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

II. Auto- and Allotriploids in *Rana nigromaculata* and *Rana brevipoda*

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(With 10 Text-figures)

INTRODUCTION

In a previous paper, the present author (1980) reported on the behavior of chromosomes at diakinesis or metaphase of the first reduction division of spermatocytes in *Rana nigromaculata, Rana brevipoda* and reciprocal hybrids between the two species. It was found that the mean number of bivalents per nucleus was 6.24 in the hybrids between female *Rana nigromaculata* and male *Rana brevipoda* and 5.94 in the reciprocal hybrids, while it was 12.95 and 12.74 in *Rana nigromaculata* and *Rana brevipoda*, respectively.

Formation of trivalents, bivalents and univalents in the first meiotic metaphase of male autotriploids has been reported by BÖÖK (1940, 1945) in *Triturus vulgaris*, by KAWAMURA (1941a) in *Rana nigromaculata* and by BEETSCHEN (1960) in *Pleurodeles waltl.* On the other hand, appearance of no or only a few trivalents besides many bivalents and univalents in the first meiotic metaphase has been reported by KAWAMURA (1952a) in male allotriploids between *Rana japonica* and *Rana ornativentris*, by BEÇAK and BEÇAK (1970) in those between female tetraploid *Odontophrynus americanus* and male diploid *Odontophrynus cultripes* and by GÜNTHER (1975a) in male triploid *Rana esculenta* which were assumed to be allotriploids between *Rana ridibunda* and *Rana lessonae.* However, no detailed analysis has so far been made on chromosomes appearing in the first meiosis of spermatocytes of auto- or allotriploid amphibians.

The present author produced many mature autotriploids in two Japanese pond frog species, *Rana nigromaculata* and *Rana brevipoda*, as well as many mature reciprocal allotriploids between these two species. The divisions of spermatocytes in these auto- and allotriploids were observed by the squash method in order to elucidate the behavior of chromosomes in the first meiosis. The results of observation will be presented here.

MATERIALS AND METHODS

Mature males and females of *Rana nigromaculata* were collected from suburbs of Hiroshima, while those of *Rana brevipoda* were collected from Konko-cho, Okayama Prefecture. Ovulation was accelerated by injecting frog pituitaries. Eggs were fertilized with sperm whose suspension had been made by crushing a piece of testis in a small quantity of tap-water. Auto- or allotriploids were produced as follows. Eggs of *Rana nigromaculata* or *Rana brevipoda* were refrigerated at 0.5 ~ 2°C for 2.5 ~ 3 hours, 20 ~ 25 minutes after they were inseminated with sperm of their own or foreign species (Nishioka, 1971, 1972). The refrigerated eggs were developed at room temperature until they became 5 to 10-month-old frogs.

Meiotic chromosomes in the testes of triploid frogs were observed by the squash method after water pretreatment (Makino and Nishimura, 1952). The detailed procedure was the same as that described in a previous paper (Okumoto, 1980). Analysis of meiotic chromosomes was performed by using enlarged photographs.

OBSERVATION

I. Autotriploids

In squash preparations of the testes of triploid *Rana nigromaculata* and triploid *Rana brevipoda*, there were a great number of meiotic figures of first spermatocytes together with a small number of mitotic figures of spermatogonia, while meiotic figures of the second reduction division were very scarce. It was found by counting the chromosomes of these meiotic or mitotic figures that most of the germ cells were triploid, although there were a small number of germ cells having aneuploid, hexaploid or more numerous chromosomes. In the present study, the chromosomes of first spermatocytes at the diakinesis or metaphase were exclusively analyzed, as trivalents, bivalents and univalents were most definitely distinguished from one another at these stages.

A total of 1195 meiotic spreads obtained from 8 male triploid *Rana nigromaculata* and 10 male triploid *Rana brevipoda* were analyzed (Table 1). Almost all of these meiotic spreads consisted of a mixture of trivalent, bivalent and univalent chromosomes, although they differed from one another in combination of these chromosomes. The meiotic spreads were divided into 13 kinds on the basis of differences in the number of trivalents which varied from 0 to 13 except one. The most numerous meiotic spreads contained 8 trivalents, while those containing 6, 7, 8 or 9 trivalents occupied more than 70% of the total number of meiotic spreads analyzed. A single meiotic spread consisted of 13 trivalents among the 1195 spreads. The meiotic spreads which were 0, 2, 3 or 13 in number of trivalents occupied less than 1% of the total number of meiotic spreads analyzed.

Each kind of meiotic spreads was generally divided into several groups on the basis of differences in combination of large and small trivalent chromosomes.

TABLE 1

Number of meiotic spreads differing in number of trivalents in male auto- and allotriploids

