

Studies on Meioses in Male Hybrids and Triploids in the *Rana nigromaculata* Group

I. Interspecific Hybrids between *Rana nigromaculata* and *Rana brevipoda*

By

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(With 8 Text-figures)

INTRODUCTION

Since the end of the last century numerous studies on amphibian chromosomes have been made by many investigators, but there are only a few reports dealing with the chromosome behavior in spermatogenesis of inter- or intraspecific hybrids. WHITE (1946) has observed the behavior of meiotic chromosomes in a sterile male hybrid between two European newt species. CALLAN and SPURWAY (1951) have reported on the behavior of meiotic chromosomes in three kinds of male interracial hybrids of *Triturus cristatus*. GÜNTHER (1975) has made a study on the meiosis in male *Rana esculenta* which have recently been considered to be natural hybrids between *Rana ridibunda* and *Rana lessonae*.

Abnormal spermatogenesis in male hybrids between two closely allied frog species has been reported by a few Japanese authors (KAWAMURA, 1943; KAWAMURA and KOBAYASHI, 1959, 1960; MORIYA, 1959, 1960). However, none of them has described the behavior of chromosomes during meiosis in these male hybrids. Recently, OHTANI (1975) has observed the constitution and behavior of lampbrush chromosomes in oocytes of female hybrids between *Rana nigromaculata* and *Rana brevipoda*.

The present author produced reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda* and observed the first meiotic divisions in the testes of male hybrids in order to elucidate the behavior of chromosomes during spermatogenesis. The results of these observations are presented in this paper.

MATERIALS AND METHODS

Male and female *Rana nigromaculata* were collected from the suburbs of Hiroshima, while male and female *Rana brevipoda* were from Konko-cho, Okayama Prefecture. Reciprocal hybrids were produced by artificial insemination between these two species. Ovulation of females was accelerated by injection of frog

pituitary suspension. Embryos and tadpoles were reared in dechlorinated tap water. Tadpoles were fed boiled spinach or chard until the metamorphosing stage. Metamorphosed frogs were fed crickets.

Meiotic chromosomes in the testes of frogs at the age of 5~10 months were examined by the squash method after water pretreatment (MAKINO and NISHIMURA, 1952). The procedure was as follows: testes were cut into pieces about one cubic millimeter in size. These pieces were immersed in distilled water for 60~90 minutes and then stained with aceto-orcein (1% orcein in 45% acetic acid) for 60~90 minutes on a slide glass. Finally, they were squashed under a cover glass after heated for 20~30 seconds and sealed with PVLB*.

Analysis of meiotic chromosomes was made using enlarged photographs.

OBSERVATION

I. Meioses in male *Rana nigromaculata* and *Rana brevipoda*

Germ cells at various stages of spermatogenesis were found in the squash preparations of testes. Mitoses of spermatogonia as well as meioses of spermatocytes showed that there were a few aneuploid cells, tetraploid cells and polyploid cells having a greater number of chromosomes than tetraploid in addition to the

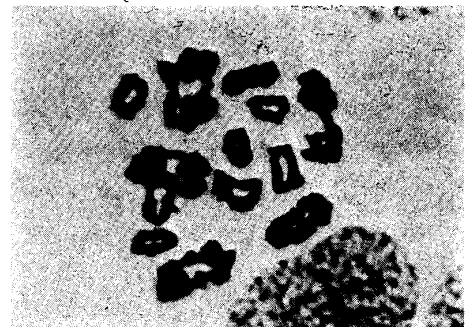
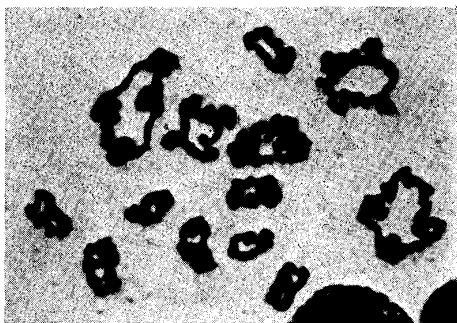


Fig. 1. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 5 large and 8 small bivalents in *Rana nigromaculata*. $\times 1050$

Fig. 2. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 5 large and 8 small bivalents in *Rana brevipoda*. $\times 1050$

* Paraffin, vaseline, lanolin, Canadian balsam=2: 1: 1: 1

TABLE 1
Frequency of various combinations of bivalents and univalents in the first meioses of male
Rana nigromaculata, *R. brevipoda* and reciprocal hybrids between these two species

