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Sex of Reciprocal Hybrids between the Hamakita (XX-XY Type) Population and the Murakami (ZW-ZZ Type) Population of *Rana rugosa*

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ABSTRACT

In *Rana rugosa*, sex chromosomes differed from populations to populations. The northern populations in eastern Japan were of the ZW-ZZ type, while the southern populations were of the XX-XY type. In these populations, chromosome pair No. 7 was always sex chromosomes. The X and W chromosomes were metacentric, while the Y and Z chromosomes were either subtelo- or submetacentric. The X and Y chromosomes were distinguished from the W and Z chromosomes, respectively, by the C-banding and LR (late replication)-banding patterns.

In the present study, eight females of the XX type and eight males of the XY-type belonging to the populations of the southern subgroup collected from Hamakita, Shizuoka Prefecture, and six females of the ZW type and seven males of the ZZ type belonging to the populations of the northern subgroup collected from Murakami, Niigata Prefecture, were used to produce reciprocal hybrids in order to examine the relationship between sex chromosomes and sex. It was found that 330 mature offspring produced from matings between females (XX) of the Hamakita population and males (ZZ) of the Murakami population were all of the XZ type in sex chromosomes. There were 175 (53.0%) females and 155 (47.0%) males. In contrast, of the 258 mature offspring produced from matings between females (ZW) of the Murakami population and males (XY) of the Hamakita population, 157 and 101 (39.1%) were females and males, respectively. Of these offspring, 140 females and 87 males were examined with regard to sex-chromosome constitution. It was found that 65 were females of the WX type and 56 were males of the ZY type. While 57 were of the WY type, 55 (96.5%) were females and the other two (3.5%) were males. Of the remaining 49 which were of the ZX type, 20 (40.8%) were females and the other 29 (59.2%) were males. These results suggested that the female-determining genes on the W chromosome of the Murakami population were more dominant than the male-determining genes on the Z chromosome of the Murakami population as well as the male-determining genes on the Y chromosome of the Hamakita population. There was clearly no difference in strength between the male-determining genes situated on the Z chromosome of the Murakami population and the female-determining genes situated on the X chromosome of the Hamakita population, because males and females were nearly the same in number on the average, when chromosome pair No. 7 was ZX chromosomes, although there was usually a difference in number between males and females. It was found, moreover, that the male-determining genes situated on the Y chromosome of the Hamakita population were more dominant than the female-determining genes situated on the X chromosome. While the female-determining genes situated on the W chromosome of the Murakami population were absolutely dominant, the male-determining genes situated on the Y chromosome of the Hamakita population were slightly more dominant than the male-determining genes situated on the Z chromosome of the Murakami population.

INTRODUCTION

Rana rugosa SCHLEGEL is distributed in Japan, Korea and northeastern China. NISHIOKA, KODAMA, SUMIDA and RYUZAKI (1993) collected 40 populations from 40 places between Hokkaido in the east and Kyushu in the west of Japan, and analyzed the enzymes and blood proteins extracted from the specimens of these populations by electrophoresis. When the genetic distances among the populations were elucidated and the phylogenetic tree was drawn by the UPGMA method, it was found that *R. rugosa* in Japan differentiated into the western group including 14 populations and the eastern group including 26 populations, and then the eastern group was differentiated into three subgroups, northern subgroup including seven populations, southern subgroup including nine populations and intermediate subgroup including 10 populations.

NISHIOKA, HANADA, MIURA and RYUZAKI (1994), in analyzing the chromosomes of 24 populations belonging to the three subgroups and the western group by the conventional GIEMSA staining, C-banding and late replication (LR)-banding methods, found that the chromosomes of *R. rugosa* were 2n=26 and each of the 12 pairs other than chromosome pair No. 7 was almost homomorphic and had no sex difference. Sex-specific differences in chromosome pair No. 7 were found among local populations. In the northern subgroup of the eastern group, chromosome pair No. 7 was female heteromorphic (ZW-ZZ type), while in the southern subgroup, it was male heteromorphic (XX-XY type). In the western group, KASHIWAGI (1993) and NISHIOKA, MIURA and SAITOH (1993) confirmed that the sex-determining mechanism was of the XX-XY type, although the sex chromosomes have not yet been distinguished. In the intermediate subgroup of the eastern group, the sex chromosomes have not yet been distinguished, nor have the sex-determining mechanisms been clarified. NISHIOKA, MIURA and SAITOH (1993) produced reciprocal hybrids between the Hirosaki population of the northern subgroup which was of the ZW-ZZ type and the Kumano population of the western group which was of the XX-XY type, although the X and Y chromosomes were not distinguished from each other. It was found that the hybrids having the W chromosome were females and those having the Z chromosome were males, regardless of the presence of the X or Y chromosome.

In the present study, the authors produced reciprocal hybrids between the Murakami population (ZW-ZZ type) of the northern subgroup and the Hamakita population (XX-XY type) of the southern subgroup in which the X and Y chromosomes were morphologically distinguished from each other. By analyzing the sex of the hybrids which had ZX or WY chromosomes, that is, the chromosomes having antagonistic sex-determining genes, the relationship of the two kinds of sex-determining genes and the evolution of the sex chromosomes having the sex-determining genes in *R. rugosa* could be clarified.

MATERIALS AND METHODS

The materials of *Rana rugosa* SCHLEGEL used in the hybridization experiments were two populations in which the sex-determining mechanisms were XX-XY type and ZW-ZZ type. The population of XX-XY type was collected from Hamakita, Shizuoka Prefecture, and included eight females and eight males. The population of ZW-ZZ type was collected from Murakami, Niigata Prefecture, and included six females and seven males. The X and W chromosomes in the two populations were metacentric and the Y and Z chromosomes were either subtelo- or submetacentric. The X and W chromosomes were completely distinguished from each other by difference in the C-banding or LR-banding pattern. The Y and Z chromosomes were also completely distinguished from each other by difference in the C-banding pattern (NISHIOKA, MIURA and SAITOH, 1993; NISHIOKA, HANADA, MIURA and RYUZAKI, 1994).

Ovulation was accelerated by injection of pituitaries of *Rana catesbeiana* into the abdominal cavity of females. Sperm suspension was obtained by crushing testes removed from each male in a small amount of distilled water. Fertilization was artificially performed on a slide glass. Tadpoles were reared by feeding boiled spinach. Frogs after metamorphosis were fed on crickets, *Gryllus bimaculatus* DE GEER (NISHIOKA and MATSUURA, 1977).

The analysis of chromosomes was all done on matured frogs. Chromosome preparations were obtained from the cells at metaphase produced by the method of blood cell culture and made by the methods of conventional Giemsa staining, C-banding and late replication (LR)-banding (NISHIOKA, MIURA and SAITOH, 1993; NISHIOKA, HANADA, MIURA and RYUZAKI, 1994).