Kind of frogs	Individual no.	No. of meioses	Number of meiotic spreads containing 0∼13 trivalents													
			0	1	2	3	4	5	6	7	8	9	10	11	12	13
NNN	78NNN1	82	0	0	0	0	1	3	7	10	15	25	10	7	4	0
	78NNN2	57	0	0	1	0	1	3	9	14	11	10	6	0	2	0
	78NNN3	135	0	0	0	1	2	9	19	24	28	28	14	8	2	0
	78NNN4	41	0	0	0	0	0	1	3	6	12	9	7	2	1	0
	78NNN5	47	0	0	0	0	1	2	5	9	8	12	5	5	0	0
	78NNN6	26	0	0	0	0	0	2	4	3	6	4	4	2	0	1
	78NNN7	108	0	0	1	1	2	6	17	17	21	25	11	5	2	0
	78NNN8	357	0	0	0	0	12	19	48	63	76	64	47	22	6	0
	Total	853	0	0	2	2	19	45	112	146	177	177	104	51	17	1
BBB	77BBB1	15	0	0	0	0	1	1	4	2	5	0	1	0	1	0
	77BBB2	32	1	0	0	0	1	2	4	11	4	6	2	1	0	0
	77BBB3	19	2	0	0	0	0	1	2	6	4	2	2	0	0	0
	77BBB4	59	0	0	0	0	2	6	12	12	11	8	6	2	0	0
	77BBB5	29	0	0	0	1	0	2	4	8	6	6	2	0	0	0
	77BBB6	16	0	0	0	0	0	3	1	2	6	0	1	3	0	0
	77BBB7	57	0	0	0	0	1	6	5	9	11	10	4	8	3	0
	77BBB8	26	0	0	0	0	0	3	3	6	4	4	3	2	1	0
	77BBB9	38	0	0	0	0	2	3	9	11	6	5	2	0	0	0
	77BBB10	51	1	0	1	0	2	4	6	10	14	8	3	1	1	0
	Total	342	4	0	1	1	9	31	50	77	71	49	26	17	6	0
NNB	78NNB1	82	23	16	22	10	7	4	0	0	0	0	0	0	0	0
	78NNB2	19	4	5	6	2	1	0	1	0	0	0	0	0	0	0
	78NNB3	33	4	13	7	4	3	1	1	0	0	0	0	0	0	0
	78NNB4	66	22	21	13	7	3	0	0	0	0	0	0	0	0	0
	78NNB5	74	21	32	15	5	1	0	0	0	0	0	0	0	0	0
	78NNB6	89	18	26	19	15	7	3	1	0	0	0	0	0	0	0
	78NNB7	30	8	12	8	2	0	0	0	0	0	0	0	0	0	0
	78NNB8	79	13	13	24	18	7	4	0	0	0	0	0	0	0	0
	78NNB9	22	5	7	7	3	0	0	0	0	0	0	0	0	0	0
	78NNB10	20	3	6	7	3	0	1	0	0	0	0	0	0	0	0
	Total	514	121	151	128	69	29	13	3	0	0	0	0	0	0	0
BBN	77BBN1	37	13	15	7	2	0	0	0	0	0	0	0	0	0	0
	77BBN2	94	33	33	20	7	1	0	0	0	0	0	0	0	0	0
	77BBN3	19	7	5	5	1	1	0	0	0	0	0	0	0	0	0
	77BBN4	18	11	4	1	2	0	0	0	0	0	0	0	0	0	0
	77BBN5	23	13	4	5	1	0	0	0	0	0	0	0	0	0	0
	77BBN6	47	19	22	4	1	1	0	0	0	0	0	0	0	0	0
	77BBN7	15	5	4	4	2	0	0	0	0	0	0	0	0	0	0
	Total	253	101	87	46	16	3	0	0	0	0	0	0	0	0	0

NNN, autotriploid *Rana nigromaculata* BBB, autotriploid *Rana brevipoda* NNB, allotriploid between female *nigromaculata* and male *brevipoda* BBN, allotriploid between female *brevipoda* and male *nigromaculata*

Bivalents were equal to univalents in number in 1105 (92.5%) of the total meiotic spreads, while univalents were more numerous than bivalents in the other 89 (7.4%) spreads. In almost all the latter, univalents were more numerous than bivalents by three. Triploid *Rana nigromaculata* somewhat differed from triploid *Rana brevipoda* in the most frequent meiotic spreads. Furthermore, the frequency

(15.5%) of meiotic spreads in which univalents were more numerous than bivalents was higher in triploid *Rana brevipoda* than that (4.2%) in triploid *Rana nigromaculata.*

1. Autotriploids of *Rana nigromaculata*

A total of 853 first meiotic spreads from eight males were analyzed (Table 1). In these meiotic spreads, there were 12 kinds of meiotic spreads which differed from one another in the number of trivalents varying from 2 to 13. The most numerous meiotic spreads were those which contained 8 or 9 trivalents in most autotriploids, although these kinds of meiotic spreads were not always the most numerous in each autotriploid. Meiotic spreads containing 8, 9 and 7 trivalents were the most numerous in three, three and one of the eight males, respectively. In the remainder, the meiotic spreads containing 8 trivalents were the same in number as those containing 9 trivalents, both being the most numerous among all the meiotic spreads.

Meiotic spreads containing 8 trivalents numbered 177 (20.8%), of which 168 contained 5 bivalents and 5 univalents in addition (Fig. 1). The other nine spreads contained 4 bivalents and 7 univalents in addition. The spreads

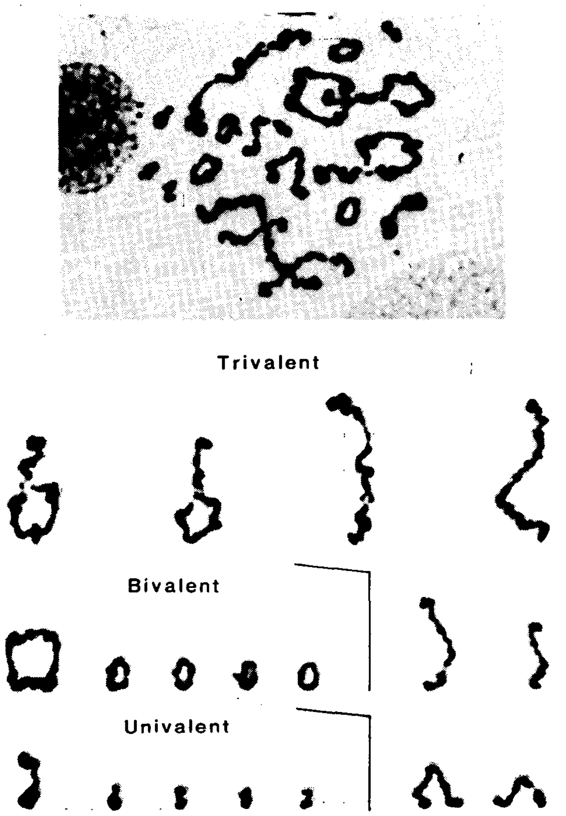

Fig. 1. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 4 large and 4 small trivalents, 5 bivalents and 5 univalents in an autotriploid male *Rana nigromaculata.* ×880