Kind of frogs	Individual no.	No. of meioses	No. of univalents														
			0	2	4	6	8	10	12	14	16	18	20	22	24	26	
NN	76NN1	34	33	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	76NN2	37	25	12	0	0	0	0	0	0	0	0	0	0	0	0	0
	76NN3	33	30	3	0	0	0	0	0	0	0	0	0	0	0	0	0
	76NN4	46	41	4	1	0	0	0	0	0	0	0	0	0	0	0	0
	76NN5	88	88	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	76NN6	64	60	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	76NN7	55	45	7	2	0	0	0	0	1	0	0	0	0	0	0	0
	77NN1	114	102	12	0	0	0	0	0	0	0	0	0	0	0	0	0
	77NN2	47	46	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	78NN1	137	137	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	78NN2	153	149	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	78NN3	265	256	9	0	0	0	0	0	0	0	0	0	0	0	0	0
	78NN4	180	174	5	1	0	0	0	0	0	0	0	0	0	0	0	0
	78NN5	112	108	3	1	0	0	0	0	0	0	0	0	0	0	0	0
	78NN6	278	275	3	0	0	0	0	0	0	0	0	0	0	0	0	0
78NN7	262	254	7	1	0	0	0	0	0	0	0	0	0	0	0	0	
Total	1905	1823	75	6	0	0	0	0	1	0	0	0	0	0	0	0	
BB	77BB1	50	50	0	0	0	0	0	0	0	0	0	0	0	0	0	
	77BB2	45	38	7	0	0	0	0	0	0	0	0	0	0	0	0	
	77BB3	50	43	7	0	0	0	0	0	0	0	0	0	0	0	0	
	77BB4	52	49	1	0	0	0	0	1	0	0	1	0	0	0	0	
	77BB5	121	41	77	3	0	0	0	0	0	0	0	0	0	0	0	
	77BB6	98	85	10	3	0	0	0	0	0	0	0	0	0	0	0	
	77BB7	342	281	56	4	1	0	0	0	0	0	0	0	0	0	0	
	77BB8	32	15	17	0	0	0	0	0	0	0	0	0	0	0	0	
	77BB9	41	38	2	0	0	0	0	0	0	0	0	0	0	0	1	
	77BB10	55	48	5	2	0	0	0	0	0	0	0	0	0	0	0	
	77BB11	76	70	6	0	0	0	0	0	0	0	0	0	0	0	0	
	77BB12	57	51	4	1	0	0	0	0	0	0	0	0	1	0	0	
	77BB13	48	37	10	1	0	0	0	0	0	0	0	0	0	0	0	
Total	1067	846	202	14	1	0	0	1	0	0	1	0	1	0	1		
NB	78NB1	188	0	0	1	5	11	15	23	32	38	26	16	11	5	5	
	78NB2	220	0	1	1	7	17	30	37	44	34	26	9	10	2	2	
	78NB3	38	1	0	3	3	4	4	5	7	5	3	3	0	0	0	
	78NB4	144	0	1	0	15	31	28	29	22	10	4	4	0	0	0	
	78NB5	124	0	1	4	2	24	21	23	19	16	5	6	3	0	0	
	78NB6	73	0	2	3	6	9	18	8	11	7	3	4	0	1	1	
	78NB7	96	0	0	0	0	3	3	7	14	12	12	21	16	6	2	
	78NB8	74	0	0	1	1	3	13	8	10	14	17	3	4	0	0	
	78NB9	38	0	0	0	0	1	3	6	3	3	8	8	5	1	0	
	78NB10	115	0	1	5	11	21	28	23	8	9	8	1	0	0	0	
Total	1110	1	6	18	50	124	163	169	170	148	112	75	49	15	10		
BN	77BN1	270	0	1	3	14	20	41	39	56	27	31	23	9	6	0	
	77BN2	39	0	0	0	4	4	5	7	8	2	2	1	3	3	0	
	77BN3	37	0	0	0	0	4	2	7	5	7	4	4	2	2	0	
	77BN4	109	0	0	0	1	5	13	15	18	16	13	15	10	2	1	
	77BN5	53	0	0	0	2	3	4	6	15	14	3	5	1	0	0	
	77BN6	49	0	0	0	1	2	3	9	7	8	9	5	5	0	0	
	77BN7	75	0	1	2	4	9	17	12	10	10	3	4	3	0	0	
Total	632	0	2	5	26	47	85	95	119	84	65	57	33	13	1		

NN, *Rana nigromaculata* BB, *Rana brevipoda*
 NB, Hybrid, NN ♀ × BB ♂ BN, Hybrid, BB ♀ × NN ♂

normal diploid cells. Only diploid cells at the diakinesis and metaphase of the first reduction division were used for chromosome analysis, because bivalent and univalent chromosomes were most clearly distinguished from each other in these cells. The number of bivalent and univalent chromosomes in each of very numerous spermatocytes was counted. The results are presented in Table 1.

A total of 2972 meiotic spreads of spermatocytes obtained from 16 male *Rana nigromaculata* and 13 male *Rana brevipoda* were observed. It was found that most of the spermatocytes contained 13 bivalents in both *Rana nigromaculata* and *Rana brevipoda* (Figs. 1 and 2). The remaining spermatocytes contained 12 bivalents and 2 univalents and, besides, there were a very few spermatocytes containing less than twelve bivalents. The male *Rana nigromaculata* differed from the male *Rana brevipoda* in the scarcity of meiotic spreads consisting of 12 bivalents and 2 univalents.

1. *Rana nigromaculata*

A total of 1905 meiotic spreads from 16 male *nigromaculata* were observed. It was found that 1823 (95.7%) of them contained 13 bivalents which consisted of 5 large and 8 small ones (Fig. 1), while 75 (3.9%) contained 12 bivalents and 2 univalents. The latter meiotic spreads were not always found in the same frequency in each of the 16 frogs. They occurred in 0~32% of the respective total number of meiotic spreads, specifically, 0~10% in 13 frogs, 11~20% in two frogs and more than 20% in one frog. The two univalents were large

TABLE 2
Numbers of ring- and rod-shaped bivalents in *Rana nigromaculata*, *Rana brevipoda* and their reciprocal hybrids

Kind of frogs	No. of bivalents	Shape of bivalents	
		Ring	Rod
NN	24672	l. 8455	l. 1004
		s. 13775	s. 1438
		t. 22230	t. 2442
BB	13599	l. 3716	l. 1384
		s. 7941	s. 558
		t. 11657	t. 1942
NB	6932	l. 284	l. 1583
		s. 1545	s. 3520
		t. 1829	t. 5103
BN	3751	l. 148	l. 846
		s. 708	s. 2049
		t. 856	t. 2895

NN, *Rana nigromaculata*

BB, *Rana brevipoda*

NB, Hybrids, NN ♀ × BB ♂

BN, Hybrids, BB ♀ × NN ♂

l., Number of large chromosomes

s., Number of small chromosomes

t., Total number

chromosomes in 57 of the 75 spreads, while they were small chromosomes in the 18 spreads. Of the meiotic spreads analyzed, the remaining seven (0.4%) differed from the others in the number of bivalents. Six of them contained 11 bivalents and 4 univalents and the remainder had 7 bivalents and 12 univalents. As a total of 24672 bivalents were found in the 1905 meiotic spreads from 16 male *nigromaculata*, the mean number of bivalents per meiotic spread was calculated to be 12.95 in *Rana nigromaculata*.