OBSERVATION

I. Development and viability

1. Hybrids between females of the Hamakita population (H) and males of the Murakami population (M)

a. Control series, Hamakita (XX)♀×Hamakita (XY)♂

In 1990 and 1992, seven matings were made between seven females (H♀, Nos. 1~3, 5~8) and six males (H♂, Nos. 1~3, 6~8) of the Hamakita population. It was found that 14.8~91.4% of 223~798 eggs, 2006 (59.7%) of 3361 eggs in total, cleaved normally. Of these eggs, 3.9~76.6%, 1040 (30.9%) eggs in total, hatched normally. Thereafter, 1.4~53.2% of eggs, 552 (16.4%) eggs in total, metamor-

TABLE 1

Development and viability of the hybrids between the Hamakita (H) and Murakami (M) populations of *Rana rugosa*

Year	Parents: Female	Parents: Male	No. of eggs	No. of normal cleavages (%)	No. of normal tail-bud embryos (%)	No. of normally hatched tadpoles (%)	No. of metamorphosed frogs (%)	Age (days) at the time of metamorphosis (Mean)
1990	H (XX), No. 1	H (XY), No. 1	798	729 (91.4)	195 (24.4)	145 (18.2)	67 (8.4)	58~80 (65.8)
	H (XX), No. 2	H (XY), No. 2	663	98 (14.8)	26 (3.9)	26 (3.9)	9 (1.4)	74~143 (99.1)
	H (XX), No. 3	H (XY), No. 3	223	185 (83.0)	156 (70.0)	151 (67.7)	59 (26.5)	79~110 (87.2)
1992	H (XX), No. 5	H (XY), No. 6	361	140 (38.8)	107 (29.6)	101 (28.0)	53 (14.7)	61~110 (72.5)
	H (XX), No. 6	H (XY), No. 7	269	236 (87.7)	222 (82.5)	206 (76.6)	143 (53.2)	63~97 (73.9)
	H (XX), No. 7	H (XY), No. 7	675	373 (55.3)	216 (32.0)	198 (29.3)	126 (18.7)	64~114 (73.7)
	H (XX), No. 8	H (XY), No. 8	372	245 (65.9)	216 (58.1)	213 (57.3)	95 (25.5)	65~128 (77.0)
	Total		3361	2006 (59.7)	1138 (33.9)	1040 (30.9)	552 (16.4)	58~143 (78.5)
1990	H (XX), No. 1	M (ZZ), No. 1	1576	429 (27.2)	269 (17.1)	247 (15.7)	134 (8.5)	61~99 (71.5)
	H (XX), No. 2	M (ZZ), No. 2	942	123 (13.1)	40 (4.2)	40 (4.2)	24 (2.5)	62~86 (68.2)
	H (XX), No. 3	M (ZZ), No. 3	308	119 (38.6)	100 (32.5)	99 (32.1)	35 (11.4)	62~89 (69.4)
	H (XX), No. 4	M (ZZ), No. 3	484	271 (56.0)	235 (48.6)	230 (47.5)	78 (16.1)	57~102 (71.5)
1992	H (XX), No. 5	M (ZZ), No. 4	625	432 (69.1)	240 (38.4)	217 (34.7)	82 (13.1)	63~383 (90.6)
	H (XX), No. 6	M (ZZ), No. 5	447	368 (82.3)	274 (61.3)	263 (58.8)	138 (30.9)	59~349 (75.7)
	H (XX), No. 7	M (ZZ), No. 6	604	168 (27.8)	96 (15.9)	88 (14.6)	30 (5.0)	64~339 (79.0)
	H (XX), No. 8	M (ZZ), No. 7	594	572 (96.3)	505 (85.0)	488 (82.2)	178 (30.0)	65~383 (83.0)
	Total		5580	2482 (44.5)	1759 (31.5)	1672 (30.0)	699 (12.5)	57~383 (76.1)
1990	M (ZW), No. 1	M (ZZ), No. 3	992	867 (87.4)	130 (13.1)	119 (12.0)	61 (6.1)	64~110 (87.8)
	M (ZW), No. 2	M (ZZ), No. 3	1333	871 (65.3)	427 (32.0)	427 (32.0)	93 (7.0)	69~117 (84.9)
	M (ZW), No. 3	M (ZZ), No. 3	1147	1020 (88.9)	511 (44.6)	417 (36.4)	157 (13.7)	65~110 (83.5)
1992	M (ZW), No. 4	M (ZZ), No. 4	139	133 (95.7)	103 (74.1)	84 (60.4)	36 (25.9)	68~97 (78.6)
	M (ZW), No. 5	M (ZZ), No. 4	393	335 (85.2)	130 (33.1)	111 (28.2)	33 (8.4)	63~112 (73.8)
	M (ZW), No. 6	M (ZZ), No. 5	551	407 (73.9)	201 (36.5)	194 (35.2)	56 (10.2)	60~342 (81.6)
	Total		4555	3633 (79.8)	1502 (33.0)	1352 (29.7)	436 (9.6)	60~342 (81.7)
1990	M (ZW), No. 1	H (XY), No. 3	1138	964 (84.7)	238 (20.9)	211 (18.5)	133 (11.7)	61~107 (80.7)
	M (ZW), No. 2	H (XY), No. 4	2516	1958 (77.8)	834 (33.1)	712 (28.3)	138 (5.5)	57~93 (66.8)
	M (ZW), No. 3	H (XY), No. 5	2164	1753 (81.0)	863 (39.9)	715 (33.0)	217 (10.0)	60~131 (74.0)
1992	M (ZW), No. 4	H (XY), No. 6	824	392 (47.6)	256 (31.1)	236 (28.6)	76 (9.2)	65~355 (91.6)
	M (ZW), No. 5	H (XY), No. 6	701	330 (47.1)	108 (15.4)	103 (14.7)	53 (7.6)	63~97 (74.0)
	M (ZW), No. 6	H (XY), No. 7	836	622 (74.4)	425 (50.8)	419 (50.1)	229 (27.4)	63~351 (78.2)
	Total		8179	6019 (73.6)	2724 (33.3)	2396 (29.3)	846 (10.3)	57~355 (77.6)

phosed normally. The number of these frogs corresponded to 27.5% of the normally cleaved eggs. These frogs metamorphosed at the age of 58~143 days, 78.5 days on the average, after fertilization (Table 1).

b. Experimental series, Hamakita (XX) ♀ × Murakami (ZZ) ♂

In 1990 and 1992, eight matings were made between eight females (H♀, Nos. 1~8) of the Hamakita population and seven males (M♂, Nos. 1~7) of the Murakami population. It was found that 13.1~96.3% of 308~1576 eggs, 2482 (44.5%) of 5580 eggs in total, cleaved normally. Of these eggs, 4.2~82.2%, 1672

(30.0%) in total, hatched normally. Thereafter, 2.5~30.9%, 699 (12.5%) eggs in total, metamorphosed normally. The number of these frogs corresponded to 28.2% of the normally cleaved eggs. This value was nearly the same as that of the control series. These frogs metamorphosed at the age of 57~383 days, 76.1 days on the average, after fertilization (Table 1).