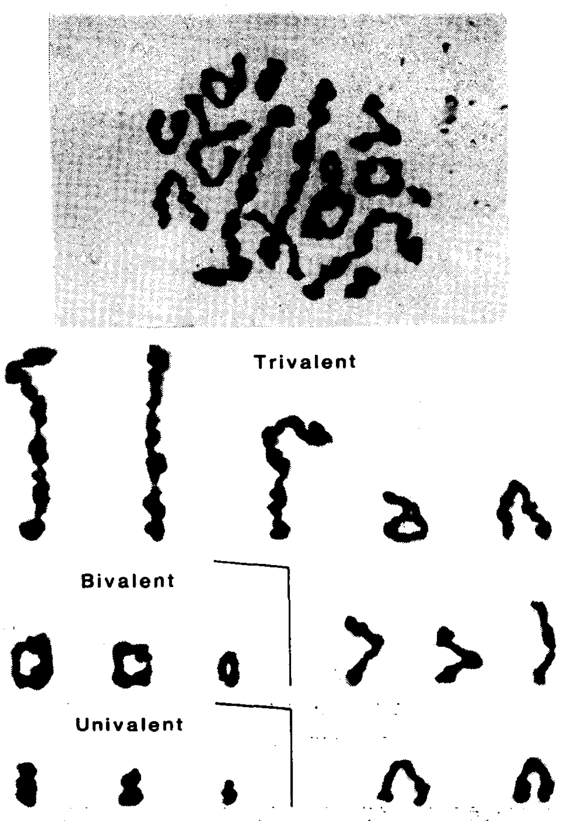

Fig. 2. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 3 large and 7 small trivalents, 3 bivalents and 3 univalents in an autotriploid male *Rana nigromaculata.* ×880

containing 8 trivalents were divided into six groups with difference in the combination of large and small trivalent chromosomes. The 8 trivalents consisted of 3 large and 5 small chromosomes in 70 spreads, 4 large and 4 small chromosomes in 48 spreads, 2 large and 6 small chromosomes in 47 spreads, 5 large and 3 small chromosomes in seven spreads, 1 large and 7 small chromosomes in four spreads and 8 small chromosomes in the remaining spread.

Meiotic spreads containing 9 trivalents numbered 177 (20.8%), of which 172 included 4 bivalents and 4 univalents in addition, while the other five included 3 bivalents and 6 univalents. The meiotic spreads containing 9 trivalents were divided into five groups. The 9 trivalents consisted of 3 large and 6 small chromosomes in 68 spreads, 4 large and 5 small chromosomes in 68 spreads, 2 large and 7 small chromosomes in 27 spreads, 5 large and 4 small chromosomes in 11 spreads and 1 large and 8 small chromosomes in the remaining three spreads.

Meiotic spreads containing 7 trivalents numbered 146 (17.1%), of which 140 contained 6 bivalents and 6 univalents in addition, while the other six contained 5 bivalents and 8 univalents. The spreads containing 7 trivalents were divided into six groups. The 7 trivalents consisted of 3 large and 4 small chromosomes in 58 spreads, 2 large and 5 small chromosomes in 48 spreads, 4 large and 3 small chromosomes in 21 spreads, 1 large and 6 small chromosomes in 15 spreads, 5 large and 2 small chromosomes in three spreads and solely 7 small chromosomes in the remainder.

Meiotic spreads containing 6 trivalents numbered 112 (13.1%), of which 105 contained 7 bivalents and 7 univalents in addition, while the other seven consisted of 6 trivalents, 6 bivalents and 9 univalents. The spreads containing 6 trivalents were divided into five groups. The 6 trivalents consisted of 2 large and 4 small chromosomes in 46 spreads, 3 large and 3 small chromosomes in 34 spreads, 1 large and 5 small chromosomes in 27 spreads, 4 large and 2 small chromosomes in four spreads and solely 6 small chromosomes in the remainder.

Meiotic spreads containing 10 trivalents numbered 104 (12.2%), of which 100 contained 3 bivalents and 3 univalents in addition (Fig. 2), while the other four contained 2 bivalents and 5 univalents. The spreads containing 10 trivalents were divided into four groups. The 10 trivalents consisted of 4 large and 6 small chromosomes in 44 spreads, 3 large and 7 small chromosomes in 32 spreads, 5 large and 5 small chromosomes in 24 spreads and 2 large and 8 small chromosomes in the remaining 4 spreads.

Meiotic spreads containing 11 trivalents numbered 51 (6.0%). All of them contained 2 bivalents and 2 univalents in addition. They were divided into three groups. The 11 trivalents consisted of 4 large and 7 small chromosomes in 23 spreads, 5 large and 6 small chromosomes in 18 spreads, 3 large and 8 small chromosomes in the remaining 10 spreads.