The bivalents were sorted into two kinds in shape. One of them was a ring-shaped bivalent in which two homologous chromosomes were united at their two ends, while the other was a rod-shaped one in which two homologous chromosomes were united at one end. A total of 22230 (90.1%) bivalents observed in *Rana nigromaculata* were ring-shaped, while the other 2442 (9.9%) bivalents were rod-shaped (Table 2).

2. *Rana brevipoda*

A total of 1067 meiotic spreads from 13 male *brevipoda* were analyzed and it was found that 846 (79.3%) of them contained 13 bivalents (Table 1) which consisted of 5 large and 8 small ones (Fig. 2). Of the other spreads, 202 (18.9%) contained 12 bivalents and 2 univalents. The frequency of this kind of meiotic spreads varied from individual to individual; it was 0~64% of the respective total number of meiotic spreads. Specifically, it was 0~10% in 6, 10~20% in 4 and more than 20% in the remaining 3 individuals. The two univalents were large chromosomes in 193 spreads.

Fourteen meiotic spreads contained 11 bivalents and 4 univalents, one contained 10 bivalents and 6 univalents, one contained 7 bivalents and 12 univalents, one contained 4 bivalents and 18 univalents, one contained 2 bivalents and 22 univalents, and the remainder contained 26 univalents. As 13599 bivalents were found in 1067 meiotic spreads from 13 male *brevipoda*, the mean number of bivalents per spread was calculated to be 12.74 in *Rana brevipoda*. A total of 11657 (85.7%) bivalents were ring-shaped, while the other 1942 were rod-shaped (Table 2).

II. Meioses in male hybrids between *Rana nigromaculata* and *Rana brevipoda*

The spermatogenesis in male hybrids between *Rana nigromaculata* and *Rana brevipoda* remarkably differed from that in male *Rana nigromaculata* or *Rana brevipoda*. In squash preparations of the testes of reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda*, a great number of meiotic figures at the diakinesis or metaphase of the first reduction division were observed. There were also some mitotic figures of spermatogonia. In contrast, second reduction divisions were scarce, and there were a few large spermatozoa which were abnormal in shape. While most of the germ cells were diploid, there were a small number of aneuploid cells, tetraploid cells and some other polyploid cells having more numerous chromosomes than tetraploid.

A total of 1742 meiotic spreads at the diakinesis or metaphase of the first reduc-

tion division obtained from 17 males of reciprocal hybrids were observed. It was found that slightly less than half of the number of meiotic chromosomes formed bivalents and the others remained unpaired. The meiotic spreads of reciprocal hybrids were sorted into 14 kinds in chromosome constitution. These kinds of meiotic spreads were as follows: the first contained 13 bivalents, the second 12 bivalents and 2 univalents, the third 11 bivalents and 4 univalents, the fourth 10 bivalents and 6 univalents, the fifth 9 bivalents and 8 univalents, the sixth 8 bivalents and 10 univalents, the seventh 7 bivalents and 12 univalents, the eighth 6 bivalents and 14 univalents, the ninth 5 bivalents and 16 univalents, the tenth 4 bivalents and 18 univalents, the eleventh 3 bivalents and 20 univalents, the twelfth 2 bivalents and 22 univalents, the thirteenth one bivalent and 24 univalents and the last 26 univalents. Moreover, each of these kinds of meiotic spreads was mostly subdivided into several groups in accordance with the difference in the combination of large and small bivalent chromosomes.

Among these meiotic spreads, those containing 6 bivalents and 14 univalents were the most numerous. The meiotic spreads containing 7 bivalents and 12 univalents, those containing 8 bivalents and 10 univalents, and those containing 5 bivalents and 16 univalents were the second, third and fourth in frequency, respectively. These four kinds occupied about 60% of the total number of meiotic spreads observed. On the other hand, there was only one meiotic spread containing 13 bivalents among the 1742 spreads from the 17 male reciprocal hybrids. The meiotic spreads containing 12 bivalents and 2 univalents were the next in fewness. Those containing 26 univalents were also very scarce. These three kinds of meiotic spreads occupied only about 1% of the total number of meiotic spreads observed. There seemed to be no essential difference in the behavior of meiotic chromosomes between reciprocal hybrids.

1. Hybrids between female *Rana nigromaculata* and male *Rana brevipoda*

A total of 1110 meiotic spreads from 10 male hybrids between female *nigromaculata* and male *brevipoda* were analyzed (Table 1). The meiotic spreads containing 6 bivalents and 14 univalents were the most numerous. This kind of meiotic spread was not always most frequently found in each male hybrid. While they were the most numerous in two of 10 males, those containing 9 bivalents and 8 univalents and those containing 8 bivalents and 10 univalents were the most numerous in two and two males, respectively. Meiotic spreads containing 5 bivalents and 16 univalents, those containing 4 bivalents and 18 univalents and those containing 3 bivalents and 20 univalents were the most numerous in one male, respectively. Meiotic spreads of the last two kinds were the most numerous in the remaining male.

