	(100%)		(100%)		(100%)		(98.0%)	(2.0%)	(93.1%)
J, No. 9	J, No. 9	234	217	208	3	202	6	193	9	161	32	158
			92.7%	(95.9%)	(1.4%)	(93.1%)	(2.8%)	(88.9%)	(4.1%)	(74.2%)	(14.7%)	(72.8%)
J, No. 10	J, No. 10	216	189	184	2	182	2	172	10	167	5	151
			87.5%	(97.4%)	(1.1%)	(96.3%)	(1.1%)	(91.0%)	(5.3%)	(88.4%)	(2.6%)	(79.9%)
J, No. 11	J, No. 11	45	43	43	0	43	0	43	0	42	1	40
			95.6%	(100%)		(100%)		(100%)		(97.7%)	(2.3%)	(93.0%)
J, No. 12	J, No. 12	57	55	53	2	53	0	53	0	49	4	40
			96.5%	(96.4%)	(3.6%)	(96.4%)		(96.4%)		(89.1%)	(7.3%)	(72.7%)
J, Nos. 1～12	J, Nos. 1～12	1519	1394	1367	18	1350	17	1309	41	1234	75	1162
			91.8%	(98.1%)	(1.3%)	(96.8%)	(1.2%)	(93.9%)	(2.9%)	(88.5%)	(5.4%)	(83.4%)

TABLE 2

Developmental capacity of hybrids between female *Rana japonica* and male *Rana tsushimensis*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos		No. of st. V tadpoles		No. of metamorphosed frogs
Female	Male			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
J, No. 1	Ts, No. 1	56	55 98.2%	51 (92.7%)	3 (5.5%)	50 (90.9%)	1 (1.8%)	46 (83.6%)	4 (7.3%)	42 (76.4%)	4 (7.3%)	0
J, No. 2	Ts, No. 2	72	70 97.2%	70 (100%)	0	67 (95.7%)	3 (4.3%)	67 (95.7%)	0	61 (87.1%)	6 (8.6%)	0
J, No. 3	Ts, No. 3	54	51 94.4%	49 (96.1%)	2 (3.9%)	48 (94.1%)	1 (2.0%)	45 (88.2%)	3 (5.9%)	42 (82.4%)	3 (5.9%)	0
J, No. 4	Ts, No. 4	69	57 82.6%	55 (96.5%)	0	55 (96.5%)	0	50 (87.7%)	5 (8.8%)	44 (77.2%)	6 (10.5%)	0
J, No. 5	Ts, No. 5	80	72 90.0%	70 (97.2%)	0	68 (94.4%)	2 (2.8%)	68 (94.4%)	0	60 (83.3%)	8 (11.1%)	0
J, No. 6	Ts, No. 6	57	52 91.2%	52 (100%)	0	52 (100%)	0	52 (100%)	0	50 (96.2%)	2 (3.8%)	0
J, No. 7	Ts, No. 7	49	46 93.9%	42 (91.3%)	2 (4.3%)	40 (87.0%)	2 (4.3%)	37 (80.4%)	3 (6.5%)	35 (76.1%)	2 (4.3%)	0
J, No. 8	Ts, No. 8	115	101 87.8%	101 (100%)	0	100 (99.0%)	1 (1.0%)	97 (96.0%)	3 (3.0%)	96 (95.0%)	1 (1.0%)	0
J, No. 9	Ts, No. 9	94	92 97.9%	90 (97.8%)	0	90 (97.8%)	0	89 (96.7%)	1 (1.1%)	85 (92.4%)	4 (4.3%)	0
J, No. 10	Ts, No. 10	66	60 90.9%	54 (90.0%)	6 (10.0%)	52 (86.7%)	2 (3.3%)	51 (85.0%)	1 (1.7%)	51 (85.0%)	0	0
J, No. 11	Ts, No. 11	40	37 92.5%	37 (100%)	0	37 (100%)	0	37 (100%)	0	24 (64.9%)	13 (35.1%)	0
J, No. 12	Ts, No. 12	32	32 100%	32 (100%)	0	32 (100%)	0	32 (100%)	0	21 (65.6%)	11 (34.4%)	0
J, Nos. 1～12	Ts, Nos. 1～12	784	725 92.5%	703 (97.0%)	13 (1.8%)	691 (95.3%)	12 (1.7%)	671 (92.6%)	20 (2.8%)	611 (84.3%)	60 (8.3%)	0

TABLE 3
Developmental capacity of haploid *Rana japonica* raised from eggs inseminated with UV-irradiated sperm of *Rana japonica*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos	
Female	Male			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
J, No. 1	UV-J, No. 1	65	63 96.9%	63 (100 %)	0	61 (96.8%)	2 (3.2%)	0	61 (96.8%)
J, No. 2	UV-J, No. 2	72	61 84.7%	59 (96.7%)	2 (3.3%)	57 (93.4%)	2 (3.3%)	0	57 (93.4%)
J, No. 3	UV-J, No. 3	40	39 97.5%	39 (100 %)	0	36 (92.3%)	3 (7.7%)	0	36 (92.3%)
J, No. 4	UV-J, No. 4	46	42 91.3%	40 (95.2%)	1 (2.4%)	35 (83.3%)	5 (11.9%)	0	35 (83.3%)
J, No. 5	UV-J, No. 5	54	49 90.7%	49 (100 %)	0	42 (85.7%)	7 (14.3%)	0	42 (85.7%)
J, No. 6	UV-J, No. 6	40	34 85.0%	34 (100 %)	0	34 (100 %)	0	0	34 (100 %)
J, No. 7	UV-J, No. 7	52	50 96.2%	47 (94.0%)	3 (6.0%)	45 (90.0%)	2 (4.0%)	0	45 (90.0%)
J, No. 8	UV-J, No. 8	46	32 70.0%	32 (100 %)	0	32 (100 %)	0	0	32 (100 %)
J, No. 9	UV-J, No. 9	54	50 92.6%	50 (100 %)	0	46 (92.0%)	4 (8.0%)	0	46 (92.0%)
J, No. 10	UV-J, No. 10	61	61 100 %	59 (96.7%)	2 (3.3%)	59 (96.7%)	0	0	59 (96.7%)
J, No. 11	UV-J, No. 11	65	55 84.6%	52 (94.5%)	3 (5.5%)	52 (94.5%)	0	0	52 (94.5%)
J, No. 12	UV-J, No. 12	54	53 98.1%	53 (100 %)	0	53 (100 %)	0	0	53 (100 %)
J, Nos. 1 ~12	UV-J, Nos. 1~12	649	589 90.8%	577 (98.0%)	11 (1.9%)	552 (93.7%)	25 (4.2%)	0	552 (93.7%)

UV-, UV-irradiated sperm

d. Gynogenetic diploids

Eggs of 12 female *Rana japonica* (J♀, Nos. 1~12) were inseminated with UV-irradiated sperm of 12 male *Rana japonica* (J♂, Nos. 1~12) or *Rana tsushimensis* (Ts♂, Nos. 1~12) and exposed to 37°C for 3 minutes in order to suppress extrusion of the second polar body. Although nearly all the eggs began to cleave, many eggs cleaved abnormally. In 12 matings with UV-irradiated sperm of male *Rana japonica*, 48.1~86.9%, average of 69.7%, of 173~482 eggs cleaved normally, while 50.9~86.9%, average of 69.6%, of 188~794 eggs did so in 12 matings with UV-irradiated sperm of male *Rana tsushimensis*.

The normally cleaved eggs derived from UV-irradiated sperm of male *Rana japonica* were very similar in development to those derived from UV-irradiated sperm of male *Rana tsushimensis*. While 23.7~94.1%, average of 57.1%, and 12.7~55.3%, average of 36.6%, of the former eggs normally hatched and metamorphosed, respectively, 29.9~95.1%, average of 56.1%, and 21.9~55.2%, average of 37.4%, of the latter eggs normally hatched and metamorphosed, re-

TABLE 4

Developmental capacity of haploid *Rana japonica* raised from eggs inseminated with UV-irradiated sperm of *Rana tsushimensis*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos	
Female	Male			Normal	Ab-normal	Normal	Ab-normal	Normal	Ab-normal
J, No. 1	UV-Ts, No. 1	60	34	33	1	21	12	0	21
			56.7%	(97.1%)	(2.9%)	(61.8%)	(35.3%)		(61.8%)
J, No. 2	UV-Ts, No. 2	66	60	58	2	55	3	0	55
			90.9%	(96.7%)	(3.3%)	(91.7%)	(5.0%)		(91.7%)
J, No. 3	UV-Ts, No. 3	62	58	56	2	49	7	0	49
			93.5%	(96.6%)	(3.4%)	(84.5%)	(12.1%)		(84.5%)
J, No. 4	UV-Ts, No. 4	64	52	43	9	42	1	0	42
			81.3%	(82.7%)	(17.3%)	(80.8%)	(1.9%)		(80.8%)
J, No. 5	UV-Ts, No. 5	74	67	67	0	62	5	0	62
			90.5%	(100 %)		(92.5%)	(7.5%)		(92.5%)
J, No. 6	UV-Ts, No. 6	75	63	61	2	57	4	0	57
			84.0%	(96.8%)	(3.2%)	(90.5%)	(6.3%)		(90.5%)
J, No. 7	UV-Ts, No. 7	91	91	88	3	84	4	0	84
			100 %	(96.7%)	(3.3%)	(92.3%)	(4.4%)		(92.3%)
J, No. 8	UV-Ts, No. 8	57	53	49	4	43	6	0	43
			93.0%	(92.4%)	(7.5%)	(81.1%)	(11.3%)		(81.1%)
J, No. 9	UV-Ts, No. 9	46	42	42	0	41	1	0	41
			91.3%	(100 %)		(97.6%)	(2.4%)		(97.6%)
J, No. 10	UV-Ts, No. 10	33	31	28	3	26	2	0	26
			93.9%	(90.3%)	(9.7%)	(83.9%)	(6.5%)		(83.9%)
J, No. 11	UV-Ts, No. 11	90	90	90	0	73	17	0	73
			100 %	(100 %)		(81.1%)	(18.9%)		(81.1%)
J, No. 12	UV-Ts, No. 12	53	53	53	0	46	7	0	46
			100 %	(100 %)		(86.8%)	(13.2%)		(86.8%)
J, Nos. 1 ~12	UV-Ts, Nos. 1~12	771	694	668	26	599	69	0	599
			90.0%	(96.3%)	(3.7%)	(86.3%)	(9.9%)		(86.3%)

UV-, UV-irradiated sperm

spectively. The 12 matings of *Rana japonica* eggs with UV-irradiated sperm of male *Rana japonica* or *Rana tsushimensis* differed from each other in developmental capacity of heat-shocked eggs (Tables 5 and 6; Figs. 2 and 3). These differences among the mating series were evidently attributable to the female parents whose recessive alleles became homozygous by diploid gynogenesis. The individuals raised from eggs of the same female parent were characteristic in type of abnormalities which they showed during development. Many of them usually died of edema, microcephaly, blisters, underdevelopment, etc. at a definite developmental stage.

i) Experimental series J. GD, No. 1

Of 203 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 1) and 194 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 1), 152 (74.9%) and 146 (75.3%) cleaved normally, respectively. Of the normally cleaved eggs in these two parts of the experimental series, 147 (96.7%) and 129 (88.4%) became normal neurulae. Thereafter,