2. Hybrids between females of the Murakami population (M) and males of the Hamakita population (H)

a. Control series, Murakami (ZW) ♀ × Murakami (ZZ) ♂

In 1990 and 1992, six matings were made between six females (M♀, Nos. 1~6) and three males (M♂, Nos. 3~5) of the Murakami population. It was found that 65.3~95.7% of 139~1333 eggs, 3633 (79.8%) of 4555 eggs in total, cleaved normally. Of these eggs, 12.0~60.4%, 1352 (29.7%) in total, hatched normally. Thereafter, 7.0~25.9%, 436 (9.6%) eggs metamorphosed normally. The number of these frogs corresponded to 12.0% of the normally cleaved eggs. These frogs metamorphosed at the age of 60~342 days, 81.7 days on the average, after fertilization (Table 1).

b. Experimental series, Murakami (ZW) ♀ × Hamakita (XY) ♂

In 1990 and 1992, six matings were made between six females (M♀, Nos. 1~6) of the Murakami population and five males (H♂, Nos. 3~7) of the Hamakita population. It was found that 47.1~84.7% of 701~2516 eggs, 6019 (73.6%) of 8179 eggs in total, cleaved normally. Of these eggs, 14.7~50.1%, 2396 (29.3%) eggs in total, hatched normally. Thereafter, 5.5~27.4%, 846 (10.3%) eggs in total, metamorphosed normally. This number of metamorphosed frogs corresponded to 14.1% of the normally cleaved eggs. These frogs metamorphosed at the age of 57~355 days, 77.6 days on the average, after fertilization (Table 1).

II. Sex ratios of hybrids

1. Hybrids between females of the Hamakita population (H) and males of the Murakami population (M)

a. Control series, Hamakita (XX) ♀ × Hamakita (XY) ♂

Of the 552 frogs produced from seven matings (H♀, Nos. 1~3, 5~8 × H♂, Nos. 1~3, 6~8), 324 died within one year at the immature stage. Of these frogs, the sex of 205 other than 119 frogs whose sex was not analyzed owing to postmortem change as they died immediately after metamorphosis was examined. The results showed that 101 and 104 frogs were females and males, respectively. The percentage of males in each mating was 29.4~61.5%, 50.7% on the average. Of the 228 mature frogs which lived more than one year after metamorphosis, 129 and 99 were females and males, respectively. The percentage of males in each mating was 30.8~56.0%, 43.4% on the average. Of the 433 total frogs whose sex

TABLE 2
Sex ratio of the hybrids between the Hamakita (H) and Murakami (M) populations of *Rana rugosa*

Year	Parents		No. of metamor-phosed frogs	Sex of immature frogs			Sex of mature frogs			Sex of all frogs examined		
	Female	Male		Total	♀	♂	Total	♀	♂	Total	♀	♂ (%)
1990	H (XX), No. 1	H (XY), No. 1	67	26	10	16	25	14	11	51	24	27 (52.9)
	H (XX), No. 2	H (XY), No. 2	9	8	5	3	—	—	—	8	5	3 (37.5)
	H (XX), No. 3	H (XY), No. 3	59	20	9	11	2	1	1	22	10	12 (54.5)
1992	H (XX), No. 5	H (XY), No. 6	53	17	12	5	25	11	14	42	23	19 (45.2)
	H (XX), No. 6	H (XY), No. 7	143	50	27	23	68	34	34	118	61	57 (48.3)
	H (XX), No. 7	H (XY), No. 7	126	40	21	19	65	45	20	105	66	39 (37.1)
	H (XX), No. 8	H (XY), No. 8	95	44	17	27	43	24	19	87	41	46 (52.9)
	Total		552	205	101	104	228	129	99	433	230	203 (46.9)
1990	H (XX), No. 1	M (ZZ), No. 1	134	23	11	12	36	22	14	59	33	26 (44.1)
	H (XX), No. 2	M (ZZ), No. 2	24	2	1	1	21	7	14	23	8	15 (65.2)
	H (XX), No. 3	M (ZZ), No. 3	35	—	—	—	26	12	14	26	12	14 (53.8)
	H (XX), No. 4	M (ZZ), No. 3	78	37	34	3	41	33	8	78	67	11 (14.1)
1992	H (XX), No. 5	M (ZZ), No. 4	82	15	11	4	52	34	18	67	45	22 (32.8)
	H (XX), No. 6	M (ZZ), No. 5	138	72	15	57	46	8	38	118	23	95 (80.5)
	H (XX), No. 7	M (ZZ), No. 6	30	16	12	4	13	8	5	29	20	9 (31.0)
	H (XX), No. 8	M (ZZ), No. 7	178	55	31	24	95	51	44	150	82	68 (45.3)
	Total		699	220	115	105	330	175	155	550	290	260 (47.3)
1990	M (ZW), No. 1	M (ZZ), No. 3	61	22	10	12	13	6	7	35	16	19 (54.3)
	M (ZW), No. 2	M (ZZ), No. 3	93	33	15	18	21	15	6	54	30	24 (44.4)
	M (ZW), No. 3	M (ZZ), No. 3	157	41	23	18	46	28	18	87	51	36 (41.4)
1992	M (ZW), No. 4	M (ZZ), No. 4	36	23	15	8	9	4	5	32	19	13 (40.6)
	M (ZW), No. 5	M (ZZ), No. 4	33	19	13	6	13	6	7	32	19	13 (40.6)
	M (ZW), No. 6	M (ZZ), No. 5	56	26	12	14	23	17	6	49	29	20 (40.8)
	Total		436	164	88	76	125	76	49	289	164	125 (43.3)
1990	M (ZW), No. 1	H (XY), No. 3	133	24	12	12	41	20	21	65	32	33 (50.8)
	M (ZW), No. 2	H (XY), No. 4	138	28	18	10	18	14	4	46	32	14 (30.4)
	M (ZW), No. 3	H (XY), No. 5	217	28	11	17	37	29	8	65	40	25 (38.5)
1992	M (ZW), No. 4	H (XY), No. 6	76	35	21	14	25	12	13	60	33	27 (45.0)
	M (ZW), No. 5	H (XY), No. 6	53	20	11	9	27	16	11	47	27	20 (42.6)
	M (ZW), No. 6	H (XY), No. 7	229	77	55	22	110	66	44	187	121	66 (35.3)
	Total		846	212	128	84	258	157	101	470	285	185 (39.4)

was analyzed, 230 and 203 were females and males, respectively. The percentage of males in each mating was 37.1~54.5%, 46.9% on the average (Table 2).

b. Experimental series, Hamakita(XX) ♀ × Murakami (ZZ) ♂

Of the 699 frogs produced from eight matings (H♀, Nos. 1~8×M♂, Nos. 1~7), 369 died within one year after metamorphosis at the immature stage. Of these frogs, the sex of 220 frogs other than 149 frogs whose sex was not analyzed owing to postmortem change as they died immediately after metamorphosis was examined. The results showed that 115 and 105 frogs were females and males, respectively. The percentage of males in each mating was 8.1~79.2%, 47.7% on the average. Of the 330 mature frogs which lived more than one year after metamorphosis, 175 and 155 were females and males, respectively. The percentage of males in each mating was 19.5~82.6%, 47.0% on the average. Of the 550 total frogs whose sex was analyzed, 290 and 260 were females and males, respectively. The percentage of males in each mating was 14.1~80.5%, 47.3% on the average (Table 2).