Meiotic spreads containing 5 trivalents numbered 45 (5.3%), of which 42 contained 8 bivalents and 8 univalents (Fig. 3), two contained 7 bivalents and 10 univalents and the remainder contained 6 bivalents and 12 univalents in addition. The spreads containing 5 trivalents were divided into four groups. The 5

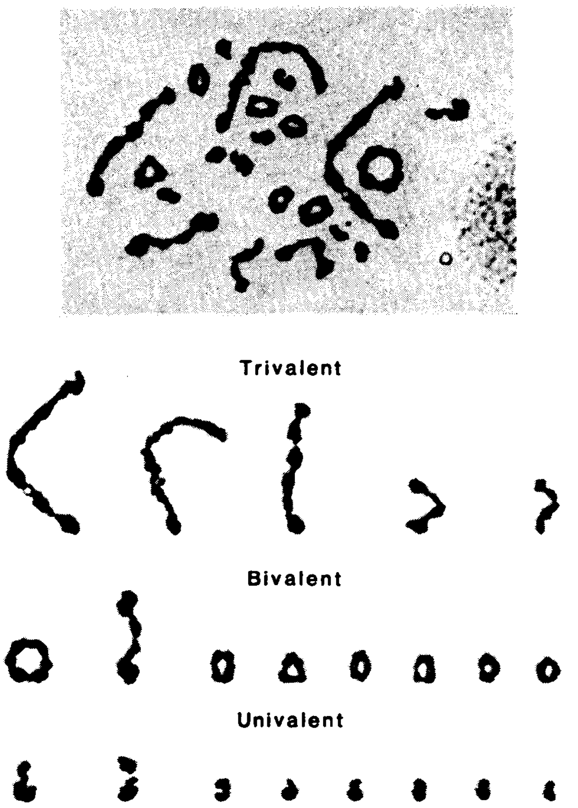

Fig. 3. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 3 large and 2 small trivalents, 8 bivalents and 8 univalents in an autotriploid male *Rana nigromaculata*. ×880

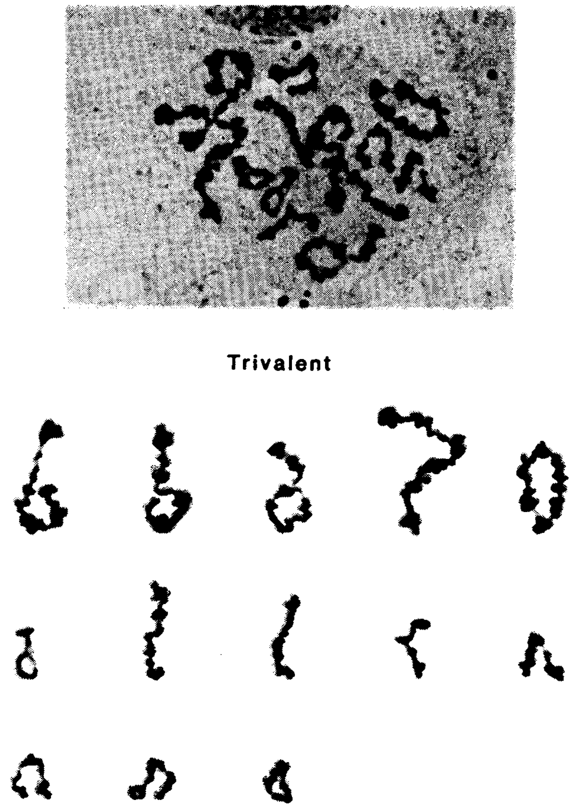

Fig. 4. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 5 large and 8 small trivalents in an autotriploid male *Rana nigromaculata*. ×880

trivalents consisted of 2 large and 3 small chromosomes in 22 spreads, 1 large and 4 small chromosomes in 12 spreads, 3 large and 2 small chromosomes in 10 spreads and solely 5 small chromosomes in the remainder.

Meiotic spreads containing 4 trivalents numbered 19 (2.2%), of which 17 contained 9 bivalents and 9 univalents in addition, while the other two contained 8 bivalents and 11 univalents. The spreads containing 4 trivalents were divided into four groups. The 4 trivalents consisted of 2 large and 2 small chromosomes in seven spreads, 1 large and 3 small chromosomes in six spreads, 3 large and 1 small chromosome in three spreads and 4 small chromosomes in the remaining three spreads.

Meiotic spreads containing 12 trivalents numbered 17 (2.0%). All of them contained 1 bivalent and 1 univalent in addition. They were divided into two groups. The 12 trivalents consisted of 5 large and 7 small chromosomes in 12 spreads and 4 large and 8 small chromosomes in five spreads.

Meiotic spreads containing 3 trivalents numbered two. These spreads included 10 bivalents and 10 univalents in addition. They were divided into two groups. The 3 trivalents consisted of 2 large and 1 small chromosome in one spread and 1 large and 2 small chromosomes in the other.

Meiotic spreads containing 2 trivalents numbered two. They contained 11 bivalents and 11 univalents in addition. The trivalents consisted of small chromosomes.

There was only one spread which contained 13 trivalents consisting of 5 large and 8 small chromosomes (Fig. 4).

As a total of 6832 trivalents were contained in 853 meiotic spreads from eight male autotriploids, the mean number of trivalents per spread was calculated to be 8.01. In these meiotic spreads, there were 37 triplets whose homologous chromosomes remained as univalents without forming trivalents or bivalents. This number of triplets corresponded to 0.3% of 11089 triplets contained in the spreads.