TABLE 5

Developmental capacity of gynogenetic diploids raised from *Rana japonica* eggs by heat-shock after insemination with UV-irradiated sperm of *Rana japonica*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos		No. of st. V tadpoles		No. of metamorphosed frogs
Female	Male			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
J, No. 1	GD-J, No. 1	203	152	147	5	132	15	121	11	99	22	84
			74.9%	(96.7%)	(3.3%)	(86.8%)	(9.9%)	(79.6%)	(7.2%)	(65.1%)	(14.5%)	(55.3%)
J, No. 2	GD-J, No. 2	359	207	197	10	176	21	142	34	115	27	95
			57.7%	(95.2%)	(4.8%)	(85.0%)	(10.1%)	(68.6%)	(16.4%)	(55.6%)	(13.0%)	(45.9%)
J, No. 3	GD-J, No. 3	173	101	96	5	62	34	60	2	56	4	45
			58.4%	(95.0%)	(5.0%)	(61.4%)	(33.7%)	(59.4%)	(2.0%)	(55.4%)	(4.0%)	(44.6%)
J, No. 4	GD-J, No. 4	313	197	184	13	154	30	73	81	43	30	25
			62.9%	(93.4%)	(6.6%)	(78.2%)	(15.2%)	(37.1%)	(41.1%)	(21.8%)	(15.2%)	(12.7%)
J, No. 5	GD-J, No. 5	419	341	234	107	216	18	104	112	101	3	99
			81.4%	(68.6%)	(31.4%)	(63.3%)	(5.3%)	(30.5%)	(32.8%)	(29.6%)	(0.9%)	(29.0%)
J, No. 6	GD-J, No. 6	316	169	164	5	162	2	159	3	154	5	90
			53.5%	(97.0%)	(3.0%)	(95.9%)	(1.2%)	(94.1%)	(1.8%)	(91.1%)	(3.0%)	(53.3%)
J, No. 7	GD-J, No. 7	229	173	165	8	142	23	127	15	107	20	92
			75.5%	(95.4%)	(4.6%)	(82.1%)	(13.3%)	(73.4%)	(8.7%)	(61.8%)	(11.6%)	(53.2%)
J, No. 8	GD-J, No. 8	482	355	312	43	169	143	84	85	74	10	69
			73.7%	(87.9%)	(12.1%)	(47.6%)	(40.3%)	(23.7%)	(23.9%)	(20.8%)	(2.8%)	(19.4%)
J, No. 9	GD-J, No. 9	375	286	285	1	180	105	145	35	128	17	58
			76.3%	(99.7%)	(0.3%)	(62.9%)	(36.7%)	(50.7%)	(12.2%)	(44.8%)	(5.9%)	(20.3%)
J, No. 10	GD-J, No. 10	413	359	344	15	328	16	207	121	194	13	139
			86.9%	(95.8%)	(4.2%)	(91.4%)	(4.5%)	(57.7%)	(33.7%)	(54.0%)	(3.6%)	(38.7%)
J, No. 11	GD-J, No. 11	415	283	280	3	275	5	266	9	188	78	153
			68.2%	(98.9%)	(1.1%)	(97.2%)	(1.8%)	(94.0%)	(3.2%)	(66.4%)	(27.6%)	(54.1%)
J, No. 12	GD-J, No. 12	206	99	99	0	97	2	67	30	64	3	46
			48.1%	(100%)		(98.0%)	(2.0%)	(67.7%)	(30.3%)	(64.6%)	(3.0%)	(46.5%)
J, Nos. 1~12	GD-J, Nos. 1~12	3903	2722	2507	215	2093	414	1555	538	1323	232	995
			69.7%	(92.1%)	(7.9%)	(76.9%)	(15.2%)	(57.1%)	(19.8%)	(48.6%)	(8.5%)	(36.6%)

GD-, Heat-shock after insemination with UV-irradiated sperm

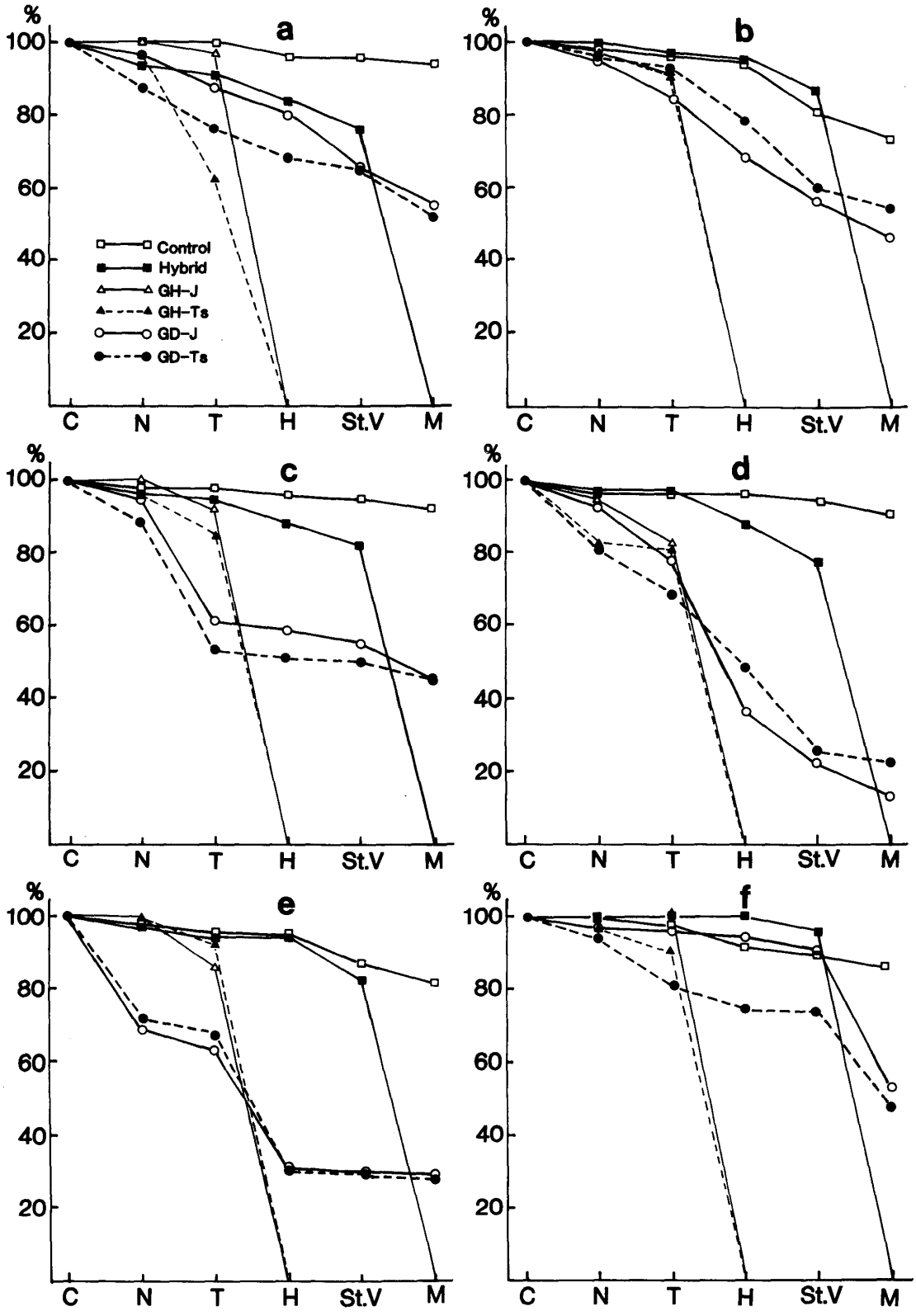

Fig. 2. Survival curves of gynogenetic diploids (GD), interspecific hybrids, gynogenetic haploids (GH) and the controls. All the individuals were raised from eggs of female *Rana japonica*. Sperm were obtained from male *Rana japonica* (J) or male *Rana tsushimensis* (Ts).

many embryos and tadpoles gradually died of edema at various developmental stages. Eventually, 84 (55.3%) and 76 (52.1%) completed metamorphosis (Fig. 2a).

ii) Experimental series J. GD, No. 2

Of the 359 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 2) and 188 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 2), 207 (57.7%) and 151 (80.3%) cleaved normally, respectively. Of the normally cleaved eggs, 197 (95.2%) and 145 (96.0%) in these two parts of the experimental series became normal neurulae. Many embryos and tadpoles gradually died of edema, and eventually 95 (45.9%) and 81 (53.6%) tadpoles completed metamorphosis (Fig. 2b).

iii) Experimental series J. GD, No. 3

Of 173 eggs inseminated with UV-irradiated sperm of male *Rana japonica* (J♂, No. 3) and 390 eggs inseminated with UV-irradiated sperm of male *Rana tsushimensis* (Ts♂, No. 3), 101 (58.4%) and 232 (59.5%) cleaved normally, respectively. Of the normally cleaved eggs in these two parts of the experimental series, 96 (95.0%) and 203 (87.5%) became nearly normal neurulae. Thereafter, 34 and 81 embryos died of microcephaly or edema at the tail-bud stage, respectively. At this stage, 62 (61.4%) normal embryos were raised from eggs inseminated with UV-irradiated sperm of the male *Rana japonica*, while 122 (52.6%) were raised from eggs inseminated with UV-irradiated sperm of the male *Rana tsushimensis*. Both kinds of embryos were almost equal to each other in subsequent development until metamorphosis. After some embryos and tadpoles died of underdevelopment or edema, 45 (44.6%) and 105 (45.3%) normally completed metamorphosis, respectively (Fig. 2c).

iv) Experimental series J. GD, No. 4

Of 313 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 4) and 694 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 4), 197 (62.9%) and 530 (76.4%) cleaved normally, respectively. Of the normally cleaved eggs in these two parts of the experimental series, 184 (93.4%) and 429 (80.9%) became neurulae. While 159 and 313 individuals died of edema, microcephaly or underdevelopment of external gills and teeth at the tail-bud, hatching and feeding tadpole stages, 25 (12.7%) and 116 (21.9%) normally completed metamorphosis (Fig. 2d).