2. Hybrids between females of the Murakami population (M) and males of the Hamakita population (H)

a. Control series, Murakami (ZW) ♀ × Murakami(ZZ) ♂

Of the 436 frogs produced from six matings (M♀, Nos. 1~6×M♂, Nos. 3~5), 311 died within one year at the immature stage. Of these frogs, the sex of 164 other than 147 frogs whose sex was not analyzed owing to postmortem change as they died immediately after metamorphosis was examined. The results showed that 88 and 76 frogs were females and males, respectively. The percentage of males in each mating was 31.6~54.5%, 46.3% on the average. Of the 125 mature frogs which lived more than one year after metamorphosis, 76 and 49 were females and males, respectively. The percentage of males in each mating was 26.1~53.8, 39.2% on the average. Of a total of 289 frogs whose sex was analyzed, 164 and 125 were females and males, respectively. The percentage of males in each mating was 40.6~54.3%, 43.3% on the average (Table 2).

b. Experimental series, Murakami (ZW) ♀ × Hamakita (XY) ♂

Of the 846 frogs produced from six matings (M♀, Nos. 1~6×H♂, Nos. 3~7), 588 died within one year after metamorphosis at the immature stage. Of these frogs, the sex of 212 frogs other than 376 frogs whose sex was not analyzed owing to postmortem change as they died immediately after metamorphosis was examined. The results showed that 128 and 84 frogs were females and males, respectively. The percentage of males in each mating was 28.6~60.7%, 39.6% on the average. Of 258 mature frogs which lived more than one year after metamorphosis, 157 and 101 were females and males, respectively. The percentage of males in each mating was 21.6~52.0%, 39.1% on the average. Of the 470 total frogs whose sex was analyzed, 285 and 185 were females and males, respectively. The percentage of males in each mating was 30.4~50.8%, 39.4% on the average (Table 2).

III. Constitution of chromosome pair No. 7 of mature hybrids and their sex

Reciprocal hybrids were produced between the Hamakita population which was of the XX-XY type and the Murakami population which was of the ZW-ZZ type in sex-determining mechanism. The chromosome pair No. 7 in mature males and females of these hybrids reared more than one year was analyzed. In these hybrids, the X and W chromosomes having female-determining genes in chromosome pair No. 7 were metacentric, while the Y and Z chromosomes having

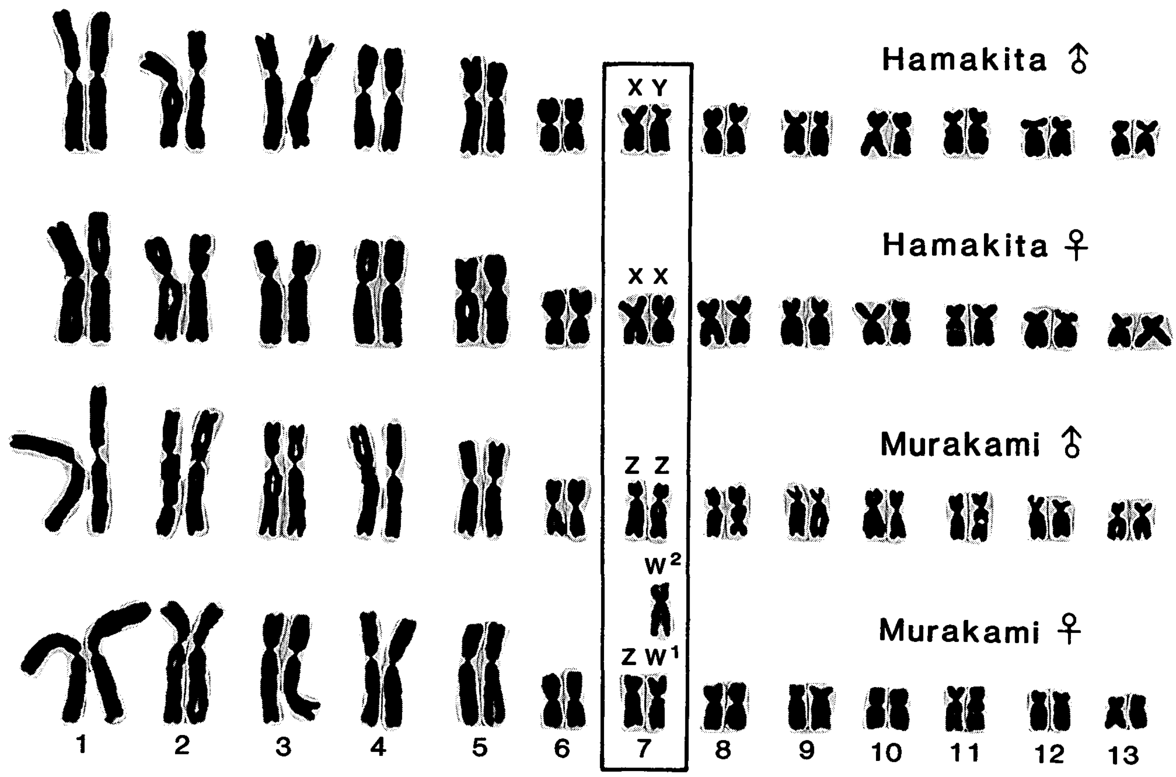

Fig. 1. Karyotypes of male and female *Rana rugosa* of the Hamakita and Murakami populations. Stained by the conventional GIEMSA staining method.