Trivalents were divided into two kinds in shape (Table 2; Fig. 5). In one of them, two of the three homologous chromosomes were conjugated with each other at their two ends and formed a ring, and the remaining chromosome was conjugated with them by its one end, while in the other kind, the three homologous chromosomes were conjugated with one another in file. The former is provisionally called ring and rod-shaped, while the latter is called long rod-shaped in the present paper. Of the 6832 trivalents, 931 (13.6%) were ring and rod-shaped, while the others (86.4%) were long rod-shaped. Bivalents were also divided into two kinds in shape (Table 2). One of them was a ring-shaped

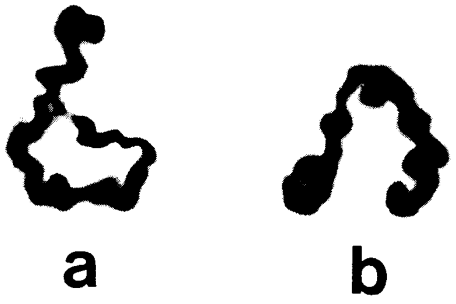

Fig. 5. Two kinds of trivalents.

a. Ring and rod-shaped trivalent in which two of the three homologous chromosomes are conjugated with each other at their two ends to form a ring and the remainder is conjugated with them at its one end.

b. Long rod-shaped trivalent in which the three homologous chromosomes are conjugated with one another in file.

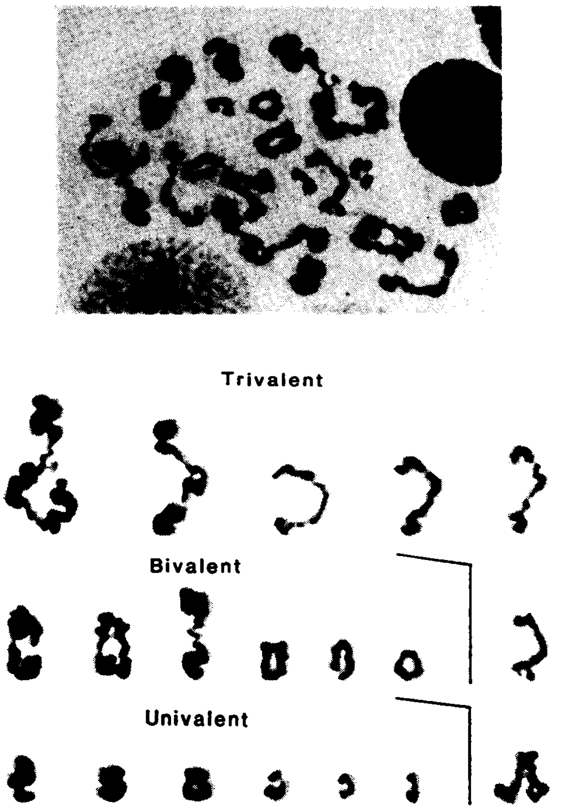

Fig. 6. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 2 large and 5 small trivalents, 6 bivalents and 6 univalents in an autotriploid male *Rana brevipoda*. ×880

bivalent in which the two homologous chromosomes were conjugated with each other at their two ends, while the other was rod-shaped bivalent in which the two homologous chromosomes were conjugated with each other in file. Of a total of 4220 bivalents found in the 853 meiotic spreads, 3542 (83.9%) were ring-shaped and the other 678 (16.1%) were rod-shaped.

2. Autotriploids of *Rana brevipoda*

A total of 342 first meiotic spreads from 10 male autotriploids were analyzed (Table 1). These meiotic spreads were divided into twelve kinds on the basis of differences in the number of trivalents. In contrast to the triploid *Rana nigromaculata*, there were no meiotic spreads containing 13 trivalents. On the other hand, there were some meiotic spreads which contained no trivalents. The most numerous meiotic spreads contained 7 trivalents in contrast to those of *Rana nigromaculata*, although this kind of meiotic spreads was not always the most numerous in each of the 10 male autotriploids. While meiotic spreads containing 7 trivalents were the most numerous in five males, those containing 8 trivalents were the most numerous in four other males. In the remaining male, meiotic spreads containing 7 trivalents were the same in number as those containing 6 trivalents and the most numerous.

Meiotic spreads containing 7 trivalents numbered 77 (22.5%), of which 70 included 6 bivalents and 6 univalents (Fig. 6), six included 5 bivalents and 8 univalents, and the remainder included 4 bivalents and 10 univalents in addition. The spreads containing 7 trivalents were divided into five groups with difference in the combination of large and small trivalent chromosomes. The 7 trivalents consisted of 2 large and 5 small chromosomes in 38 spreads, 3 large and 4 small chromosomes in 25 spreads, 4 large and 3 small chromosomes in seven spreads, 1 large and 6 small chromosomes in six spreads and 5 large and 2 small chromsomes in the remaining spread.

Meiotic spreads containing 8 trivalents numbered 71 (20.8%), of which 62 included 5 bivalents and 5 univalents in addition, while the other nine included 4 bivalents and 7 univalents. The spreads containing 8 trivalents were divided into six groups. The 8 trivalents consisted of 3 large and 5 small chromosomes in 29 spreads, 4 large and 4 small chromosomes in 17 spreads, 2 large and 6 small chromosomes in 16 spreads, 1 large and 7 small chromosomes in six spreads, 5 large and 3 small chromosomes in two spreads and 8 small chromosomes in the remainder.

Meiotic spreads containing 6 trivalents numbered 50 (14.6%), of which 34 included 7 bivalents and 7 univalents in addition, while the other 16 included 6 bivalents and 9 univalents. The spreads containing 6 trivalents were divided into five groups. The 6 trivalents consisted of 2 large and 4 small chromosomes in 23 spreads, 1 large and 5 small chromosomes in 15 spreads, 3 large and 3 small chromosomes in nine spreads, 4 large and 2 small chromosomes in two spreads and 6 small chromosomes in the remainder.

Meiotic spreads containing 9 trivalents numbered 49 (14.3%), of which 46 included 4 bivalents and 4 univalents in addition, while the other three included 3 bivalents and 6 univalents. The spreads containing 9 trivalents were divided into four groups. The 9 trivalents consisted of 3 large and 6 small chromosomes in 21 spreads, 2 large and 7 small chromosomes in 14 spreads, 4 large and 5 small chromosomes in 11 spreads and 5 large and 4 small chromosomes in the remaining three spreads.