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a. Series of female No. 1 and male No. 1
b. Series of female No. 2 and male No. 2
c. Series of female No. 3 and male No. 3
d. Series of female No. 4 and male No. 4
e. Series of female No. 5 and male No. 5
f. Series of female No. 6 and male No. 6

C — Cleavage N — Neurula stage T — Tail-bud stage H — Hatching St. V — TAYLOR and KOLLROS' stage V M — Completion of metamorphosis

TABLE 6

Developmental capacity of gynogenetic diploids raised from *Rana japonica* eggs by heat-shock after insemination with UV-irradiated sperm of *Rana tsushimensis*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos		No. of st. V tadpoles		No. of metamorphosed frogs
Female	Male			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
J, No. 1	GD–Ts, No. 1	194	146	129	17	111	18	99	12	95	4	76
			75.3%	(88.4%)	(11.6%)	(76.0%)	(12.3%)	(67.8%)	(8.2%)	(65.1%)	(2.7%)	(52.1%)
J, No. 2	GD–Ts, No. 2	188	151	145	6	141	4	118	23	91	27	81
			80.3%	(96.0%)	(4.0%)	(93.4%)	(2.6%)	(78.1%)	(15.2%)	(60.3%)	(17.9%)	(53.6%)
J, No. 3	GD–Ts, No. 3	390	232	203	29	122	81	118	4	116	2	105
			59.5%	(87.5%)	(12.5%)	(52.6%)	(34.9%)	(50.9%)	(1.7%)	(50.0%)	(0.9%)	(45.3%)
J, No. 4	GD–Ts, No. 4	694	530	429	101	365	64	257	108	132	125	116
			76.4%	(80.9%)	(19.1%)	(68.9%)	(12.1%)	(48.5%)	(20.4%)	(24.9%)	(23.6%)	(21.9%)
J, No. 5	GD–Ts, No. 5	794	542	391	151	366	25	162	204	157	5	150
			68.3%	(72.1%)	(27.9%)	(67.5%)	(4.6%)	(29.9%)	(37.6%)	(29.0%)	(0.9%)	(27.7%)
J, No. 6	GD–Ts, No. 6	325	261	246	15	210	36	195	15	193	2	123
			80.3%	(94.3%)	(5.7%)	(80.5%)	(13.8%)	(74.7%)	(5.7%)	(73.9%)	(0.8%)	(47.1%)
J, No. 7	GD–Ts, No. 7	200	154	151	3	141	10	109	32	96	13	85
			77.0%	(98.1%)	(1.9%)	(91.6%)	(6.5%)	(70.8%)	(20.8%)	(62.3%)	(8.4%)	(55.2%)
J, No. 8	GD–Ts, No. 8	511	260	221	39	119	102	83	36	78	5	69
			50.9%	(85.0%)	(15.0%)	(45.8%)	(39.2%)	(31.9%)	(13.8%)	(30.0%)	(1.9%)	(26.5%)
J, No. 9	GD–Ts, No. 9	516	324	275	49	199	76	172	27	154	18	103
			62.8%	(84.9%)	(15.1%)	(61.4%)	(23.5%)	(53.1%)	(8.3%)	(47.5%)	(5.6%)	(31.8%)
J, No. 10	GD–Ts, No. 10	435	378	314	64	286	28	171	115	163	8	109
			86.9%	(83.1%)	(16.9%)	(75.7%)	(7.4%)	(45.2%)	(30.4%)	(43.1%)	(2.1%)	(28.8%)
J, No. 11	GD–Ts, No. 11	430	326	326	0	314	12	310	4	245	65	165
			75.8%	(100%)		(96.3%)	(3.7%)	(95.1%)	(1.2%)	(75.2%)	(19.9%)	(50.6%)
J, No. 12	GD–Ts, No. 12	510	307	306	1	304	2	230	74	219	11	168
			60.2%	(99.7%)	(0.3%)	(99.0%)	(0.7%)	(74.9%)	(24.1%)	(71.3%)	(3.6%)	(54.7%)
J, Nos. 1～12	GD–Ts, Nos. 1～12	5187	3611	3136	475	2678	458	2024	654	1739	285	1350
			69.6%	(86.8%)	(13.2%)	(74.2%)	(12.7%)	(56.1%)	(18.1%)	(48.2%)	(7.9%)	(37.4%)

GD–, Heat-shock after insemination with UV-irradiated sperm

v) Experimental series J. GD, No. 5

Of 419 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 5) and 794 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 5), 341 (81.4%) and 542 (68.3%) cleaved normally, respectively. Of the normally cleaved eggs in these two parts of the experimental series, 234 (68.6%) and 391 (72.1%) became normal neurulae, while 107 (31.4%) and 151 (27.9%) became abnormal embryos having an unclosed yolk plug. At the hatching stage, 104 (30.5%) embryos derived from UV-irradiated sperm of the male *Rana japonica* and 162 (29.9%) embryos derived from UV-irradiated sperm of the male *Rana tsushimensis* were normal, while 112 and 204 others had an extremely curved body, respectively. In the two parts, 99 (29.0%) and 150 (27.7%) tadpoles completed metamorphosis (Fig. 2e).

vi) Experimental series J. GD, No. 6

Of 316 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 6) and 325 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 6), 169 (53.5%) and 261 (80.3%) cleaved normally, respectively. The other eggs inseminated with *Rana japonica* sperm mostly cleaved abnormally. Of the normally cleaved eggs in the two parts of the experimental series, 164 (97.0%) and 246 (94.3%) became normal neurulae. Thereafter, these embryos mostly developed normally until the metamorphosing stage, although a small number of embryos died of various abnormalities. At stages XVII~XX, 64 out of 154 tadpoles derived from UV-irradiated sperm of the male *Rana japonica* and 70 out of 193 tadpoles derived from UV-irradiated sperm of the male *Rana tsushimensis* suddenly died of edema, while the remaining 90 (53.3%) and 123 (47.1%) completed metamorphosis (Fig. 2f).

vii) Experimental series J. GD, No. 7

Of 229 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 7) and 200 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 7), 173 (75.5%) and 154 (77.0%) cleaved normally, respectively. Of the normally cleaved eggs in these two parts of the experimental series, 165 (95.4%) and 151 (98.1%) became normal neurulae. At the tail-bud, hatching and feeding tadpole stages, many individuals gradually died of edema, and 92 (53.2%) and 85 (55.2%) tadpoles attained completion of metamorphosis (Fig. 3a).

viii) Experimental series J. GD, No. 8

Of 482 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 8) and 511 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 8), 355 (73.7%) and 260 (50.9%) cleaved normally, respectively. In these two parts of the experimental series, 43 and 39 embryos died of edema at the neurula stage and 143 and 102 others also died of edema at the tail-bud stage. Of the normally cleaved eggs, 169 (47.6%) and 119 (45.8%) became normal tail-bud embryos in the two experimental parts. Thereafter,

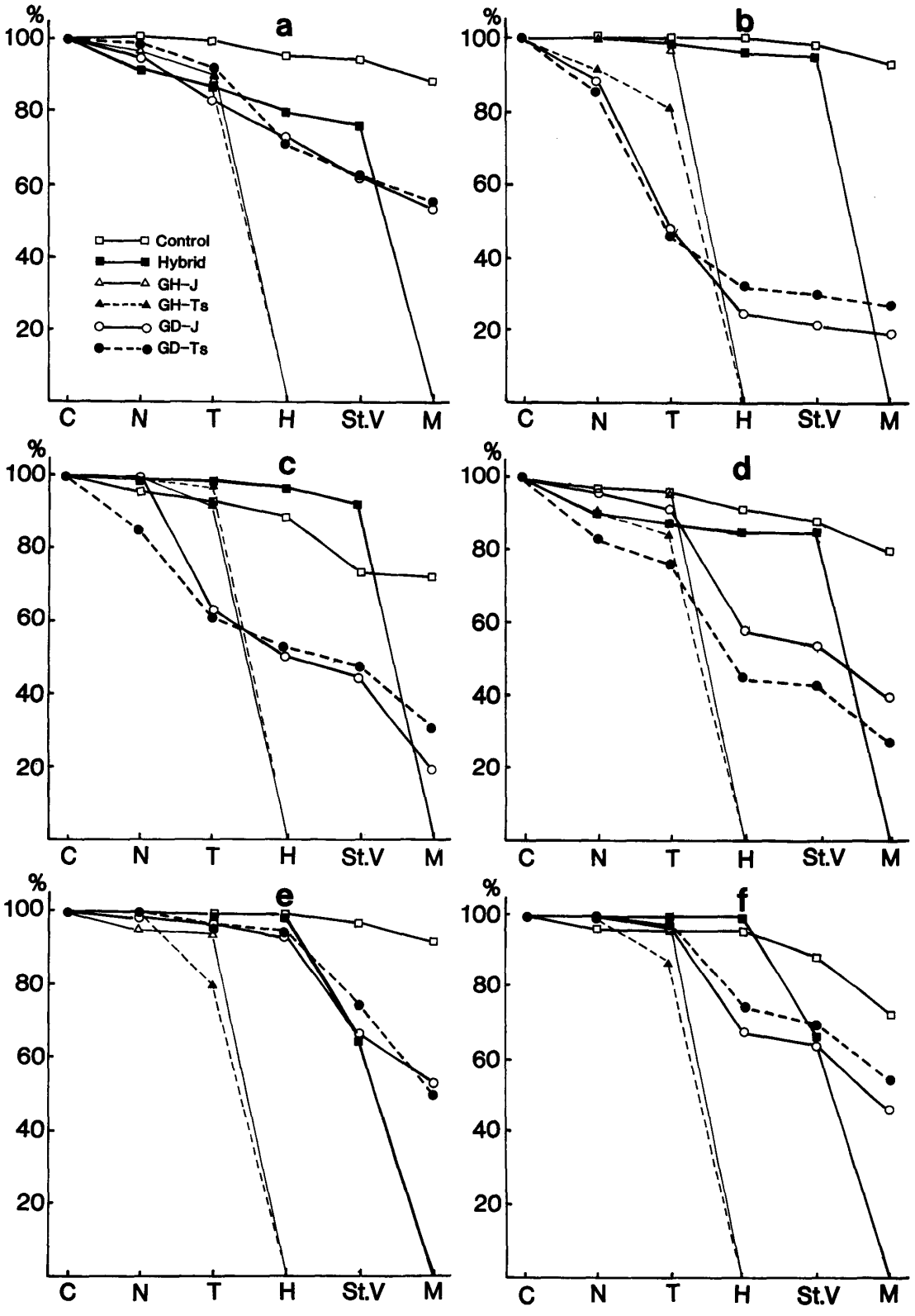

Fig. 3. Survival curves of gynogenetic diploids (GD), interspecific hybrids, gynogenetic haploids (GH) and the controls. All the individuals were raised from eggs of female *Rana japonica*. Sperm were obtained from male *Rana japonica* (J) or male *Rana tsushimensis* (Ts).