As stated above, meiotic spreads containing 6 bivalents and 14 univalents were the most numerous among those observed in the ten male hybrids, that is 170 (15.3%). These meiotic spreads were further subdivided into 5 groups on the basis of differences in the combination of large and small bivalent chromosomes. The results of observation showed that the six bivalents consisted of one large and

5 small chromosomes in 63 spreads, two large and 4 small chromosomes in 59 spreads, 3 large and 3 small chromosomes in 23 spreads, 6 small chromosomes in 23 spreads, and 4 large and 2 small chromosomes in the remaining 2 spreads.

Meiotic spreads containing 7 bivalents and 12 univalents numbered 169 (15.2%) and were subdivided into five groups on the basis of differences in the combinations of large and small bivalent chromosomes. It was found that the seven bivalents consisted of 2 large and 5 small chromosomes in 71 spreads, one large and 6 small chromosomes in 58 spreads, 3 large and 4 small chromosomes in 28 spreads, 4 large and 3 small chromosomes in 6 spreads, and 7 small chromosomes in the remaining 6 spreads. Meiotic spreads containing 8 bivalents and 10 univalents numbered 163 (14.7%) and were subdivided into 6 groups. The eight bivalents consisted of 2 large and 6 small chromosomes in 65 spreads, 3 large and 5 small chromosomes in 62 spreads, one large and 7 small chromosomes in 26 spreads, 4 large and 4 small chromosomes in 7 spreads, 8 small chromosomes in 2 spreads, and 5 large and 3 small chromosomes in the remaining one spread.

Meiotic spreads containing 5 bivalents and 16 univalents numbered 148 (13.3%) and were subdivided into five groups. The five bivalents consisted of one large and 4 small chromosomes in 72 spreads, 2 large and 3 small chromosomes in 35 spreads, 5 small chromosomes in 30 spreads, 3 large and 2 small chromosomes in 10 spreads, and 4 large and one small chromosome in the remaining one spread. Meiotic spreads containing 9 bivalents and 8 univalents numbered 124 (11.2%) and were subdivided into 4 groups. The nine bivalents consisted of 3 large and 6 small chromosomes in 57 spreads, 2 large and 7 small chromosomes in 39 spreads, 4 large and 5 small chromosomes in 15 spreads (Fig. 3), and one large and 8 small chromosomes in the remaining 13 spreads. Meiotic spreads containing 4 bivalents and 18 univalents numbered 112 (10.1%) and were subdivided into four groups. The four bivalents consisted of one large and 3 small chromosomes in 60 spreads, 4 small chromosomes in 29 spreads, 2 large and 2 small chromosomes in 21 spreads, and 3 large and one small chromosome in the remaining 2 spreads. Meiotic spreads containing 3 bivalents and 20 univalents numbered 75 (6.8%) and were subdivided into 4 groups. The three bivalents consisted of 3 small chromosomes in 33 spreads, one large and 2 small chromosomes in 31 spreads, 2 large and one small chromosome in 10 spreads, and 3 large chromosomes in the remaining one spread.

Meiotic spreads containing 10 bivalents and 6 univalents numbered 50 (4.5%) and were divided into 4 groups. The ten bivalents consisted of 3 large and 7 small chromosomes in 26 spreads, 2 large and 8 small chromosomes in 14 spreads, 4 large and 6 small chromosomes in 9 spreads, and 5 large and 5 small chromosomes in the remaining one spread. Meiotic spreads containing 2 bivalents and 22 univalents numbered 49 (4.4%) and were divided into 3 groups. The two bivalents consisted of small chromosomes in 30 spreads, one large and one small chromosome in 17 spreads, and 2 large chromosomes in the remaining two spreads. Meiotic spreads containing 11 bivalents and 4 univalents numbered 18 (1.6%) and were divided into three groups. The eleven bivalents consisted of

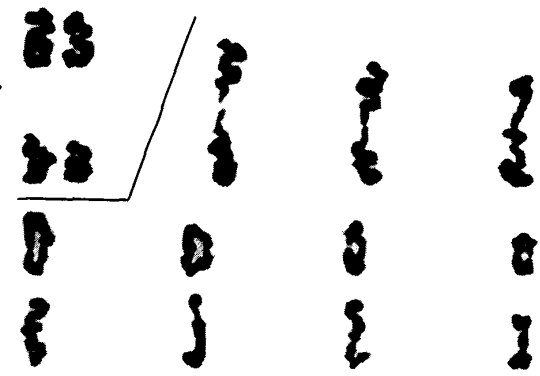
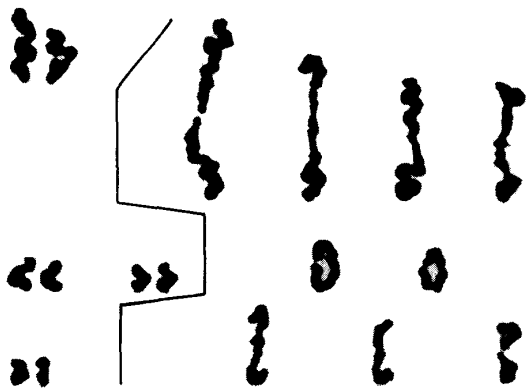
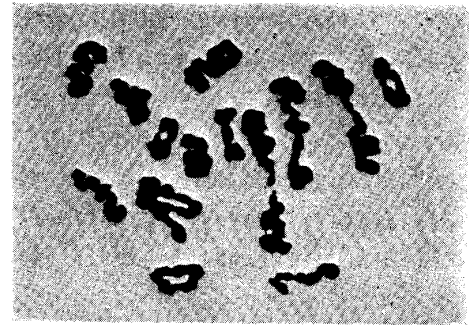
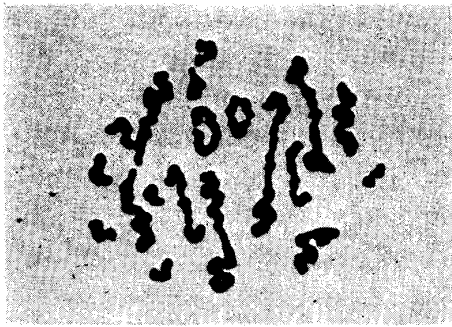