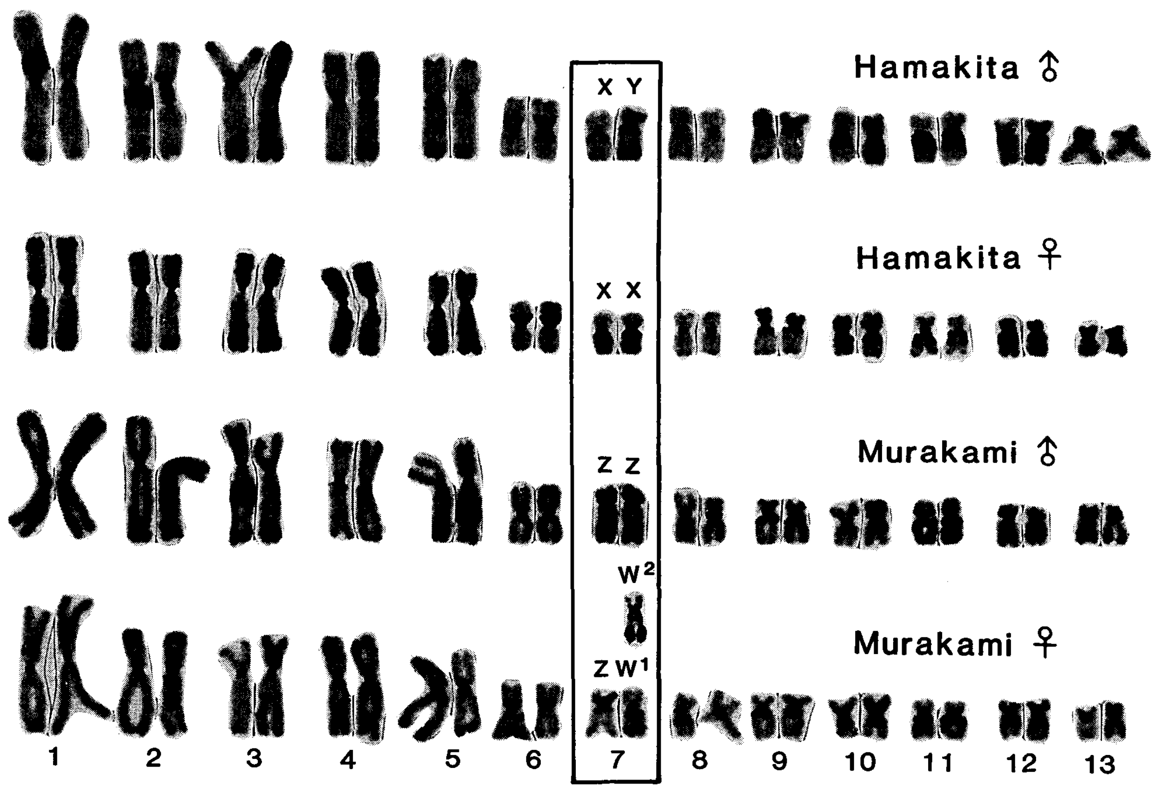

Fig. 2. Karyotypes of male and female *Rana rugosa* of the Hamakita and Murakami populations. Stained by the C-banding method.

TABLE 3

Constitution of chromosome pair No. 7 of the mature hybrids between the Hamakita (H) and Murakami (M) populations in *Rana rugosa*

Year	Parents		No. of mature frogs	Sex	No. of analyzed frogs	Type of chromosome pair No. 7							
	Female	Male				XX	XY	ZW	ZZ	WX	WY	ZX	ZY
1990	H (XX), No. 1	H (XY), No. 1	25	Female	14	14	0	—	—	—	—	—	—
				Male	11	0	11	—	—	—	—	—	—
	H (XX), No. 3	H (XY), No. 3	2	Female	1	1	0	—	—	—	—	—	—
				Male	1	0	1	—	—	—	—	—	—
1992	H (XX), No. 5	H (XY), No. 6	25	Female	11	11	0	—	—	—	—	—	—
				Male	14	0	14	—	—	—	—	—	—
	H (XX), No. 6	H (XY), No. 7	68	Female	34	34	0	—	—	—	—	—	—
				Male	34	0	34	—	—	—	—	—	—
	H (XX), No. 7	H (XY), No. 7	65	Female	45	45	0	—	—	—	—	—	—
				Male	20	0	20	—	—	—	—	—	—
	H (XX), No. 8	H (XY), No. 8	43	Female	24	24	0	—	—	—	—	—	—
				Male	19	0	19	—	—	—	—	—	—
	Total		228	Female	129	129	0	—	—	—	—	—	—
				Male	99	0	99	—	—	—	—	—	—
1990	H (XX), No. 1	M (ZZ), No. 1	36	Female	22	—	—	—	—	—	—	22	—
				Male	14	—	—	—	—	—	—	14	—
	H (XX), No. 2	M (ZZ), No. 2	21	Female	7	—	—	—	—	—	—	7	—
				Male	14	—	—	—	—	—	—	14	—
	H (XX), No. 3	M (ZZ), No. 3	26	Female	12	—	—	—	—	—	—	12	—
				Male	14	—	—	—	—	—	—	14	—
	H (XX), No. 4	M (ZZ), No. 3	41	Female	33	—	—	—	—	—	—	33	—
				Male	8	—	—	—	—	—	—	8	—
1992	H (XX), No. 5	M (ZZ), No. 4	52	Female	34	—	—	—	—	—	—	34	—
				Male	18	—	—	—	—	—	—	18	—
	H (XX), No. 6	M (ZZ), No. 5	46	Female	8	—	—	—	—	—	—	8	—
				Male	38	—	—	—	—	—	—	38	—
	H (XX), No. 7	M (ZZ), No. 6	13	Female	8	—	—	—	—	—	—	8	—
				Male	5	—	—	—	—	—	—	5	—
	H (XX), No. 8	M (ZZ), No. 7	95	Female	51	—	—	—	—	—	—	51	—
				Male	44	—	—	—	—	—	—	44	—
	Total		330	Female	175	—	—	—	—	—	—	175	—
				Male	155	—	—	—	—	—	—	155	—
1990	M (ZW), No. 1	M (ZZ), No. 3	13	Female	6	—	—	6	0	—	—	—	—
				Male	7	—	—	0	7	—	—	—	—
	M (ZW), No. 2	M (ZZ), No. 3	21	Female	15	—	—	15	0	—	—	—	—
				Male	6	—	—	0	6	—	—	—	—
	M (ZW), No. 3	M (ZZ), No. 3	46	Female	28	—	—	28	0	—	—	—	—
				Male	18	—	—	0	18	—	—	—	—
1992	M (ZW), No. 4	M (ZZ), No. 4	9	Female	4	—	—	4	0	—	—	—	—
				Male	5	—	—	0	5	—	—	—	—
	M (ZW), No. 5	M (ZZ), No. 4	13	Female	6	—	—	6	0	—	—	—	—
				Male	7	—	—	0	7	—	—	—	—
	M (ZW), No. 6	M (ZZ), No. 5	23	Female	17	—	—	17	0	—	—	—	—
				Male	6	—	—	0	6	—	—	—	—
	Total		125	Female	76	—	—	76	0	—	—	—	—
				Male	49	—	—	0	49	—	—	—	—
1990	M (ZW), No. 1	H (XY), No. 3	41	Female	11	—	—	—	—	6	5	0	0
				Male	13	—	—	—	—	0	1	4	8
	M (ZW), No. 2	H (XY), No. 4	18	Female	6	—	—	—	—	2	3	1	0
				Male	2	—	—	—	—	0	1	0	1
	M (ZW), No. 3	H (XY), No. 5	37	Female	29	—	—	—	—	14	9	6	0
				Male	8	—	—	—	—	0	0	4	4
1992	M (ZW), No. 4	H (XY), No. 6	25	Female	12	—	—	—	—	7	5	0	0
				Male	12	—	—	—	—	0	0	4	8
	M (ZW), No. 5	H (XY), No. 6	27	Female	16	—	—	—	—	7	6	3	0
				Male	11	—	—	—	—	0	0	2	9
	M (ZW), No. 6	H (XY), No. 7	110	Female	66	—	—	—	—	29	27	10	0
				Male	41	—	—	—	—	0	0	15	26
	Total		258	Female	140	—	—	—	—	65	55	20	0
				Male	87	—	—	—	—	0	2	29	56

XZ is included in ZX.

male-determining genes were either subtelo- or submetacentric. Thus, these two kinds of chromosomes could be completely distinguished from each other by the conventional GIEMSA staining method (Fig. 1). Although the X and W chromosomes were difficult to be distinguished from each other by the conventional GIEMSA staining method, they could be distinguished from each other by the C-banding and LR (late replication)-banding methods (Figs. 2, 3). The subtelo- or submetacentric Y and Z chromosomes were also completely distinguished from each other by the C-banding and LR-banding methods. Thus, the X, Y, W and Z chromosomes which were the sex chromosomes of the two populations were completely distinguished from one another.