85 and 36 embryos became microcephalic or edematous at the hatching stage. Their body was dorsally curved and their external gills were extremely ill-developed. Eventually, 84 (23.7%) and 83 (31.9%) embryos hatched normally, and 69 (19.4%) and 69 (26.5%) tadpoles attained completion of metamorphosis (Fig. 3b).

ix) Experimental series J. GD, No. 9

Of 375 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 9) and 516 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 9), 286 (76.3%) and 324 (62.8%) cleaved normally, respectively. Of the normally cleaved eggs in these two parts of the experimental series, 285 (99.7%) and 275 (84.9%) became normal neurulae, while 49 derived from UV-irradiated sperm of the male *Rana tsushimensis* were abnormal neurulae having an unclosed yolk plug. At the tail-bud stage, 105 and 76 embryos in the two experimental parts died of edema at the same time and their body was dorsally curved and underdeveloped. Many other individuals died of edema at the hatching or feeding tadpole stage. Although 128 and 154 tadpoles in the two experimental parts developed normally until the metamorphosing stage, 70 and 51 of these tadpoles suddenly became edematous and died during metamorphosis. Eventually, 58 (20.3%) and 103 (31.8%) tadpoles attained completion of metamorphosis (Fig. 3c).

x) Experimental series J. GD, No. 10

Of 413 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 10) and 435 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 10), 359 (86.9%) and 378 (86.9%) cleaved normally, respectively. Of the normally cleaved eggs in these two parts of the experimental series, 344 (95.8%) and 314 (83.1%) became normal neurulae, while 64 others derived from sperm of the male *Rana tsushimensis* were abnormal neurulae having an unclosed yolk plug. At the hatching stage, 121 out of 328 tadpoles and 115 out of 286 tadpoles in the two experimental parts died of emaciation having ill-developed external gills, while 207 (57.7%) and 171 (45.2%) hatched normally. At the feeding tadpole stage, 55 and 54 individuals died of underdevelopment, and 13 and 8 died of edema. Thereafter, 139 (38.7%) and 109 (28.8%) tadpoles completed metamorphosis in the two experimental parts (Fig. 3d).

xi) Experimental series J. GD, No. 11

Of 415 eggs inseminated with UV-irradiated sperm of a male *Rana japonica*

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a. Series of female No. 7 and male No. 7
b. Series of female No. 8 and male No. 8
c. Series of female No. 9 and male No. 9
d. Series of female No. 10 and male No. 10
e. Series of female No. 11 and male No. 11
f. Series of female No. 12 and male No. 12

C — Cleavage N — Neurula stage T — Tail-bud stage H — Hatching St. V — TAYLOR and KOLLROS' stage V M — Completion of metamorphosis

(J♂, No. 11) and 430 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 11), 283 (68.2%) and 326 (75.8%) normally cleaved, respectively. Nearly all of the normally cleaved eggs developed normally during the embryonic stage, and 266 (94.0%) and 310 (95.1%) of them in the two parts of the experimental series hatched normally. However, 113 and 145 tadpoles died of edema before metamorphosis, while 153 (54.1%) and 165 (50.6%) attained completion of metamorphosis in the two experimental parts (Fig. 3e).

xii) Experimental series J. GD, No. 12

Of 206 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 12) and 510 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 12), 99 (48.1%) and 307 (60.2%) cleaved normally, respectively, while most of the other eggs cleaved abnormally. Of the normally cleaved eggs in the two parts of the experimental series, 97 (98.0%) and 304 (99.0%) became normal tail-bud embryos. At the hatching stage, 30 out of 97 and 74 out of 304 embryos died of edema. Thereafter, 21 and 62 individuals also died of edema during the tadpole stage. Eventually, 46 (46.5%) and 168 (54.7%) tadpoles completed metamorphosis in the two experimental parts (Fig. 3f).

2. Individuals produced from female *Rana tsushimensis*

a. Control *Rana tsushimensis*

In five matings between five females (Ts♀, Nos. 1～5) and five males (Ts♂, Nos. 13～17), 81.6～98.0%, average of 91.0%, of 51～78 eggs cleaved normally. The normally cleaved eggs were almost normal in development and 81.0～90.3%, average of 86.6%, of them attained completion of metamorphosis, while the others died of various abnormalities (Table 7).

b. Diploid hybrids

Eggs of five female *Rana tsushimensis* (Ts♀, Nos. 1～5) were inseminated with sperm of five male *Rana japonica* (J♂, Nos. 13～17). In the five crosses, 79.2～90.6%, average of 83.3%, of 48～86 eggs cleaved normally. The normally cleaved eggs were almost normal in development during the embryonic stage, although the differentiation of external gills was somewhat inferior to that of the controls. Of the normally cleaved eggs, 78.5～100%, average of 86.6%, hatched normally. When the hybrids attained the feeding tadpole stage, they did not eat so actively as the controls did and began to retard in growth like the reciprocal hybrids. After the age of about 40 days, they gradually became emaciated and died without attaining the metamorphosing stage (Table 8).

c. Gynogenetic haploids

Eggs of five female *Rana tsushimensis* (Ts♀, Nos. 1～5) were inseminated with UV-irradiated sperm of five male *Rana tsushimensis* (Ts♂, Nos. 13～17). In the five matings, 87.7～95.3%, average of 91.5%, of 43～65 eggs cleaved normally.

TABLE 7

Developmental capacity of the control *Rana tsushimensis*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos		No. of st. V tadpoles		No. of metamorphosed frogs
Female	Male			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Ts, No. 1	Ts, No. 13	76	62	62	0	62	0	61	1	60	1	56
			81.6%	(100%)		(100%)		(98.4%)	(1.6%)	(96.8%)	(1.6%)	(90.3%)
Ts, No. 2	Ts, No. 14	61	58	57	1	55	2	55	0	53	2	47
			95.1%	(98.3%)	(1.7%)	(94.8%)	(3.4%)	(94.8%)		(91.4%)	(3.4%)	(81.0%)
Ts, No. 3	Ts, No. 15	78	71	70	1	70	0	70	0	69	1	64
			91.0%	(98.6%)	(1.4%)	(98.6%)		(98.6%)		(97.2%)	(1.4%)	(90.1%)
Ts, No. 4	Ts, No. 16	51	50	50	0	49	1	48	1	48	0	45
			98.0%	(100%)		(98.0%)	(2.0%)	(96.0%)	(2.0%)	(96.0%)		(90.0%)
Ts, No. 5	Ts, No. 17	78	72	71	1	67	4	65	2	61	4	59
			92.3%	(98.6%)	(1.4%)	(93.1%)	(5.6%)	(90.3%)	(2.8%)	(84.7%)	(5.6%)	(81.9%)
Ts, Nos. 1～5	Ts, Nos. 13～17	344	313	310	3	303	7	299	4	291	8	271
			91.0%	(99.0%)	(1.0%)	(96.8%)	(2.2%)	(95.5%)	(1.3%)	(93.0%)	(2.6%)	(86.6%)

TABLE 8

Developmental capacity of hybrids between female *Rana tsushimensis* and male *Rana japonica*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos		No. of st. V tadpoles		No. of metamorphosed frogs
Female	Male			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Ts, No. 1	J, No. 13	79	65	61	4	60	1	51	9	46	5	0
			82.3%	(93.8%)	(6.2%)	(92.3%)	(1.5%)	(78.5%)	(13.8%)	(70.8%)	(7.7%)	
Ts, No. 2	J, No. 14	64	51	50	1	47	3	41	6	40	1	0
			79.7%	(98.0%)	(2.0%)	(92.2%)	(5.9%)	(80.4%)	(11.8%)	(78.4%)	(2.0%)	
Ts, No. 3	J, No. 15	86	72	67	5	65	2	58	7	52	6	0
			83.7%	(93.1%)	(6.9%)	(90.3%)	(2.8%)	(80.6%)	(9.7%)	(72.2%)	(8.3%)	
Ts, No. 4	J, No. 16	64	58	58	0	58	0	58	0	43	15	0
			90.6%	(100%)		(100%)		(100%)		(74.1%)	(25.9%)	
Ts, No. 5	J, No. 17	48	38	38	0	38	0	38	0	28	10	0
			79.2%	(100%)		(100%)		(100%)		(73.7%)	(26.3%)	
Ts, Nos. 1～5	J, Nos. 13～17	341	284	274	10	268	6	246	22	209	37	0
			83.3%	(96.5%)	(3.5%)	(94.4%)	(2.1%)	(86.6%)	(7.7%)	(73.6%)	(13.0%)	

TABLE 9

Developmental capacity of haploid *Rana tsushimensis* raised from eggs inseminated with UV-irradiated sperm of *Rana tsushimensis*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos	
Female	Male			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Ts, No. 1	UV-Ts, No. 13	54	50 92.6%	49 (98.0%)	1 (2.0%)	47 (94.0%)	2 (4.0%)	0	47 (94.0%)
Ts, No. 2	UV-Ts, No. 14	65	57 87.7%	52 (91.2%)	2 (3.5%)	52 (91.2%)	0	0	52 (91.2%)
Ts, No. 3	UV-Ts, No. 15	43	41 95.3%	40 (97.6%)	1 (2.4%)	36 (87.8%)	4 (9.6%)	0	36 (87.8%)
Ts, No. 4	UV-Ts, No. 16	57	52 91.2%	52 (100 %)	0	52 (100 %)	0	0	52 (100 %)
Ts, No. 5	UV-Ts, No. 17	64	59 92.2%	59 (100 %)	0	55 (93.2%)	4 (6.8%)	0	55 (93.2%)
Ts, Nos. 1 ~5	UV-Ts, Nos. 13~17	283	259 91.5%	252 (97.3%)	4 (1.5%)	242 (93.4%)	10 (3.9%)	0	242 (93.4%)

UV-, UV-irradiated sperm

TABLE 10

Developmental capacity of haploid *Rana tsushimensis* raised from eggs inseminated with UV-irradiated sperm of *Rana japonica*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos	
Female	Male			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Ts, No. 1	UV-J, No. 13	61	53 86.9%	51 (96.2%)	2 (3.8%)	50 (94.3%)	1 (1.9%)	0	50 (94.3%)
Ts, No. 2	UV-J, No. 14	75	54 72.0%	52 (96.3%)	2 (3.7%)	49 (90.7%)	3 (5.6%)	0	49 (90.7%)
Ts, No. 3	UV-J, No. 15	82	71 86.6%	67 (94.4%)	4 (5.6%)	65 (91.5%)	2 (2.8%)	0	65 (91.5%)
Ts, No. 4	UV-J, No. 16	49	44 89.8%	44 (100 %)	0	44 (100 %)	0	0	44 (100 %)
Ts, No. 5	UV-J, No. 17	69	56 81.2%	56 (100 %)	0	56 (100 %)	0	0	56 (100 %)
Ts, Nos. 1 ~5	UV-J, Nos. 13~17	336	278 82.7%	270 (97.1%)	8 (2.9%)	264 (95.0%)	6 (2.2%)	0	264 (95.0%)

UV-, UV-irradiated sperm

Nearly all the normally cleaved eggs developed almost normally until the hatching stage and became typical hybrid-type tadpoles (Table 9).

Eggs of the same five female *Rana tsushimensis* were inseminated with UV-irradiated sperm of five male *Rana japonica* (J♂, Nos. 13~17). In the five matings, 72.0~89.8%, average of 82.7%, of 49~82 eggs cleaved normally. Nearly all the normally cleaved eggs became typical haploid-type tadpoles (Table 10).

d. Gynogenetic diploids

Eggs of five female *Rana tsushimensis* (Ts♀, Nos. 1~5) were inseminated with

TABLE 11 Developmental capacity of gynogenetic diploids raised from *Rana tsushimensis* eggs by heat-shock after insemination with UV-irradiated sperm of *Rana tsushimensis*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos		No. of st. V tadpoles		No. of metamorphosed frogs
Female	Male			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Ts, No. 1	GD–Ts, No. 13	205	111 54.1%	109 (98.2%)	2 (1.8%)	107 (96.4%)	2 (1.8%)	88 (79.3%)	19 (17.1%)	86 (77.5%)	2 (1.8%)	72 (64.9%)
Ts, No. 2	GD–Ts, No. 14	231	180 77.9%	173 (96.1%)	7 (3.9%)	160 (88.9%)	13 (7.2%)	153 (85.0%)	7 (3.9%)	83 (46.1%)	70 (38.9%)	79 (43.9%)
Ts, No. 3	GD–Ts, No. 15	270	164 60.7%	133 (81.1%)	1 (0.6%)	130 (79.3%)	3 (1.8%)	125 (76.2%)	5 (3.0%)	123 (75.0%)	2 (1.2%)	87 (53.0%)
Ts, No. 4	GD–Ts, No. 16	274	157 57.3%	135 (86.0%)	22 (14.0%)	133 (84.7%)	2 (1.3%)	126 (80.3%)	7 (4.5%)	64 (40.8%)	62 (39.5%)	32 (20.4%)
Ts, No. 5	GD–Ts, No. 17	411	333 81.0%	321 (96.4%)	5 (1.5%)	235 (70.6%)	86 (25.8%)	201 (60.4%)	34 (10.2%)	174 (52.3%)	27 (8.1%)	142 (42.6%)
Ts. Nos. 1～5	GD–Ts, Nos. 13～17	1391	945 67.9%	871 (92.2%)	37 (3.9%)	765 (81.0%)	106 (11.2%)	693 (73.3%)	72 (7.6%)	530 (56.1%)	163 (17.2%)	412 (43.6%)