Fig. 3. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 4 large and 5 small bivalents and 8 univalents in a male hybrid, *Rana nigromaculata* ♀ × *R. brevipoda* ♂. ×1050

Fig. 4. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 3 large and 8 small bivalents and 4 univalents in a male hybrid, *Rana nigromaculata* ♀ × *R. brevipoda* ♂. ×1050

4 large and 7 small chromosomes in 9 spreads, 3 large and 8 small chromosomes in 8 spreads (Fig. 4), and 5 large and 6 small chromosomes in the remaining one spread. Meiotic spreads containing one bivalent and 24 univalents numbered 15 (1.4%) and were divided into two groups. The single bivalent was a small chromosome in 12 spreads, while it was a large chromosome in the other 3 spreads. Meiotic spreads containing 26 univalents (Fig. 5) numbered 10 (0.9%). Meiotic spreads containing 12 bivalents and 2 univalents numbered 6 (0.5%) and were divided into two groups. The twelve bivalents consisted of 4 large and 8 small chromosomes in 5 spreads, and 5 large and 7 small chromosomes in the other spread. There was only one spread which contained 13 bivalents alone (Fig. 6).

As a total of 6932 bivalents were contained in the 1110 meiotic spreads from the 10 male hybrids between female *nigromaculata* and male *brevipoda*, the mean number of bivalents per spread was calculated to be 6.24 in this kind of hybrids. Of the bivalents, 1829 (26.4%) were ring-shaped, while the other 5103 (73.6%) were rod-shaped (Table 2).

2. Hybrids between female *Rana brevipoda* and male *Rana nigromaculata*

A total of 632 meiotic spreads from 7 male hybrids between female *brevipoda*

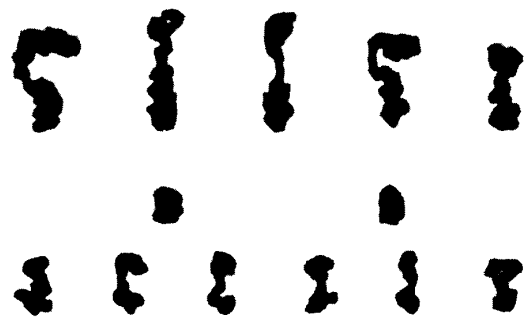
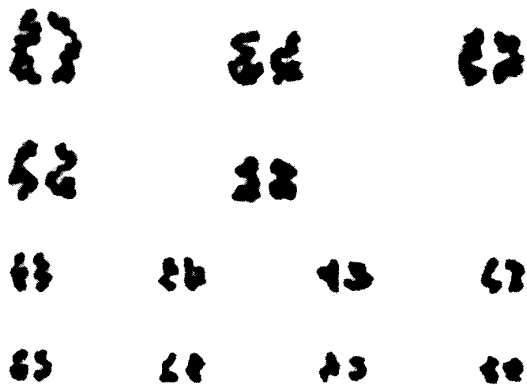
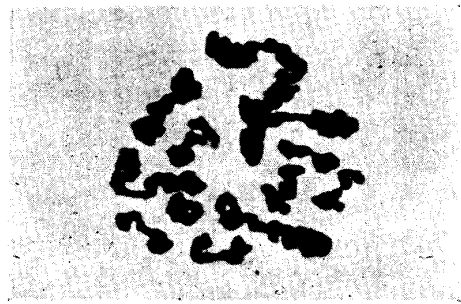
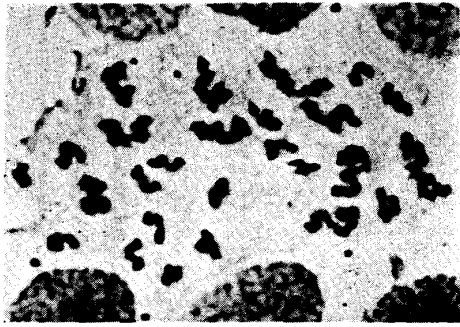


Fig. 5. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 26 univalents in a male hybrid, *Rana nigromaculata* ♀ × *R. brevipoda* ♂. × 1050

Fig. 6. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 5 large and 8 small bivalents in a male hybrid, *Rana nigromaculata* ♀ × *R. brevipoda* ♂. × 1050

and male *nigromaculata* were observed (Table 1). Although meiotic spreads containing 6 bivalents and 14 univalents were the most numerous in these male hybrids, they were not always the most numerous in each male. This kind of meiotic spread was the most numerous in four males, while in another male, meiotic spreads containing 8 bivalents and 10 univalents were the most numerous. In still another male, those containing 5 bivalents and 16 univalents and those containing 7 bivalents and 12 univalents were the most numerous. In the remaining male hybrid, meiotic spreads containing 4 bivalents and 18 univalents and those containing 7 bivalents and 12 univalents were the most numerous.