The hybrids obtained from matings, XX♀×ZZ♂, were of the XZ type, while the hybrids obtained from the matings, ZW♀×XY♂, had four kinds of chromosomal constitutions, WX, WY, ZX and ZY types (Fig. 4). Of these four types, the WX type having only female-determining genes and the ZY type having only male-determining genes were assumed to become females and males, respectively. In contrast, the sex of the WY or ZX type which had both female- and male-determining genes was examined (Table 3).

1. Mature hybrids between females of the Hamakita population (H) and males of the Murakami population (M)

a. Control series, H(XX)♀, Nos. 1 and 3, 5~8×H(XY)♂, Nos. 1 and 3, 6~8

A total of 228 offspring produced from six matings was alive more than one year after metamorphosis and matured. When the constitution of chromosome pair No. 7 was examined in 129 females of the foregoing offspring, it was found that chromosome pair No. 7 was XX type constructed of a pair of homozygous metacentric X chromosomes. In contrast, each of the other 99 males had a metacentric X chromosome and a subtelo- or submetacentric Y chromosome, that is, chromosome pair No. 7 consisted of heterozygous XY chromosomes (Table 3).

b. Experimental series, H(XX)♀, Nos. 1~8×M(ZZ)♂, Nos. 1~7

A total of 330 hybrids produced from eight matings was alive more than one year after metamorphosis and matured. When the constitution of chromosome pair No. 7 was examined, it was found that all the hybrids were of the XZ type constructed of the X and Z chromosomes. Of these hybrids, produced from a mating, H(XX)♀, No. 4×M(ZZ)♂, No. 3, a small number were males, that is, 8 (19.5%) of 41 offspring were males, while in the hybrids produced from a mating, H(XX)♀, No. 6×M(ZZ)♂, No. 5, there were very numerous males, that is, 38 (82.6%) of 46 offspring were males. Of 330 offspring produced from all the eight matings, 175 were females and 155(47.0%) were males. It appeared that the XZ hybrids became males in some cases, while they became females in the other cases. Thus, the sex determination in the hybrids of the XZ type seemed to be unstable (Table 3; Fig. 5).

2. Mature hybrids between females of the Murakami population (M) and males of the Hamakita population (H)

a. Control series, M(ZW)♀, Nos. 1~6×M(ZZ)♂, Nos. 3~5

A total of 125 offspring produced from six matings was alive more than one year after metamorphosis and matured. Of these offspring, 76 were females and 49 were males. When the constitution of chromosome pair No. 7 in the 76 females was examined, it was found that it was always the heterozygous ZW type constructed of a submetacentric Z chromosome and a metacentric W chromosome. In contrast, the constitution of chromosome pair No. 7 in all the 49 males was ZZ type constructed of a pair of homozygous submetacentric Z chromosomes (Table 3).

b. Experimental series, M(ZW)♀, Nos. 1~6×H(XY)♂, Nos. 3~7

A total of 258 hybrids produced from six matings was alive more than one year after metamorphosis and matured. When the sex-chromosome constitution of chromosome pair No. 7 in these hybrids was examined, it was found that there were four types, WX, WY, ZX and ZY. The analyses of chromosomes were made in 227 frogs including 140 females and 87 males, as the other 31 frogs died before the chromosome analyses or had poor mitotic figures. As expected, 65

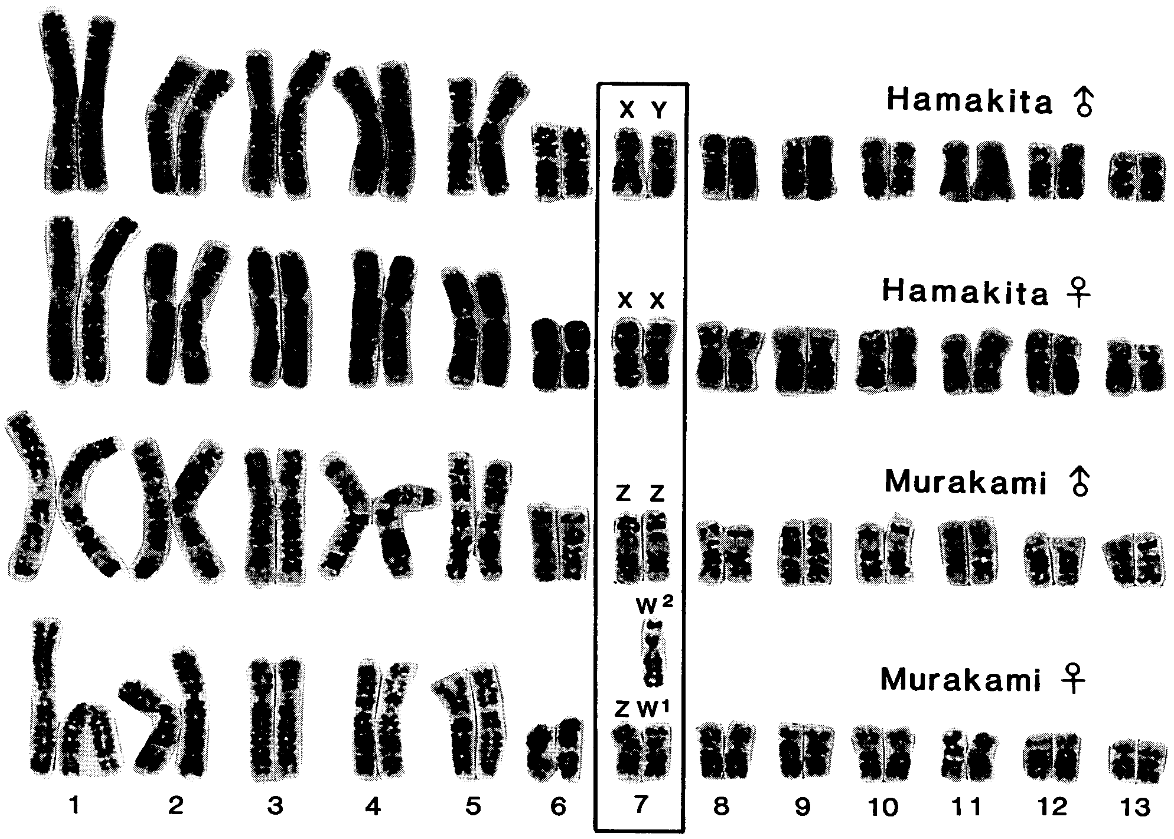

Fig. 3. Karyotypes of male and female *Rana rugosa* of the Hamakita and Murakami populations. Stained by the late replication (LR)-banding method.