GD–, Heat-shock after insemination with UV-irradiated sperm of *Rana tsushimensis*

TABLE 12 Developmental capacity of gynogenetic diploids raised from *Rana tsushimensis* eggs by heat-shock after insemination with UV-irradiated sperm of *Rana japonica*

Parents		No. of eggs	No. of normally cleaved eggs	No. of neurulae		No. of tail-bud embryos		No. of hatching embryos		No. of st. V tadpoles		No. of metamorphosed frogs
Female	Male			Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Ts, No. 1	GD–J, No. 13	182	93 51.1%	90 (96.8%)	3 (3.2%)	85 (91.4%)	5 (5.4%)	70 (75.3%)	15 (16.1%)	67 (72.0%)	3 (3.2%)	53 (57.0%)
Ts, No. 2	GD–J, No. 14	499	284 56.9%	270 (95.1%)	14 (4.9%)	256 (90.1%)	14 (4.9%)	213 (75.0%)	43 (15.1%)	108 (38.0%)	105 (37.0%)	91 (32.0%)
Ts, No. 3	GD–J, No. 15	276	153 55.4%	152 (99.3%)	1 (0.7%)	145 (94.8%)	7 (4.6%)	139 (90.8%)	6 (3.9%)	133 (86.9%)	6 (3.9%)	87 (56.9%)
Ts, No. 4	GD–J, No. 16	410	197 48.0%	197 (100%)	0	191 (97.0%)	6 (3.0%)	178 (90.4%)	13 (6.6%)	59 (29.9%)	119 (60.4%)	49 (24.9%)
Ts, No. 5	GD–J, No. 17	620	409 66.0%	405 (99.0%)	4 (1.0%)	286 (69.9%)	119 (29.1%)	241 (58.9%)	45 (11.0%)	235 (57.5%)	6 (1.5%)	189 (46.2%)
Ts, Nos. 1～5	GD–J, Nos. 13～17	1987	1136 57.2%	1114 (98.1%)	22 (1.9%)	963 (84.8%)	151 (13.3%)	841 (74.0%)	122 (10.7%)	602 (53.0%)	239 (21.0%)	469 (41.3%)

GD–, Heat-shock after insemination with UV-irradiated sperm of *Rana japonica*

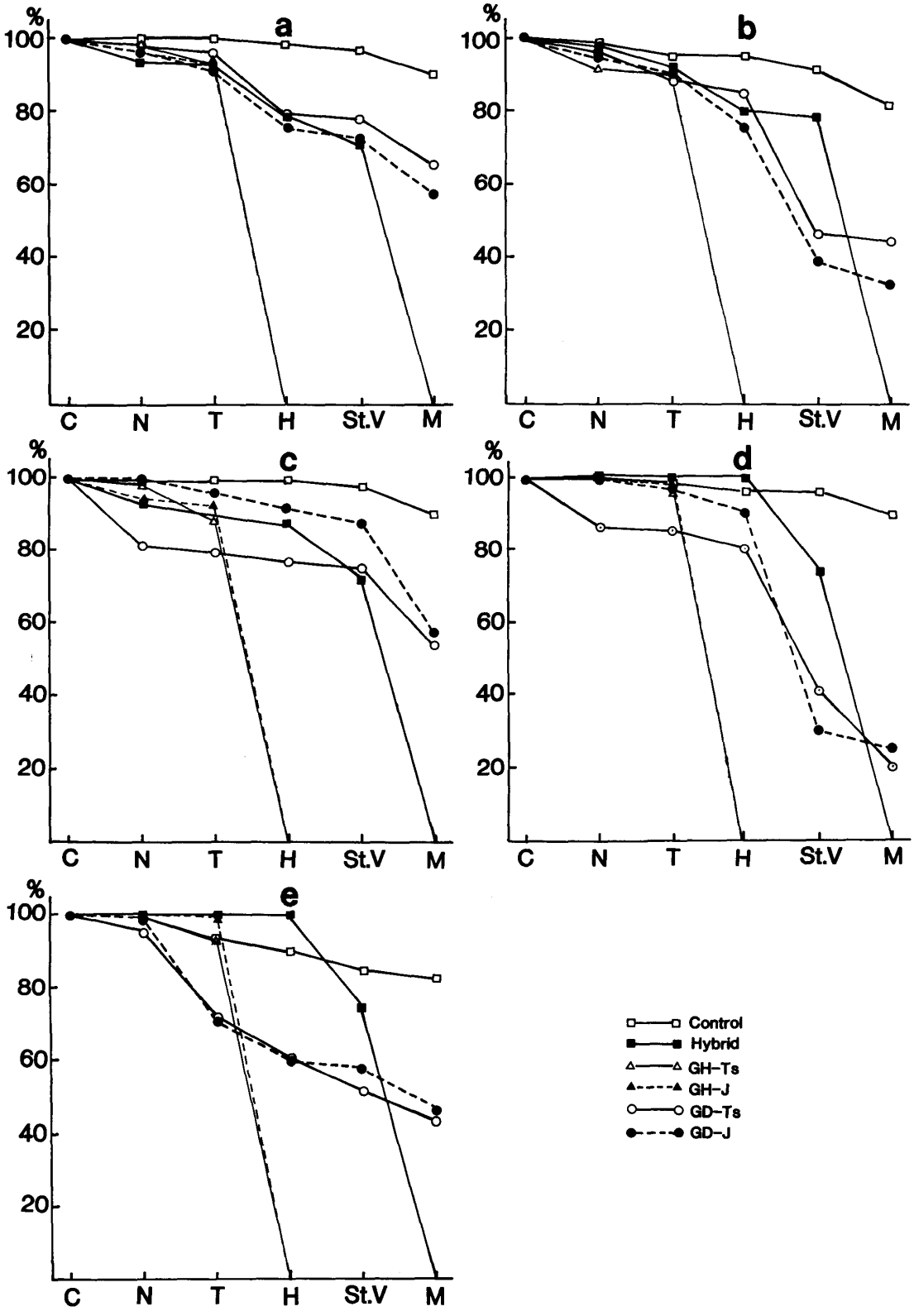

Fig. 4. Survival curves of gynogenetic diploids (GD), interspecific hybrids, gynogenetic haploids (GH) and the controls. All the individuals were raised from eggs of female *Rana tsushimensis*. Sperm were obtained from male *Rana tsushimensis* (Ts) or male *Rana japonica* (J).

UV-irradiated sperm of five male *Rana tsushimensis* (Ts♂, Nos. 13~17) or *Rana japonica* (J♂, Nos. 13~17) and exposed to 36°C for 3 minutes in order to suppress extrusion of the second polar body. Although the rate of cleavage was very high, many eggs cleaved abnormally owing to effects of high temperature. When inseminated with UV-irradiated sperm of the five male *Rana tsushimensis*, 54.1~81.0%, average of 67.9%, of 205~411 eggs cleaved normally, while 48.0~66.0%, average of 57.2%, of 182~620 eggs cleaved normally when inseminated with UV-irradiated sperm of *Rana japonica* (Tables 11 and 12). In total, 2081 (61.6%) out of 3378 eggs cleaved normally.

The normally cleaved eggs derived from UV-irradiated sperm of male *Rana tsushimensis* were very similar in development to those derived from UV-irradiated sperm of male *Rana japonica*. While 60.4~85.0%, average of 73.3%, and 20.4~64.9%, average of 43.6%, of the former normally hatched and completed metamorphosis, respectively, 58.9~90.8%, average of 74.0%, and 24.9~57.0%, average of 41.3%, of the latter normally hatched and completed metamorphosis, respectively. Many embryos and tadpoles in each of the five matings of *Rana tsushimensis* eggs with UV-irradiated *Rana tsushimensis* or *Rana japonica* sperm usually died of the same abnormality such as edema, microcephaly or underdevelopment occurring at a definite developmental stage. Such an abnormality was probably attributable to the genetic character of the female parent whose recessive alleles became homozygous by diploid gynogenesis. Color mutants were also produced from a female by diploid gynogenesis.

i) Experimental series Ts. GD, No. 1

Of 205 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 13) and 182 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 13), 111 (54.1%) and 93 (51.1%) normally cleaved, respectively. Of the normally cleaved eggs in these two parts of the experimental series, 19 and 15 died of edema at the hatching stage. Immediately before metamorphosis, 14 and 14 tadpoles also died of edema in the same way. A few others died of various abnormalities. Eventually, 72 (64.9%) and 53 (57.0%) tadpoles attained completion of metamorphosis in the two experimental parts (Fig. 4a).

ii) Experimental series Ts. GD, No. 2

Of 231 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 14) and 499 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 14), 180 (77.9%) and 284 (56.9%) normally cleaved,

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a. Series of female No. 1 and male No. 1
b. Series of female No. 2 and male No. 2
c. Series of female No. 3 and male No. 3
d. Series of female No. 4 and male No. 4
e. Series of female No. 5 and male No. 5

C — Cleavage N — Neurula stage T — Tail-bud stage H — Hatching St. V — TAYLOR and KOLLROS' stage V M — Completion of metamorphosis

respectively. Of the normally cleaved eggs in the former part of the experimental series, 70 died of underdevelopment at the feeding tadpole stage (stage V), while 101 in total died during the embryonic and tadpole stages. In the latter part of the experimental series, 43 of the normally cleaved eggs died of ill-development of external gills at the hatching stage, 105 others died of underdevelopment at the feeding tadpole stage (stage V) and in addition 45 others died of some other abnormalities during the embryonic and tadpole stages. In the two experimental parts, 79 (43.9%) and 91 (32.0%) tadpoles completed metamorphosis (Fig. 4b).

iii) Experimental series Ts. GD, No. 3

Of 270 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 15) and 276 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 15), 164 (60.7%) and 153 (55.4%) normally cleaved, respectively. Of the normally cleaved eggs in these two parts of the experimental series, 36 and 46 died of edema immediately before metamorphosis, while 77 and 66 in total, respectively, died during the embryonic and tadpole stages. Eventually, 87 (53.0%) and 87 (56.9%) tadpoles attained completion of metamorphosis.