As stated above, meiotic spreads containing 6 bivalents and 14 univalents were the most numerous in the 7 male hybrids; they numbered 119 (18.8%) and were subdivided into 4 groups on the basis of differences in the combination of large and small bivalent chromosomes. The six bivalents consisted of one large and 5 small chromosomes in 54 spreads (Fig. 7), 2 large and 4 small chromosomes in 44 spreads, 3 large and 3 small chromosomes in 13 spreads, and 6 small chromosomes in the remaining 8 spreads.

Meiotic spreads containing 7 bivalents and 12 univalents numbered 95 (15.0%) and were subdivided into 5 groups. The seven bivalents consisted of 2 large and 5 small chromosomes in 44 spreads, one large and 6 small chromosomes in 25

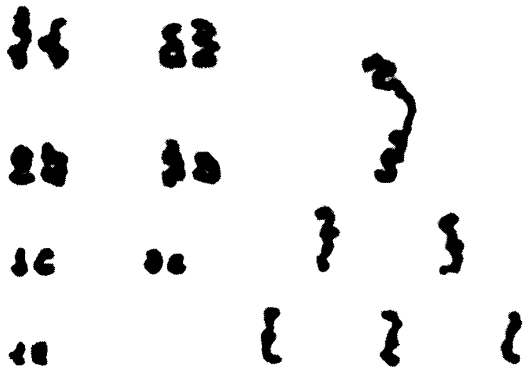
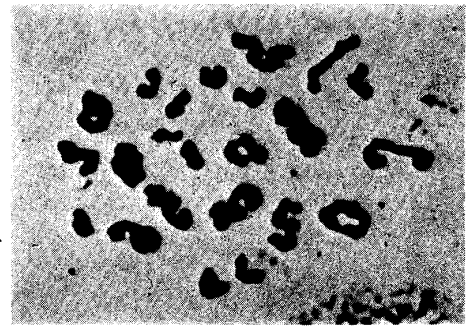
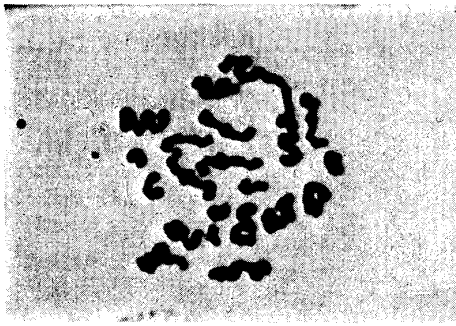


Fig. 7. Spread of a spermatocyte at the first meiosis and the chromosome complement containing one large and 5 small bivalents and 14 univalents in a male hybrid, *Rana brevipoda* ♀ × *R. nigromaculata* ♂. ×1050

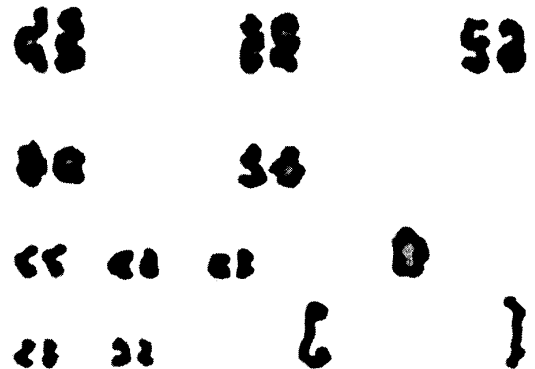


Fig. 8. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 3 small bivalents and 20 univalents in a male hybrid, *Rana brevipoda* ♀ × *R. nigromaculata* ♂. ×1050

spreads, 3 large and 4 small chromosomes in 21 spreads, 4 large and 3 small chromosomes in 3 spreads, and 7 small chromosomes in the remaining 2 spreads. Meiotic spreads containing 8 bivalents and 10 univalents numbered 85 (13.4%) and were divided into 5 groups. The eight bivalents consisted of 2 large and 6 small chromosomes in 33 spreads, 3 large and 5 small chromosomes in 25 spreads, one large and 7 small chromosomes in 16 spreads, 4 large and 4 small chromosomes in 9 spreads, and 8 small chromosomes in the remaining 2 spreads.

Meiotic spreads containing 5 bivalents and 16 univalents numbered 84 (13.3%) and were divided into 5 groups. The five bivalents consisted of one large and 4 small chromosomes in 35 spreads, 2 large and 3 small chromosomes in 28 spreads, 5 small chromosomes in 17 spreads, 3 large and 2 small chromosomes in 3 spreads, and 4 large and one small chromosome in the remaining one spread. Meiotic spreads containing 4 bivalents and 18 univalents numbered 65 (10.3%) and were divided into 3 groups. The four bivalents consisted of one large and 3 small chromosomes in 27 spreads, 4 small chromosomes in 25 spreads, and 2 large and 2 small chromosomes in the remaining 13 spreads. Meiotic spreads containing 3 bivalents and 20 univalents numbered 57 (9.0%) and were divided into 3 groups. The three bivalents consisted of one large and 2 small chromosomes in 28 spreads, 3 small chromosomes in 27 spreads (Fig. 8), and 2 large and

one small chromosome in the remaining 2 spreads.

Meiotic spreads containing 9 bivalents and 8 univalents numbered 47 (7.4%) and were divided into 4 groups. The nine bivalents consisted of 2 large and 7 small chromosomes in 22 spreads, 3 large and 6 small chromosomes in 18 spreads, 4 large and 5 small chromosomes in 4 spreads, and one large and 8 small chromosomes in the remaining 3 spreads. Meiotic spreads containing 2 bivalents and 22 univalents numbered 33 (5.2%) and were divided into 3 groups. The two bivalents were all small chromosomes in 22 spreads, one large and one small chromosome in 9 spreads, and 2 large chromosomes in the remaining 2 spreads. Meiotic spreads containing 10 bivalents and 6 univalents numbered 26 (4.1%) and were divided into 3 groups. The ten bivalents consisted of 3 large and 7 small chromosomes in 18 spreads, 4 large and 6 small chromosomes in 6 spreads, and 2 large and 8 small chromosomes in the remaining 2 spreads. Meiotic spreads containing one bivalent and 24 univalents numbered 13 (2.1%). The single bivalent was a small chromosome in 10 spreads, while it was a large chromosome in the other 3 spreads.