Meiotic spreads containing 5 trivalents numbered 31 (9.1%), of which 24 included 8 bivalents and 8 univalents, four included 7 bivalents and 10 univalents, two included 5 bivalents and 14 univalents, and the remaining one included 6 bivalents and 12 univalents in addition. The spreads containing 5 trivalents were divided into five groups. The 5 trivalents consisted of 1 large and 4 small chromosomes in 11 spreads, 2 large and 3 small chromosomes in 10 spreads, 3 large and 2 small chromosomes in six spreads, 5 small chromosomes in three spreads and 4 large and 1 small chromosome in the remainder.

Meiotic spreads containing 10 trivalents numbered 26 (7.6%), of which 22 included 3 bivalents and 3 univalents in addition, while the other four spreads included 2 bivalents and 5 univalents. The spreads containing 10 trivalents were divided into four groups. The 10 trivalents consisted of 4 large and 6 small chromosomes in 11 spreads, 3 large and 7 small chromosomes in seven spreads, 2 large and 8 small chromosomes in five spreads and 5 large and 5 small chromosomes in the remaining three spreads.

Meiotic spreads containing 11 trivalents numbered 17 (5.0%), of which 16 included 2 bivalents and 2 univalents in addition, while the other spread included 1 bivalent and 4 univalents. The spreads containing 11 trivalents were divided into three groups. The 11 trivalents consisted of 4 large and 7 small chromosomes in 12 spreads, 3 large and 8 small chromosomes in three spreads and 5 large and 6 small chromosomes in the remaining two spreads.

Meiotic spreads containing 4 trivalents numbered nine (2.6%), of which seven included 9 bivalents and 9 univalents in addition, while the other two consisted of 4 trivalents, 8 bivalents and 11 univalents. The spreads containing 4 trivalents were divided into four groups. The 4 trivalents consisted of 2 large and 2 small chromosomes in four spreads, 1 large and 3 small chromosomes in three spreads, 3 large and 1 small chromosome in one spread and 4 small chromosomes in the remaining one spread.

Meiotic spreads containing 12 trivalents numbered six. All of them contained 1 bivalent and 1 univalent beside the trivalents. The 12 trivalents consisted of 4 large and 8 small chromosomes in the six spreads.

Meiotic spreads containing no trivalent numbered four, of which two contained 8 bivalents and 23 univalents, one contained 1 bivalent and 37 univalents, and the remainder consisted solely of 39 univalents.

Meiotic spread containing 3 trivalents numbered one. This spread contained 10 bivalents and 10 univalents beside the trivalents. The 3 trivalents consisted of 1 large and 2 small chromosomes.

There was one spread which contained 2 trivalents, 11 bivalents and 11

TABLE 2

Number of trivalents and bivalents differing in conjugating manner of homologous chromosomes in male auto- and allotriploids

Kind of frogs	Individual no.	No. of meioses	No. of trivalents				No. of bivalents			
			Ring and rod-shaped		Long rod-shaped		Ring-shaped		Rod-shaped	
			Large	Small	Large	Small	Large	Small	Large	Small
NNN	78NNN1	82	38	63	227	373	129	195	15	24
	78NNN2	57	28	17	139	251	91	151	26	30
	78NNN3	135	65	56	345	600	228	365	36	56
	78NNN4	41	39	38	99	170	55	114	12	4
	78NNN5	47	29	40	124	191	69	132	11	13
	78NNN6	26	24	27	65	98	34	71	7	11
	78NNN7	108	47	57	260	482	171	266	61	57
	78NNN8	357	178	185	852	1625	599	872	152	163
	Total	853	448	483	2111	3790	1376	2166	320	358
BBB	77BBB1	15	8	14	31	56	16	31	19	18
	77BBB2	32	23	15	60	134	41	63	30	35
	77BBB3	19	10	22	30	67	28	34	16	18
	77BBB4	59	32	59	118	227	75	133	64	48
	77BBB5	29	14	19	68	114	32	74	25	25
	77BBB6	16	14	19	33	60	18	35	15	14
	77BBB7	57	38	60	118	253	68	111	58	28
	77BBB8	26	15	17	65	110	32	56	18	23
	77BBB9	38	5	18	82	162	44	84	52	37
	77BBB10	51	24	35	100	214	62	110	63	40
	Total	342	183	278	705	1397	416	731	360	286
NNB	78NNB1	82	1	6	38	93	279	441	82	100
	78NNB2	19	1	8	0	24	47	74	33	43
	78NNB3	33	0	1	10	51	129	177	24	29
	78NNB4	66	1	6	17	56	266	420	42	39
	78NNB5	74	1	2	14	64	320	470	32	46
	78NNB6	89	7	10	36	105	326	510	69	79
	78NNB7	30	2	0	8	24	115	186	23	27
	78NNB8	79	1	5	41	116	265	374	75	109
	78NNB9	22	0	1	1	28	67	110	34	34
	78NNB10	20	1	2	9	22	73	120	16	14
	Total	514	15	33	182	583	1887	2882	430	520
BBN	77BBN1	37	0	2	8	25	90	187	71	65
	77BBN2	94	0	11	12	75	259	496	151	132
	77BBN3	19	0	3	6	13	48	114	37	20
	77BBN4	18	0	1	4	7	55	100	27	32
	77BBN5	23	0	2	3	12	62	132	44	31
	77BBN6	47	1	4	9	23	151	278	60	63
	77BBN7	15	0	0	4	14	46	78	19	22
	Total	253	1	23	46	169	711	1385	409	365

univalents. The 2 trivalents consisted of small chromosomes.