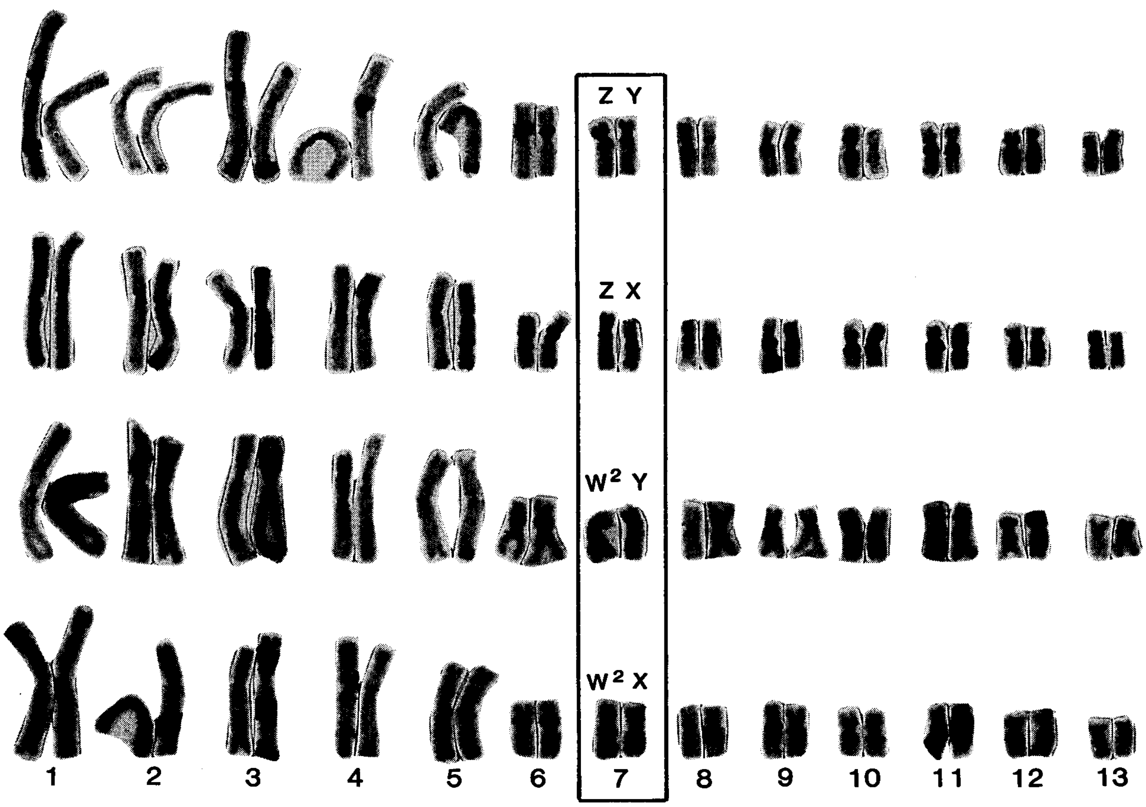

Fig. 4. Karyotypes of hybrids between females of the Murakami population (ZW-ZZ type) and males of the Hamakita population (XX-XY type). Stained by the C-banding method.

WX type frogs were all females, and 56 ZY type frogs were all males. Of the 57 WY type frogs, 55 were females and two were males. Of the 49 ZX type frogs, 20 were females and 29 were males. The sex ratio of ZX type frogs was unstable as that found in the XZ type frogs which were produced from matings, H(XX)♀×M(ZZ)♂ (Table 3; Figs. 4, 5).

DISCUSSION

The presence of two types of sex-determining mechanisms in one and the same species of vertebrates was first discovered in a Mexican small freshwater fish, *Platypoecilus maculatus*, by GORDON in 1944 and 1947. According to his reports, the wild stocks of this species were XX-XY type, while the domesticated stocks of the same species had been found to be ZZ-ZW type by BELLAMY (1922), GORDON (1927) and others. GORDON assumed that the nature of the W chromosome is not homologous to any X chromosome of the platyfish and may be traced to a *Xiphophorus hellerii* chromosome which has introgressed through intergenetic hybridization into the genetic structure of the domesticated platyfish. The sex-determining mechanism of the ZW-ZZ type was thereafter discovered in several freshwater fishes, for example, in *Gambusia affinis* (CHEN and EBELING, 1968; YOSHIDA and HAYASHI, 1970; ITAHASHI and KAWASE, 1975; OJIMA, 1982), *Gambusia gaigei*, *G. hurtadoi* and *G. nobilis* (CAMPOS and HUBBS, 1971), and *Mollienesia*

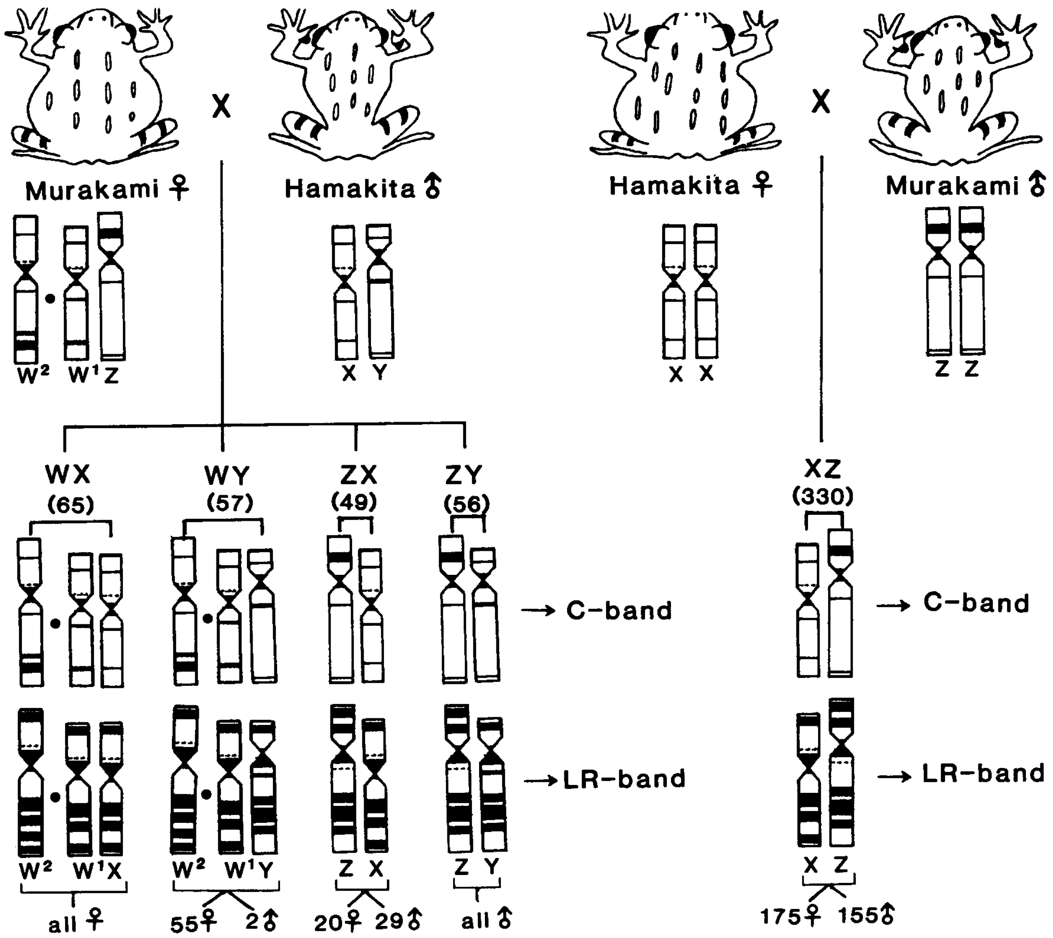

Fig. 5. Sex-chromosome constitutions of mature reciprocal hybrids between the Murakami and Hamakita populations and their sex. Stained by the C- and LR-banding methods.

sphenops (Rishi, 1976). The XX-XY type was found in *Xiphophorus xiphidium* (Foerster and Anders, 1977). On the other hand, the presence of two types of sex-determining mechanisms, XX-XY type and ZW-ZZ type, in one and the same species was found in *Rana rugosa* by Nishioka, Miura and Saitoh (1993). In contrast to the platyfish observed by Gordon (1944, 1947), each of the two types of *R. rugosa* in sex-determining mechanisms was collected from different localities.