Meiotic spreads containing 11 bivalents and 4 univalents numbered 5 (0.8%) and were divided into 3 groups. The eleven bivalents consisted of 4 large and 7 small chromosomes in 3 spreads, 3 large and 8 small chromosomes in one spread, and 5 large and 6 small chromosomes in the remaining spread. Meiotic spreads containing 12 bivalents and 2 univalents numbered 2 (0.3%). The twelve bivalents consisted of 4 large and 8 small chromosomes in these two spreads. Only one meiotic spread contained 26 univalents alone. There was no meiotic spread containing 13 bivalents.

As a total of 3751 bivalents were contained in the 632 meiotic spreads from the 7 male hybrids between female *brevipoda* and male *nigromaculata*, the mean number of bivalents per spread was calculated to be 5.94 in this kind of hybrids. While 856 (22.8%) bivalents were of ring-shape, the other 2895 (77.2%) were of rod-shape (Table 2).

DISCUSSION

1. Meiotic chromosomes of *Rana nigromaculata* and *Rana brevipoda*

The number of chromosomes of *Rana nigromaculata* was reported by IRIKI (1932) to be 26 in spermatogonia and 13 in primary spermatocytes. The karyotypes of *Rana nigromaculata* and *Rana brevipoda* were clarified by NISHIOKA (1972). Each genome of these two species consisted of 5 large and 8 small chromosomes. In the present study, it was found that 1823 (95.7%) of 1905 meiotic spreads in *Rana nigromaculata* and 846 (79.3%) of 1067 meiotic spreads in *Rana brevipoda* contained 13 bivalents. Of the remaining meiotic spreads, 75 (3.9%) in *nigromaculata* and 202 (18.9%) in *brevipoda* contained 12 bivalents and 2 univalents, and six in *nigromaculata* and 14 in *brevipoda* contained 11 bivalents and 4 univalents. A similar finding was made by WHITE (1946) in *Triturus cristatus*. While there

were usually 12 bivalents in primary spermatocytes, a small number of meiotic spreads contained 11 bivalents and 2 univalents.

On the other hand, a large bivalent chromosome peculiar in shape and behavior was found in the first meiotic divisions of some spermatocytes in *Hyla arborea japonica*, *Rana rugosa* and *Rana nigromaculata* by IRIKI (1930, 1932), *Rhacophorus schlegelii*, *Rana chensinensis* and *Bufo sachalinensis* by MAKINO (1932a, b), *Bufo bufo japonicus* by MINOUCHI and IRIKI (1931), and five *Bufo* species from North America by WITSCHI (1933). This chromosome was of open V-shape and stood vertically to the equatorial plate at metaphase, in contrast to the other bivalents which were of ring-shape or dumbbell-shape, and preceded to the poles before the others at the anaphase. The present author observed that the two univalents found in the first meiotic division were large chromosomes in 57 of 75 spreads of *nigromaculata* and 193 of 202 spreads of *brevipoda*. It is very probable that the two univalents correspond to the peculiar chromosome observed by the earlier authors, and moreover, that the spermatocytes containing two small univalent chromosomes and those containing four or more univalents became abnormal during spermatogenesis and degenerated sooner or later, or became abnormal spermatozoa which were deficient in reproductive capacity.

2. Meiotic chromosomes of hybrids between *Rana nigromaculata* and *Rana brevipoda*

MORIYA (1951, 1960) reported that reciprocal hybrids between the two closely allied species, *Rana nigromaculata* and *Rana brevipoda* were easily produced by artificial fertilization, and that male hybrids were almost completely sterile, while female hybrids were fertile to a large extent. These findings have been repeatedly confirmed by KAWAMURA and NISHIOKA (1975, 1977, 1978).

The chromosome constitution in the first meiotic division in male hybrids between *Rana nigromaculata* and *Rana brevipoda* differed distinctly from that in males of the parental species. In reciprocal hybrids, there were 14 kinds of chromosome constitutions in which the bivalents varied from 0 to 13 in number. Among various meiotic spreads, those containing 6 bivalents and 14 univalents were the highest in frequency. When the bivalents increased or decreased in number, the meiotic spreads gradually decreased in frequency. The meiotic spreads containing 5, 6, 7 or 8 bivalents occupied about 60% of the total number of meiotic spreads analyzed in reciprocal hybrids. The mean number of bivalents per nucleus was 6.24 in the hybrids between female *nigromaculata* and male *brevipoda* while 5.94 in the reciprocal hybrids. These findings are very similar to those by WHITE (1946) in the hybrids between female *Triturus marmoratus* and male *Triturus cristatus carnifex*. According to WHITE, the chromosomes of these two newt species were $2n=24$, and the meiotic spreads at the "metaphase-anaphase" of the first reduction division contained bivalents and univalents. There were 8 kinds of meiotic spreads in which the bivalents varied from 2 to 9 in number. The meiotic spreads containing 4, 5 or 6 bivalents were the most numerous among them. They occupied more than 75% of the total number of meiotic spreads

examined. The bivalents per cell were 5.14 in mean number.