As a total of 2563 trivalents were contained in 342 meiotic spreads from 10 male autotriploids, the mean number of trivalents per spread was calculated to be 7.49. In these meiotic spreads, there were 90 triplets whose homologous chromosomes remained as univalents without forming trivalents or bivalents. This number of triplets corresponded to 2.0% of 4446 triplets contained in the spreads. Of the

2563 trivalents, 461 (18.0%) were ring and rod-shaped, while the other 2102 (82.0%) were long rod-shaped. Of a total of 1793 bivalents found in 342 meiotic spreads, 1147 (64.0%) were ring-shaped and the other 646 (36.0%) were rod-shaped (Table 2).

II. Allotriploids

In squash preparations of the testes of reciprocal allotriploids between *Rana nigromaculata* and *Rana brevipoda*, there were a great number of meiotic figures of the first spermatocytes and a small number of mitotic figures of spermatogonia besides abnormal spermatozoa. Meiotic figures of the second spermatocytes were scarce. While most of the germ cells were triploid, there were a small number of aneuploid, hexaploid and higher polyploid cells.

A total of 767 meiotic spreads obtained from 10 male allotriploids between female *nigromaculata* and male *brevipoda* and seven male allotriploids between female *brevipoda* and male *nigromaculata* were analyzed (Table 1). These meiotic spreads mainly contained bivalents and univalents, and there were usually no or a few trivalents. These meiotic spreads were divided into seven kinds on the basis of differences in number of trivalents varying from 0 to 6 in each spread. The most numerous meiotic spreads contained one trivalent, while those containing no trivalent were the second in abundance. These two kinds of meiotic spreads occupied about 60% of the total number of meiotic spreads analyzed. On the other hand, meiotic spreads containing 6 trivalents were the fewest, while those containing 5 and 4 trivalents were the second and the third in fewness, respectively. These three kinds of meiotic spreads occupied only about 6% of the total number of meiotic spreads. These kinds of meiotic spreads could generally be divided into several groups on the basis of differences in combination of large and small trivalent chromosomes. The numbers of bivalents were equal to those of univalents in 538 (70.1%) meiotic spreads, while univalents were more numerous than bivalents in the other 229 (29.9%) spreads. In about 70% of the latter spreads, univalents were more numerous than bivalents by three.

The allotriploids between female *nigromaculata* and male *brevipoda* somewhat differed from the reciprocal allotriploids in the most frequent kind of first meiotic spreads. Furthermore, the frequency of meiotic spreads in which univalents were more numerous than bivalents was fairly lower (22.0%) in the former allotriploids than that (48.5%) in the reciprocal allotriploids.

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

A total of 514 first meiotic spreads obtained from 10 male allotriploids were analyzed (Table 1). They were divided into seven kinds on the basis of differences in number of trivalents, which varied from 0 to 6 in each spread. The most numerous meiotic spreads were those containing 1 trivalent. However, this kind of meiotic spreads was not always the most numerous in each of the 10 male allotriploids. While it was the most numerous in four allotriploids, meiotic spreads containing 2 trivalents were the most numerous in three others. These two kinds of meiotic spreads were equal in number and the most numerous in one allotriploid. In the remaining two, the meiotic spreads containing no trivalent were the most numerous.

Meiotic spreads containing 1 trivalent numbered 151 (29.4%), of which 128 included 12 bivalents and 12 univalents in addition (Fig. 7). Of the other spreads, 17 included 11 bivalents and 14 univalents, four included 10 bivalents and 16 univalents, one included 9 bivalents and 18 univalents, and the remainder included 8 bivalents and 20 univalents in addition. The meiotic spreads containing 1 trivalent were divided into two groups with differences in combination of large and small trivalent chromosomes. The single trivalent was a small chromosome in 115 spreads, while it was a large chromsome in the other 36 spreads.

Meiotic spreads containing 2 trivalents numbered 128 (24.9%), of which 94 included 11 bivalents and 11 univalents (Fig. 8), 29 included 10 bivalents and 13 univalents, three included 9 bivalents and 15 univalents, one included 8 bivalents and 17 univalents and the remainder included 7 bivalents and 19 univalents in addition. The spreads containing 2 trivalents were divided into three groups.

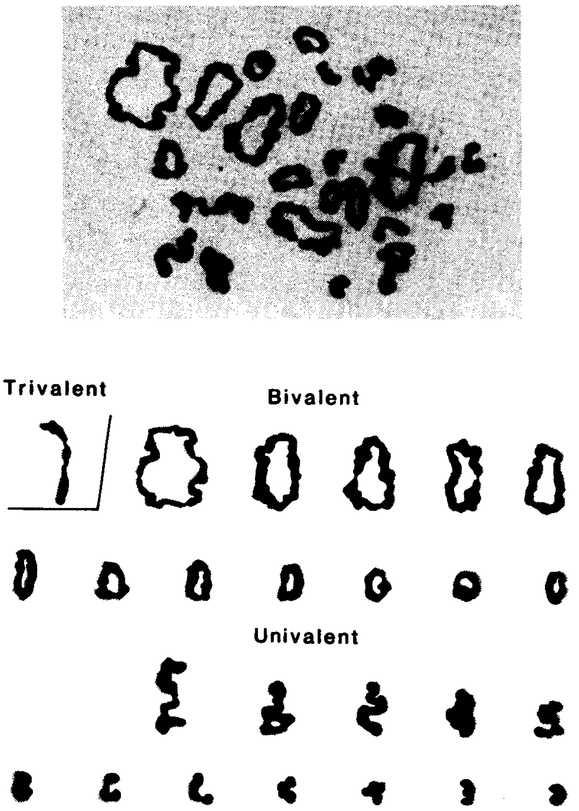

Fig. 7. Spread of a spermatocyte at the first meiosis and the chromosome complement containing one small trivalent, 12 bivalents and 12 univalents in an allotriploid male produced from a female *Rana nigromaculata* and a male *Rana brevipoda*. ×880