Of 123 feeding tadpoles at stage V derived from UV-irradiated sperm of the male *Rana tsushimensis* and 133 derived from UV-irradiated sperm of the male *Rana japonica,* 58 and 65 were black-eyed mutants, respectively. Of these mutants, 33 and 43 normally completed metamorphosis, while 54 and 44 wild-type tadpoles did so. The remaining tadpoles in the two parts of the experimental series died of edema (Fig. 4c).

iv) Experimental series Ts. GD, No. 4

Of 274 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 16) and 410 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 16), 157 (57.3%) and 197 (48.0%) normally cleaved, respectively. While 126 (80.3%) and 178 (90.4%) of the normally cleaved eggs in these two parts of the experimental series hatched normally, 62 and 119 tadpoles suddenly ceased eating at stage V and died soon thereafter of emaciation. Eventually, 32 (20.4%) and 49 (24.9%) tadpoles attained completion of metamorphosis (Fig. 4d).

v) Experimental series Ts. GD, No. 5

Of 411 eggs inseminated with UV-irradiated sperm of a male *Rana tsushimensis* (Ts♂, No. 17) and 620 eggs inseminated with UV-irradiated sperm of a male *Rana japonica* (J♂, No. 17), 333 (81.0%) and 409 (66.0%) cleaved normally, respectively. While nearly all of the normally cleaved eggs became normal neurulae, 191 and 220 individuals in these two parts of the experimental series died of edema, underdevelopment or deformity at the embryonic or tadpole stage. Eventually, 142 (42.6%) and 189 (46.2%) tadpoles completed metamorphosis (Fig. 4e).

II. Sex of gynogenetic diploids

1. Control *Rana japonica*

The metamorphosed frogs produced from 12 matings between 12 females (J♀, Nos. 1～12) and 12 males (J♂, Nos. 1～12) were mostly preserved shortly after metamorphosis in order to examine their sex. While all the metamorphosed frogs produced from matings Nos. 1, 2 and 8 were killed, a small part of those produced from each of the other nine matings was reared until sexual maturity. The sex of the control *Rana japonica* was determined in 1134 out of 1162 metamorphosed frogs. In the remaining frogs, the sex was not determined, as they died before attaining sexual maturity in most cases.

It was found that of 900 juvenile frogs produced from 12 matings, 449 were females with normal ovaries, five were females with underdeveloped ovaries, two were hermaphrodites transforming from females to males and 444 were males (Table 13). Of 234 mature frogs produced from nine matings, 119 were females and 115 were males. When the two hermaphrodites were counted as males, as they were destined to become males sooner or later, 573 out of 1134 frogs in total produced from 12 matings were females and the other 561 (49.5%) were males.

TABLE 13

Number and sex of the control *Rana japonica*

Series	No. of metamorphosed frogs	Juvenile frogs					Mature frogs			All frogs examined			
		Total	♀	♀u	♀⇄♂	♂	Total	♀	♂	Total	♀	♂	% (♂)
J. Cont, No. 1	103	103	50	1	0	52	—	—	—	103	51	52	50.5
J. Cont, No. 2	97	97	47	3	0	47	—	—	—	97	50	47	48.5
J. Cont, No. 3	95	72	35	0	0	37	19	12	7	91	47	44	48.4
J. Cont, No. 4	112	85	44	0	0	41	23	10	13	108	54	54	50.0
J. Cont, No. 5	69	41	24	0	0	17	25	14	11	66	38	28	42.4
J. Cont, No. 6	105	77	37	0	0	40	25	13	12	102	50	52	51.0
J. Cont, No. 7	98	68	34	0	0	34	27	12	15	95	46	49	51.6
J. Cont, No. 8	94	94	45	1	2	46	—	—	—	94	46	48	51.1
J. Cont, No. 9	158	114	57	0	0	57	42	22	20	156	79	77	49.4
J. Cont, No. 10	151	96	49	0	0	47	46	23	23	142	72	70	49.3
J. Cont, No. 11	40	10	9	0	0	10	21	10	11	40	19	21	52.5
J. Cont, No. 12	40	34	18	0	0	16	6	3	3	40	21	19	47.5
Total	1162	900	449	5	2	444	234	119	115	1134	573	561	49.5

♀u, Female with underdeveloped ovaries

♂, Hermaphrodite with gonads transforming into testes

2. Gynogenetic diploids in *Rana japonica*

a. Frogs from UV-irradiated sperm of *Rana japonica*

The metamorphosed frogs raised from eggs of 12 female *Rana japonica* (J♀, Nos. 1～12) after inseminating with UV-irradiated sperm of 12 male *Rana japonica* (J♂, Nos. 1～12) in 12 matings (Nos. 1～12) were mostly preserved shortly after metamorphosis in order to examine their sex. While all the meta-

morphosed frogs produced from matings Nos.1, 2 and 8 were killed at this stage, a small part of those produced from each of the other nine matings was reared until sexual maturity. The sex of the gynogenetic diploids was determined in 984 out of 995 metamorphosed frogs (Table 14). The sex of the remaining frogs could not be clarified, as they died before reaching sexual maturity in most cases.

Of 794 juvenile frogs produced from 12 matings (Nos. 1～12), 669 were females with normal ovaries, 33 were females with underdeveloped ovaries, 23 were hermaphrodites and 69 were males. Of 190 mature frogs, 163 were females and 27 were males. When the hermaphrodites were counted as males, 865 out of 984 gynogenetic diploids in total produced from 12 matings by using UV-irradiated sperm of male *Rana japonica* were females and only 119 (12.1%) were males.

b. Frogs from UV-irradiated sperm of *Rana tsushimensis*

The metamorphosed frogs raised from eggs of 12 *Rana japonica* (J♀, Nos. 1～

TABLE 14

Number and sex of gynogenetic diploids raised from *Rana japonica* eggs by heat-shock after insemination with UV-irradiated sperm of *Rana japonica* (J) or *Rana tsushimensis* (Ts)

Series	No. of meta-mor-phosed frogs	Juvenile frogs					Mature frogs			All frogs examined			
		Total	♀	♀u	⚥	♂	Total	♀	♂	Total	♀	♂	% (♂)
J. GD (J), No. 1	84	84	78	1	2	3	—	—	—	84	79	5	6.0
J. GD (J), No. 2	95	95	85	5	3	2	—	—	—	95	90	5	5.3
J. GD (J), No. 3	45	24	18	3	1	2	21	20	1	45	41	4	8.9
J. GD (J), No. 4	25	21	17	2	0	2	4	3	1	25	22	3	12.0
J. GD (J), No. 5	99	75	66	4	1	4	15	14	1	90	84	6	6.7
J. GD (J), No. 6	90	65	43	10	5	7	23	19	4	88	72	16	18.2
J. GD (J), No. 7	92	35	33	0	0	2	57	51	6	92	84	8	8.7
J. GD (J), No. 8	69	69	49	3	5	12	—	—	—	69	52	17	24.6
J. GD (J), No. 9	58	34	27	0	1	6	24	20	4	58	47	11	19.0
J. GD (J), No. 10	139	133	127	2	1	3	6	5	1	139	134	5	3.6
J. GD (J), No. 11	153	121	89	3	4	25	32	24	8	153	116	37	24.2
J. GD (J), No. 12	46	38	37	0	0	1	8	7	1	46	44	2	4.3
Total	995	794	669	33	23	69	190	163	27	984	865	119	12.1
J. GD (Ts), No. 1	76	76	68	4	1	3	—	—	—	76	72	4	5.3
J. GD (Ts), No. 2	81	81	75	2	3	1	—	—	—	81	77	4	4.9
J. GD (Ts), No. 3	105	63	55	3	2	3	42	39	3	105	97	8	7.6
J. GD (Ts), No. 4	116	56	45	5	2	4	57	50	7	113	100	13	11.5
J. GD (Ts), No. 5	150	101	86	1	2	12	48	43	5	149	130	19	12.8
J. GD (Ts), No. 6	123	82	65	7	5	5	40	37	3	122	109	13	10.7
J. GD (Ts), No. 7	85	65	60	2	2	1	20	20	0	85	82	3	3.5
J. GD (Ts), No. 8	69	69	48	3	2	16	—	—	—	69	51	18	26.1
J. GD (Ts), No. 9	103	42	37	0	1	4	61	46	15	103	83	20	19.4
J. GD (Ts), No. 10	109	103	95	2	1	5	6	5	1	109	102	7	6.4
J. GD (Ts), No. 11	165	102	74	1	4	23	57	46	11	159	118	41	25.8
J. GD (Ts), No. 12	168	73	70	0	0	3	95	91	4	168	161	7	4.2
Total	1350	913	778	30	25	80	426	377	49	1339	1181	157	11.7

♀u, Female with underdeveloped ovaries

⚥, Hermaphrodite with gonads transforming into testes

12) after inseminating with UV-irradiated sperm of 12 male *Rana tsushimensis* (Ts♂, Nos. 1～12) in 12 matings (Nos. 1～12) were mostly preserved shortly after metamorphosis in order to examine their sex. While all the metamorphosed frogs produced from matings Nos. 1, 2 and 8 were killed at this stage, a small part of those produced from each of the other three matings was reared until sexual maturity. The sex of the gynogenetic diploids was determined in 1339 out of 1350 metamorphosed frogs, while that of the remaining frogs was not clarified, as they died before sexual maturity in most cases.

Of 913 juvenile frogs produced from 12 matings (Nos. 1～12), 778 were females with normal ovaries, 30 were females with underdeveloped ovaries, 25 were hermaphrodites and 80 were males. Of 426 mature frogs, 377 were females and 49 were males. When the hermaphrodites were counted as males, 1181 out of 1339 gynogenetic diploids in total produced from 12 matings by using UV-irradiated sperm of male *Rana tsushimensis* were females and only 157 (11.7%) were males (Table 14).

3. Control *Rana tsushimensis*

The metamorphosed frogs produced from five matings between five females (Ts♀, Nos. 1～5) and five males (Ts♂, Nos. 13～17) were mostly preserved shortly after metamorphosis in order to examine their sex. While all the metamorphosed frogs produced from matings Nos. 1 and 2 were killed, a small part of those produced from the other three matings was reared until sexual maturity. The sex of the control *Rana tsushimensis* was determined in all of 271 metamorphosed frogs.

TABLE 15
Number and sex of the control *Rana tsushimensis*

Series	No. of metamorphosed frogs	Juvenile frogs					Mature frogs			All frogs examined			
		Total	♀	♀u	⚥	♂	Total	♀	♂	Total	♀	♂	% (♂)
Ts, No. 1	56	56	27	0	0	29	—	—	—	56	27	29	51.8
Ts, No. 2	47	47	23	0	0	24	—	—	—	47	23	24	51.1
Ts, No. 3	64	32	17	0	0	15	32	15	17	64	32	32	50.0
Ts, No. 4	45	3	1	0	0	2	42	21	21	45	22	23	51.1
Ts, No. 5	59	50	24	0	0	26	9	4	5	59	28	31	52.5
Total	271	188	92	0	0	96	83	40	43	271	132	139	51.3

♀u, Female with underdeveloped ovaries
⚥, Hermaphrodite with gonads transforming into testes

Of 188 juvenile frogs produced from five matings, 92 were females with normal ovaries and 96 were males. Neither females with underdeveloped ovaries nor hermaphrodites were observed among the juvenile frogs. Of 83 mature frogs produced from matings Nos. 3, 4 and 5, 40 were females and 43 were males. Of 271 metamorphosed frogs in total produced from five matings, 132 were females and 139 (51.3%) were males (Table 15).

4. Gynogenetic diploids in *Rana tsushimensis*

a. Frogs from UV-irradiated sperm of *Rana tsushimensis*

The metamorphosed frogs raised from eggs of five female *Rana tsushimensis* (Ts♀, Nos. 1～5) after inseminating with UV-irradiated sperm of five male *Rana tsushimensis* (Ts♂, Nos. 13～17) in five matings (Nos. 1～5) were mostly preserved shortly after metamorphosis in order to examine their sex. While all the metamorphosed frogs produced from matings Nos. 1 and 2 were killed at this stage, a small part of those produced from each of the other three matings was reared until sexual maturity. The sex of the gynogenetic diploids was determined in 401 out of 412 metamorphosed frogs in total.