Rana esculenta distributed widely in Europe are recently assumed by many authors to be natural hybrids between *Rana ridibunda* and *Rana lessonae* (BERGER, 1964; GÜNTHER, 1967, 1973; TUNNER, 1973; UZZELL and BERGER, 1975; etc.). According to GÜNTHER (1975), the meioses of male *esculenta* were abnormal in various degrees. The main differences in meioses of males between *esculenta* and the hybrids between *nigromaculata* and *brevipoda* were as follows: first, while there were normal meiotic figures in various frequency in almost all individuals of *esculenta*, there was only one normal meiotic figure in 1742 spreads from 17 hybrids between *nigromaculata* and *brevipoda*. Second, while many first meiotic spreads having 13 univalents were found in *esculenta*, there were no such meiotic spreads in reciprocal hybrids between *nigromaculata* and *brevipoda*. Third, nearly all of the first meioses in male hybrids between *nigromaculata* and *brevipoda* contained a mixture of univalents and bivalents, while those of male *esculenta* were normal, or abnormal in a greater or lesser degrees, that is, they showed aneuploidy, polyploidy, breakage, fragmentation or unstable pairing of chromosomes, homologous trivalents, heterogous bi-, tri-, tetra- or multivalents, or some other abnormalities. These differences in meioses of males between *esculenta* and the hybrids of the two Japanese pond frog species may be attributable to the fact that *esculenta* are not simple interspecific hybrids, but are complicated backcrosses.

The meioses of male hybrids between *nigromaculata* and *brevipoda* differed largely in the behavior of chromosomes from that of female hybrids. According to OHTANI (1975) who studied on the lampbrush chromosomes in the eggs of female *nigromaculata*, *brevipoda*, and reciprocal hybrids between them, the eggs of the hybrids usually had 13 bivalents. In contrast, various number from none to all of the 13 homologous pairs of chromosomes did not form bivalents in the primary spermatocytes of male hybrids. Moreover, there seemed to be no homologous chromosomes which were more prone to form bivalents than the others. In other words, the pairing of homologous chromosomes seemed to occur at random. This is assumed from the facts that there were as many as 14 kinds of meiotic spreads in a number of bivalents and that the meiotic spreads containing the same number of bivalents were sorted into several groups on the basis of differences in the combination of large and small bivalents.

There were two kinds of bivalents, ring-shape and rod-shape, in the meiotic spreads at the diakinesis or metaphase of male *nigromaculata*, *brevipoda* and reciprocal hybrids. While the ring-shaped bivalents were far more numerous than the rod-shaped ones in *nigromaculata* and *brevipoda*, the rod-shaped bivalents were more numerous than the ring-shaped ones in the hybrids. This finding together with the finding that the hybrids contained far more numerous univalents than the parental species may correspond well with the findings by WHITE (1946), CALLAN and SPURWAY (1951) and OHTANI (1975) that the frequency of chiasmata per nucleus was remarkably lower in interspecific or interracial hybrids of newts or frogs than that in the parental species.

MORIYA (1960) stated that the primary spermatocytes of reciprocal hybrids

between *nigromaculata* and *brevipoda* mostly degenerated at the anaphase of the first reduction division, owing to abnormalities such as non-disjunction of chromosomes and formation of chromosome bridges. A few of them could develop into large, abnormal spermatozoa, probably without passing through the second maturation division. It was found in the present study that all but one of the meiotic spreads in reciprocal hybrids between *nigromaculata* and *brevipoda* contained 2~26 univalents. The abnormality in the spermatogenesis of these hybrids must be attributable to the occurrence of univalents.

SUMMARY

1. The behavior of chromosomes at the diakinesis or metaphase of the first reduction division of spermatocytes in *Rana nigromaculata*, *Rana brevipoda* and reciprocal hybrids between the two species was observed by the squash method after water pretreatment.

2. In *Rana nigromaculata* and *Rana brevipoda*, each pair of homologous chromosomes usually formed bivalents in the first reduction division. Thus, the meiotic spreads mostly contained 13 bivalents. Most of the others contained 12 bivalents and 2 univalents. The mean number of bivalents per nucleus was 12.95 in *Rana nigromaculata*, while 12.74 in *Rana brevipoda*.

3. In reciprocal hybrids between *nigromaculata* and *brevipoda*, a little less than half of the pairs of homologous chromosomes formed bivalents, while the others remained as univalents. There were 14 kinds of meiotic spreads in the combination of bivalents and univalents. The number of bivalents contained in each kind of meiotic spreads varied from 0 to 13. Of the meiotic spreads, those containing 6 bivalents and 14 univalents were the highest in frequency. When the bivalents increased or decreased in number, the meiotic spreads gradually decreased in frequency. The four most numerous kinds of meiotic spreads containing 5, 6, 7 or 8 bivalents occupied about 60% of the total number of meiotic spreads analyzed. The three fewest kinds of meiotic spreads containing 0, 12 or 13 bivalents occupied only about 1% of the total number. The mean number of bivalents per nucleus was 6.24 in the hybrids between female *nigromaculata* and male *brevipoda*, while 5.94 in the reciprocal hybrids. Each of the 14 kinds of meiotic spreads was subdivided into several kinds on the basis of differences in the combination of large and small bivalent chromosomes. It was assumed that the pairing of homologous chromosomes occurred at random in the hybrids.

4. There were two kinds of bivalents in shape, that is, ring-shaped and rod-shaped. While the ring-shaped bivalents were far more numerous than the rod-shaped ones in *nigromaculata* and *brevipoda*, the rod-shaped bivalents were far more numerous than the ring-shaped ones in the hybrids between them.

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