The phylogenetic tree of *R. rugosa* drawn by Nishioka, Kodama, Sumida and Ryuzaki (1993) on the basis of the genetic distances among the 40 populations showed that this species was first divided into the eastern and western groups. Then, the eastern group was divided into three subgroups, the northern, intermediate and southern subgroups. In the western group and the three subgroups, it was found that chromosome pair No. 7 of the 13 chromosome pairs in total was really or probably sex chromosomes. Although in chromosome pair No. 7 of the intermediate subgroup, the sex chromosomes were not morphologically distinguished from each other and the sex-determining mechanism was obscure, it was recently confirmed by Nishioka and Hanada (unpublished) that the sex-determining mechanism was probably of the XX-XY type, as the offspring of males whose sex was reversed from females by injection of testosterone propionate were almost females. Thus, it was found that *R. rugosa* belonging to the intermediate and southern subgroups of the eastern group and the western group were of the XX-XY type in the sex-determining mechanism on the basis that chromosome pair No. 7 had undergone a specific change.

NISHIOKA, HANADA, MIURA and RYUZAKI (1994) compared 13 chromosome pairs of the northern, southern and intermediate subgroups of the eastern group and the western group of *R. rugosa* with one another, and found that 12 chromosome pairs other than chromosome pair No. 7 had a sex-specific change. In the northern subgroup (ZW-ZZ type), the Z chromosome was subtelo- or submetacentric and divided into five types, Z^A, Z^B, Z^C, Z^D and Z^O, by C-banding and LR-banding patterns, while the W chromosome was metacentric and divided into two types, W^1 and W^2, by the two banding patterns. In the southern subgroup, the X chromosome was metacentric and very similar to W^1 chromosome of the northern subgroup by the C-banding and LR-banding patterns. The Y chromosome was either subtelo- or submetacentric and divided into two types, Y^A and Y^B, by the C-banding and LR-banding patterns. In the western group, the X and Y chromosomes were not morphologically distinguished from each other. Chromosome pair No. 7 was either submeta- or subtelocentric and divided into two types, 7^A and 7^B, by the C-banding and LR-banding patterns. These 7^A and 7^B chromosomes were very similar to Y^A and Y^B chromosomes in the southern subgroup, respectively. In the intermediate subgroup of the eastern group, the sex-determining chromosome was named 7^C. This chromosome was considered to have been derived from the 7^B type chromosome of the western group by a pericentric inversion. The X chromosome in the southern subgroup and the W^1 chromosome in the northern subgroup were considered to have been produced from the 7^C type chromosome by a pericentric inversion. In order to produce the W^1 or X chromosome from the Z^B or 7^B chromosome, it was necessary to perform two pericentric inversions.

To clarify the difference between the two types of sex-determining mechanisms, NISHIOKA, MIURA and SAITOH (1993) produced reciprocal hybrids between the Hirosaki population (ZW-ZZ type) of the northern subgroup and the Kumano population (XX-XY type) of the western group, in which the X and Y chromosomes were not morphologically distinguished from each other. The results showed that the hybrids having the W chromosome were females and those having the Z chromosome were males, regardless of the presence of the X or Y chromosome.

In the present study, NISHIOKA and HANADA produced reciprocal hybrids between the Hamakita population (XX-XY type) belonging to the southern subgroup and the Murakami population (ZW-ZZ type) of the northern subgroup of the eastern group, in order to examine the relationship between sex chromosomes and sex. When females (XX) of the Hamakita population were mated with males (ZZ) of the Murakami population, all the offspring were XZ in sex chromosomes and produced nearly the same numbers of females and males, as the

X and Z chromosomes had female- and male-determining genes, respectively. In contrast, when females (ZW) of the Murakami population were mated with males (XY) of the Hamakita population, a total of 227 offspring was produced and their sexes and the sex-chromosome constitutions were found to be as follows. The offspring included four kinds of sex chromosome constitutions, ZX, ZY, WX and WY, as expected, and there were nearly the same number of individuals in each kind of sex-chromosome constitutions. More precisely, the number and sexes of frogs, the ZX including 49 consisted of 20 females and 29 males, the ZY including 56 males, the WX including 65 females and the WY including 57 consisted of 55 females and two males, in total 227 consisted of 140 females and 87 males.

As the Z and Y chromosomes had male-determining genes and the W and X chromosomes had female-determining genes, it seemed to be natural that the ZY and WX became males and females, respectively. The fact that the ZX in the sex chromosome combination produced females and males in nearly the same number agreed with the result of the reciprocal matings between female (XX) of the Hamakita population and male (ZZ) of the Murakami population. The XZ offspring included 53.0% females and 47.0% males. The result that the sex-chromosome constitution of WY produced 55 females and two males seems to show that the female-determining genes contained in the W chromosome were very powerful, as compared with the male-determining genes contained in the Y chromosome.

These results suggested that the female-determining genes on the W chromosome of the Murakami population were more dominant than the male-determining genes on the Z chromosome of the Murakami population as well as the male-determining genes on the Y chromosome of the Hamakita population. There was clearly no difference in strength between the male-determining genes situated on the Z chromosome of the Murakami population and the female-determining genes situated on the X chromosome of the Hamakita population, because males and females were nearly the same in number on the average, when chromosome pair No. 7 was ZX chromosomes, although there was usually a difference in number between males and females. It was found, moreover, that the male-determining genes situated on the Y chromosome of the Hamakita population were more dominant than the female-determining genes situated on the X chromosome. While the female-determining genes situated on the W chromosome of the Murakami population were absolutely dominant, the male-determining genes situated on the Y chromosome of the Hamakita population were slightly more dominant than the male-determining genes situated on the Z chromosome of the Murakami population.

These results were very similar to those obtained by Nishioka, Miura and Saitoh (1993) by reciprocal matings between the Hirosaki population (ZW-ZZ type) of the northern subgroup and the Kumano population (XX-XY type) of the western group. In these matings, the hybrids having the W chromosome were females and those having the Z chromosome were males, regardless of the presence of the X or Y chromosome. This suggested that the female-determining genes in the W chromosome and the male-determining genes in the Z chromosome were not the same in potency in different regions.

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