Of 340 juvenile frogs produced from five matings (Nos. 1～5), 291 were females with normal ovaries, 11 were females with underdeveloped ovaries, 10 were hermaphrodites and 28 were males. Of 61 mature frogs produced from three (Nos. 3, 4 and 5) of the five matings, 56 were females and five were males. Four of these mature frogs were black-eyed mutants consisting of three females and one male. They were produced from mating No. 3. When the hermaphrodites were counted as males, 358 out of 401 gynogenetic diploids in total produced from five matings by using UV-irradiated sperm of male *Rana tsushimensis* were females and only 43 (10.7%) were males (Table 16).

TABLE 16

Number and sex of gynogenetic diploids raised from *Rana tsushimensis* eggs by heat-shock after insemination with UV-irradiated sperm of *Rana tsushimensis* (Ts) or *Rana japonica* (J)

Series	No. of meta-mor-phosed frogs	Juvenile frogs					Mature frogs			All frogs examined			
		Total	♀	♀u	⚥	♂	Total	♀	♂	Total	♀	♂	%(♂)
Ts. GD (Ts), No. 1	72	72	60	4	3	5	—	—	—	72	64	8	11.1
Ts. GD (Ts), No. 2	79	79	73	3	2	1	—	—	—	79	76	3	3.8
Ts. GD (Ts), No. 3	87	72	51	3	2	16	4*	3*	1*	76	57	19	25.0
Ts. GD (Ts), No. 4	32	25	24	0	0	1	7	6	1	32	30	2	6.3
Ts. GD (Ts), No. 5	142	92	83	1	3	5	50	47	3	142	131	11	7.7
Total	412	340	291	11	10	28	61	56	5	401	358	43	10.7
Ts. GD (J), No. 1	53	53	36	5	5	7	—	—	—	53	41	12	22.6
Ts. GD (J), No. 2	91	91	86	1	3	1	—	—	—	91	87	4	4.4
Ts. GD (J), No. 3	87	74	58	5	4	7	13(5*)	8(2*)	5(3*)	87	71	16	18.3
Ts. GD (J), No. 4	49	37	35	0	0	2	12	12	0	49	47	2	4.1
Ts. GD (J), No. 5	189	96	87	0	3	6	93	89	4	189	176	13	6.9
Total	469	351	302	11	15	23	118	109	9	469	422	47	10.0

♀u, Female with underdeveloped ovaries

⚥, Hermaphrodite with gonads transforming into testes

* black-eyed mutant

b. Frogs from UV-irradiated sperm of *Rana japonica*

The metamorphosed frogs raised from eggs of five female *Rana tsushimensis* (Ts♀, Nos. 1～5) after inseminating with UV-irradiated sperm of five male

Rana japonica (J♂, Nos. 13～17) in five matings (Nos. 1～5) were mostly preserved shortly after metamorphosis in order to examine their sex. While all the metamorphosed frogs produced from two matings (Nos. 1 and 2) were killed at this stage, a small part of those produced from the other three matings was reared until sexual maturity. The sex of the gynogenetic diploids was determined in 469 metamorphosed frogs in total.

Of 351 juvenile frogs produced from five matings (Nos. 1～5), 302 were females with normal ovaries, 11 were females with underdeveloped ovaries, 15 were hermaphrodites and 23 were males. Of 118 mature frogs produced from three matings (Nos. 3, 4 and 5), 109 were females and nine were males. Five of 13 mature frogs produced from mating No. 3 were black-eyed mutants and consisted of two females and three males. When the hermaphrodites were counted as males, 422 out of 469 gynogenetic diploids in total produced from five matings by using UV-irradiated sperm of male *Rana japonica* were females and only 47 (10.0%) were males (Table 16).

DISCUSSION

1. Production of gynogenetic diploids

It is well-known since GOLDSCHMIDT (1920) and PARMENTER (1925) reported on the chromosomes of parthenogenetically developed frogs and old tadpoles that these individuals are usually diploids. Although the production of parthenogenetic diploids was a promising tool for detecting recessive mutational alleles contained in natural frog populations, it was difficult to obtain such animals abundantly. In the season of 1919, LOEB (1921) pricked about 55000 eggs obtained from 26 female *Rana pipiens* with a fine needle and produced 244 (0.44%) hatching or hatched tadpoles of which 145 died within 3 weeks. Of these eggs, 13500 which were obtained from two females and punctured immediately after the frogs arrived at the laboratory produced 135 (1%) hatching tadpoles, of which 92 (0.68%) lived for more than 3 weeks. Only seven out of 41500 eggs obtained from the remaining 24 females became tadpoles and could live for more than 3 weeks. PARMENTER (1925) observed the sex and chromosomes of the tadpoles produced by LOEB (1921) in 1919. There were 34 tadpoles. Although only one of them metamorphosed at the age of 4 months, this died 5 months after metamorphosis. The other tadpoles remained at approximately uniform size without protruding the forelegs until they were killed at various ages. Thus, a total of 21 parthenogenetic frogs raised by LOEB during several years were obtained from among hundreds of thousands of pricked eggs.

PARMENTER (1933) examined the chromosome number of 29 individuals produced parthenogenetically by himself from *Rana pipiens* and *Rana palustris* eggs. Ten of these individuals were diploids. While five diploids were abnormal embryos or edematous tadpoles, the other five became metamorphosed frogs. Although he did not describe the number of eggs pricked as well as the number of

females used in the experiments, it is estimated from the records in Table 1 of his article that about 12000 uterine eggs from 12 females of the two species were pricked in obtaining the above parthenogenetic individuals. If this estimation is true, the five metamorphosed frogs correspond to 0.04% of the pricked eggs. In 1935, KAWAMURA (1936) pricked a total of 11842 uterine eggs obtained from 15 female *Rana nigromaculata* with a platinum needle and obtained 179 embryos which lived for more than four days. Six of these embryos attained completion of metamorphosis and were diploids. This number corresponds to 0.05% of the pricked eggs. In 1936 and 1937, KAWAMURA (1939) obtained 86 parthenogenetic diploids from about 164000 uterine eggs of 151 female *Rana nigromaculata*. Of these diploids, 18 lived for more than 50 days and only 12 metamorphosed. This number of metamorphosed frogs corresponds to 0.007% of the pricked eggs. In 1940, KAWAMURA (1949) repeated the same experiment and produced 31 diploid tadpoles from 62600 uterine eggs. Eighteen of these tadpoles, 0.03% of the pricked eggs, completed metamorphosis. During the experiment of this year, four albino tadpoles happened to be produced together with two wild-type ones from a female parent (TOKUNAGA, 1949). These parthenogenetic tadpoles fortunately completed metamorphosis except one albino. KAWAMURA (1949) obtained six parthenogenetic frogs in 1938 and 1939 by pricking uterine eggs of *Rana japonica* and also TCHOU et al. (1938, 1940) produced two mature females from *Rana nigromaculata* eggs by pricking, but the number of pricked eggs in these experiments has not been reported.

MORIWAKI (1957) refrigerated 63732 unfertilized eggs of *Rana japonica* after pricking with a glass needle and obtained 41 (0.064%) diploid frogs, of which 16 (0.025%) attained sexual maturity. Refrigeration was made with the aim of suppressing the second polar body formation and of making the egg diploid by fusion of the second polar body nucleus with the female pronucleus. VOLPE and DASGUPTA (1962) produced gynogenetic diploids in *Rana pipiens* by exposing eggs to 37°C for 4 minutes after inseminated with sperm of *Scaphiopus holbrooki*. The percentages of insemination in the experimental crosses between female *Rana pipiens* and male *Scaphiopus holbrooki* were low, varying from 28 to 54%. Of 811 cleaved eggs in eight experimental series, 70 (8.6%) became gynogenetic diploids at the tail-bud stage and 17 (2.1%) attained completion of metamorphosis.

On the basis of the findings by SIMON (1930) and DALCQ (1930) that UV-irradiation of sperm incapacitated their nucleus, abundant gynogenetic diploids were produced in our laboratory from *Rana nigromaculata* eggs by refrigeration after insemination with UV-irradiated sperm of *Rana pipiens* or *Rana brevipoda* in 1964, 1965, 1966 and 1968 (KAWAMURA and NISHIOKA, 1977). It was found that 1463 (30%) of 4877 eggs obtained from 26 females became metamorphosed frogs in the experimental series, while 86% of the control eggs inseminated with untreated sperm attained the same stage. Similar experiments were made every year from 1967 to 1970 by RICHARDS and NACE (1978) in *Rana pipiens*. They produced a total of 1269 gynogenetic frogs from eggs of 106 females by heat-

shock after insemination with UV-irradiated sperm of *Rana clamitans* or *Rana pipiens*. According to NACE, RICHARDS and ASHER (1970), the efficacy of this treatment was variable and the percentage of viable embryos produced from more than 65 females ranged from zero to 34. In our laboratory, third-generation offspring were also produced from female *Rana brevipoda* by repeating gynogenetic reproduction three times during the period between 1966 and 1970 (KAWAMURA and NISHIOKA, 1977).

In the present study, gynogenetic diploid frogs were produced from eggs of 12 female *Rana japonica* and five female *Rana tsushimensis* by heat-shock after insemination with UV-irradiated sperm of male *Rana japonica* or *Rana tsushimensis*. Of the control *Rana japonica* and *Rana tsushimensis* eggs inseminated with sperm of their own species, 91.8% and 91.0% cleaved normally, respectively. Of these normally cleaved eggs, 83.4% and 86.6% attained completion of metamorphosis, respectively. No metamorphosed frogs were produced from reciprocal crosses between these two species. No normally hatched tadpoles were obtained from eggs of both species by insemination with UV-irradiated sperm of male *Rana japonica* or *Rana tsushimensis*, although more than 82% of eggs cleaved normally. While 2722 (69.7%) of 3903 *Rana japonica* eggs cleaved normally by heat-shock after insemination with UV-irradiated sperm of male *Rana japonica*, 3611 (69.6%) of 5187 eggs of the same species did so by the same treatment after inseminating with UV-irradiated sperm of male *Rana tsushimensis*. While 36.6% of the normally cleaved eggs in the series of male *Rana japonica* became metamorphosed frogs, 37.4% of the normally cleaved eggs in the series of male *Rana tsushimensis* did so. On the other hand, 945 (67.9%) of 1391 *Rana tsushimensis* eggs cleaved normally by heat-shock after insemination with UV-irradiated sperm of male *Rana tsushimensis*, while 1136 (57.2%) of 1987 eggs of the same species did so by the same treatment after insemination with UV-irradiated sperm of male *Rana japonica*. While 43.6% of the normally cleaved eggs in the series of male *Rana tsushimensis* became metamorphosed frogs, 41.3% of the normally cleaved eggs in the series of male *Rana japonica* did so. These findings seem to indicate that the UV-irradiated sperm of the foreign species did not differ from those of the own species in producing gynogenetic diploids. Besides, it was confirmed that about one-fourth of uterine eggs became gynogenetic diploids on the whole by the method of heat-shock after insemination with UV-irradiated sperm in *Rana japonica* and *Rana tsushimensis*, although percentages of gynogeneitc diploid frogs remarkably differ with female parents.

2. Sex of gynogenetic frogs

According to PARMENTER (1925) only three of 21 metamorphosed frogs of *Rana pipiens* which were obtained parthenogenetically by LOEB during several years were females. Of 34 parthenogenetic tadpoles of the same species produced by LOEB in 1919 and examined by PARMENTER, 12 were males, two were hermaphrodites, 18 were females and two were of doubtful sex. PARMENTER has assumed that the normal male is heterogametic and that overripeness is a factor in produc-

ing both sexes of parthenogenetic frogs and tadpoles. KAWAMURA (1949) reported that 30 out of 38 diploid parthenogenetic frogs raised from *Rana nigromaculata* eggs in 1935, 1936, 1937 and 1940 were females with nearly normal ovaries. Four others were females with degenerating or underdeveloped ovaries, two others were males with rudimentary testes and the remaining two were males with nearly normal testes. On the other hand, of the six young parthenogenetic frogs raised from *Rana japonica* eggs in 1938 and 1939, two were females with nearly normal ovaries, three were females with degenerating or underdeveloped ovaries and one was a male with nearly normal testes. Of 16 mature parthenogenetic frogs produced by MORIWAKI (1957) from *Rana japonica* eggs by cold-treatment after pricking, nine were females and seven were males.

KAWAMURA and NISHIOKA (1977) reported on the sex of gynogenetic diploids produced in several Japanese anurans. Of 1462 gynogenetic frogs raised from eggs of 26 female *Rana nigromaculata*, 1387 were females and 75 (5.1%) were males including individuals transforming into males. Of 1798 gynogenetic diploids produced from *Rana brevipoda* eggs, 1773 were females and 25 (1.4%) were males including 11 individuals transforming into males. In the controls of these gynogenetic diploids produced from *Rana nigromaculata* and *Rana brevipoda*, males and females were nearly equal in number. Of 1269 gynogenetic diploids produced by RICHARDS and NACE (1978) between 1967 and 1970 from eggs of 106 female *Rana pipiens*, 967 were females and 302 (23.8%) were males including 35 hermaphrodites, while 227 females and 240 (51.4%) were males among the biparental controls.

Gynogenetic diploids of *Rana japonica* were first produced in 1967 in the authors' laboratory by heat-shock of eggs after insemination with UV-irradiated sperm of the same species. Of 445 juvenile frogs raised from eggs of five females, 401 were females and 44 (9.9%) were males including individuals transforming into males (KAWAMURA and NISHIOKA, 1977). In the following year, gynogenetic diploids were produced from 12 *Rana japonica* and five *Rana tsushimensis* by heat-shock of eggs after inseminating with UV-irradiated sperm of the two species. The sex of a part of these gynogenetic frogs was briefly reported after examining their gonads immediately after metamorphosis, although the sex ratio of 13 mature frogs from series, Ts. GD (J), No. 3 raised from *Rana tsushimensis* eggs by inseminating with UV-irradiated sperm of *Rana japonica* was especially included (KAWAMURA and NISHIOKA, 1977). The sex of all the gynogenetic frogs and the controls obtained in 1968 is described in the present paper.

Of 1134 juvenile and mature frogs raised from *Rana japonica* eggs as the controls, 573 were females and 561 (49.5%) were males including two individuals transforming into males. Of 984 juvenile and mature gynogenetic frogs raised from *Rana japonica* eggs by inseminating with UV-irradiated sperm of the same species, 865 were females and 119 (12.1%) were males including 23 individuals transforming into males, while of 1339 juvenile and mature gynogenetic frogs raised from *Rana japonica* eggs by inseminating with UV-irradiated sperm of *Rana tsushimensis*, 1181 were females and 157 (11.7%) were males including 25

individuals transforming into males. On the other hand, of 271 juvenile and mature frogs raised from *Rana tsushimensis* eggs as the controls, 132 were females and 139 (51.3%) were males. Of 401 juvenile and mature gynogenetic frogs raised from *Rana tsushimensis* eggs by inseminating with UV-irradiated sperm of the same species, 358 were females and 43 (10.7%) were males including 10 individuals transforming into males, while of 469 juvenile and mature gynogenetic frogs raised from *Rana tsushimensis* eggs by inseminating with UV-irradiated sperm of *Rana japonica,* 422 were females and 47 (10.0%) were males including 15 individuals transforming into males. These findings evidently show that the male is heterogametic in these two brown frog species and that the UV-irradiated sperm of *Rana japonica* are equivalent to those of *Rana tsushimensis* in producing sex-reversed genetic females.

Percentages of sex-reversed genetic females among gynogenetically produced diploids remarkably differed with females from which eggs were obtained. It was 3.5~26.1% in 12 female *Rana japonica* and 3.8~25.0% in five female *Rana tsushimensis.* However, it is also evident that the percentages of sex-reversed genetic females differed as a whole with species. In the present study, about 12% and 10% of the respective total number of gynogenetic diploids were males in *Rana japonica* and *Rana tsushimensis,* respectively. In *Rana nigromaculata,* 15.4% were males, while only 1.4% were males in *Rana brevipoda.* In *Rana pipiens,* about 24% of gynogenetically produced diploids were males including hermaphrodites (RICHARDS and NACE, 1978). In contrast to these species, sex reversal did not occur in gynogenetic diploids raised from eggs of *Hyla arborea japonica* and *Bombina orientalis* by cold-treatment after inseminating with UV-irradiated sperm of their own species.

It is well-known that overripeness of uterine eggs, artificial parthenogenesis, interspecific hybridization, polyploidy, sex-hormones or high temperature give rise to sex reversal in various anuran species. Many studies on sex reversal caused by such factors have been reported by KAWAMURA and his collaborators since 1939 (cf. KAWAMURA and NISHIOKA, 1977). If the sex reversal induced by sex hormone administration is excluded, this phenomenon always co-exists with any developmental abnormalities created by the above agents. Sex reversal seems to occur as a by-product of such developmental abnormalities in a definite direction, for example, from female to male in many species of Ranidae. Thus, sex reversal in anurans seems to be controlled by two categories. One is the degree of stability in sex determination and sex differentiation. This seems to be a genetic character peculiar to each species or population. The other is the severity of developmental abnormalities caused by various kinds of factors. The difference in male percentage between parthenogenetically and gynogenetically developed diploids in *Rana japonica, Rana nigromaculata* or *Rana pipiens* seems to be attributable to that in the severity of developmental abnormalities.

The method of diploid gynogenesis utilized in the present study seems to be suitable for producing diploids which are almost homozygous for every locus. At the same time, this method seems to be appropriate in estimating the degree

of stability in sex determination and sex differentiation of each species or population, as the severity of developmental abnormalities caused by this method itself is comparatively slight in spite of the fact that the animals usually become homozygous for any injurious recessive genes contained in the eggs.

SUMMARY

1. Gynogenetic diploids were produced from eggs of 12 female *Rana japonica* and five female *Rana tsushimensis* by exposing these eggs to high temperature after inseminating with UV-irradiated sperm of male *Rana japonica* or *Rana tsushimensis*. They were compared in developmental capacity with the control *Rana japonica* or *Rana tsushimensis*, hybrids between female *Rana japonica* and male *Rana tsushimensis* and gynogenetic haploids of the two species.

2. While 93.9% and 83.4% of 1394 normally cleaved eggs of 12 female *Rana japonica* mated with 12 male *Rana japonica* normally hatched and metamorphosed, respectively, 92.6% and 0% of 725 normally cleaved eggs of the same 12 females mated with 12 male *Rana tsushimensis* did so, respectively. No gynogenetic haploids produced from eggs of the same 12 females by inseminating with UV-irradiated sperm of the 12 male *Rana japonica* or the 12 male *Rana tsushimensis* hatched normally.

3. In contrast to the finding that more than 90% of eggs of the 12 female *Rana japonica* cleaved normally by inseminating with non-treated or UV-irradiated sperm of the 12 male *Rana japonica* or the 12 male *Rana tsushimensis*, 69.7% of 3903 and 69.6% of 5187 eggs of the same 12 females did so by exposing to 37°C for 3 minutes after inseminating with UV-irradiated sperm of the 12 male *Rana japonica* and the 12 male *Rana tsushimensis*, respectively. Of the normally cleaved eggs in the seires which were heat-shocked after inseminating with UV-irradiated sperm of the male *Rana japonica*, 57.1% and 36.6% normally hatched and metamorphosed, respectively, while in the series which were heat-shocked after inseminating with UV-irradiated sperm of the male *Rana tsushimensis*, 56.1% and 37.4% of the normally cleaved eggs normally hatched and metamorphosed, respectively.

4. While 95.5% and 86.6% of 344 normally cleaved eggs of five female *Rana tsushimensis* mated with five male *Rana tsushimensis* hatched and metamorphosed normally, respectively, 86.6% and 0% of 341 normally cleaved eggs of the same five females mated with five male *Rana japonica* did so, respectively. No gynogenetic haploids produced from eggs of the same five females by inseminating with UV-irradiated sperm of the five male *Rana tsushimensis* or the five male *Rana japonica* hatched normally.

5. In contrast to the finding that more than 82% of eggs of the five female *Rana tsushimensis* cleaved normally by inseminating with non-treated or UV-irradiated sperm of the five male *Rana tsushimensis* or the five male *Rana japonica*, 67.9% of 1391 and 57.2% of 1987 eggs of the same five females did so by exposing to 36°C for 3 minutes after inseminating with UV-irradiated sperm of the five

male *Rana tsushimensis* and the five male *Rana japonica*, respectively. Of the normally cleaved eggs in the series which were heat-shocked after inseminating with UV-irradiated sperm of the male *Rana tsushimensis*, 73.3% and 43.6% normally hatched and metamorphosed, respectively, while in the series which were heat-shocked after inseminating with UV-irradiated sperm of the male *Rana japonica*, 74.0% and 41.3% of the normally cleaved eggs normally hatched and metamorphosed, respectively.

6. Of 984 gynogenetic diploids raised from *Rana japonica* eggs by inseminating with UV-irradiated sperm of *Rana japonica*, 865 were females and 119 (12.1%) were males, while of 1339 gynogenetic diploids raised from *Rana japonica* eggs after inseminating with UV-irradiated sperm of *Rana tsushimensis*, 1181 were females and 157 (11.7%) were males. In the control *Rana japonica*, 573 frogs were females and 561 (49.5%) were males.

7. Of 401 gynogenetic diploids raised from *Rana tsushimensis* eggs after inseminating with UV-irradiated sperm of *Rana tsushimensis*, 358 were females and 43 (10.7%) were males, while of 469 gynogenetic diploids raised from *Rana tsushimensis* eggs by inseminating with UV-irradiated sperm of *Rana japonica*, 422 were females and 47 (10.0%) were males. In the control *Rana tsushimensis*, 132 were females and 139 (51.3%) were males.

8. In *Rana japonica* and *Rana tsushimensis*, the male is considered to be heterogametic on the basis of the finding that an overwhelming majority of gynogenetic diploids is females. Thus, the male gynogenetic diploids are assumed to be sex-reversed genetic females. Sex reversal in gynogenetic diploids seems to occur as a by-product of some developmental abnormality.

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