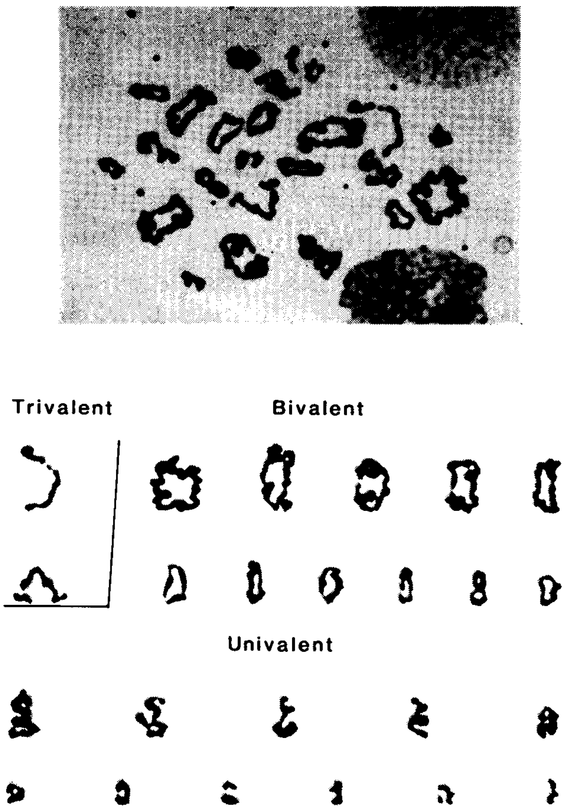

Fig. 8. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 2 small trivalents, 11 bivalents and 11 univalents in an allotriploid male produced from a female *Rana nigromaculata* and a male *Rana brevipoda*. ×880

The 2 trivalents were small chromosomes in 69 spreads, 1 large and 1 small chromosome in 54 spreads and 2 large chromosomes in the remaining five spreads.

Meiotic spreads containing no trivalent numbered 121 (23.5%), of which 88 contained 13 bivalents and 13 univalents, 22 spreads contained 12 bivalents and 15 univalents (Fig. 9), eight spreads contained 11 bivalents and 17 univalents, two spreads contained 9 bivalents and 21 univalents, and the remainder contained 10 bivalents and 19 univalents.

Meiotic spreads containing 3 trivalents numbered 69 (13.4%), of which 54 included 10 bivalents and 10 univalents, 13 included 9 bivalents and 12 univalents and the remaining two included 7 bivalents and 16 univalents in addition. The spreads containing 3 trivalents were divided into three groups. The 3 trivalents consisted of 3 small chromosomes in 33 spreads, 1 large and 2 small chromosomes in 27 spreads and 2 large and 1 small chromosome in the remaining nine spreads.

Meiotic spreads containing 4 trivalents numbered 29 (5.6%), of which 25 included 9 bivalents and 9 univalents in addition, while the other four included 8 bivalents and 11 univalents. The spreads containing 4 trivalents were divided into three groups. The 4 trivalents consisted of 1 large and 3 small chromosomes in 11 spreads, 2 large and 2 small chromosomes in nine spreads and 4 small chromosomes in the remaining nine spreads.

Meiotic spreads containing 5 trivalents numbered 13 (2.5%), of which 10 included 8 bivalents and 8 univalents in addition, while the other three included 7 bivalents and 10 univalents. The spreads containing 5 trivalents were divided into four groups. The 5 trivalents consisted of 2 large and 3 small chromosomes in six spreads, 1 large and 4 small chromosomes in three spreads, 5 small chromosomes in three other spreads and 3 large and 2 small chromosomes in the remaining one spread.

Meiotic spreads containing 6 trivalents numbered three (0.6%), of which two included 7 bivalents and 7 univalents in addition, while the other included 6 bivalents and 9 univalents. These three spreads differed from one another in combination of large and small trivalent chromosomes. The 6 trivalents consisted of 2 large and 4 small chromosomes in one spread, 3 large and 3 small chromosomes in another spread and 6 small chromosomes in the remainder.

As a total of 813 trivalents were found in 514 meiotic spreads from 10 male allotriploids between female *nigromaculata* and male *brevipoda*, the mean number of trivalents per spread was calculated to be 1.58. In these meiotic spreads, there were 150 triplets whose homologous chromosomes remained as univalents without forming trivalents or bivalents. This number of triplets corresponded to 2.2% of 6682 triplets contained in the spreads. Of these trivalents, 48 (5.9%) were ring and rod-shaped, while the other 765 (94.1%) were long rod-shaped. Of a total of 5719 bivalents found in the 514 meiotic spreads, 4769 (83.4%) were ring-shaped, while the other 950 (16.6%) were rod-shaped (Table 2).

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

A total of 253 meiotic spreads obtained from seven male allotriploids were analyzed (Table 1). In these meiotic spreads, there were five kinds containing 0~4 trivalents. The most numerous meiotic spreads contained no trivalent, in contrast to those of the reciprocal allotriploids. However, this kind of meiotic spreads was not always the most numerous in each of the seven males. While this kind was the most numerous in four allotriploids, the meiotic spreads containing 1 trivalent were the most numerous in two others. These two kinds of meiotic spreads were the same in number and the most numerous in the remaining allotriploid.

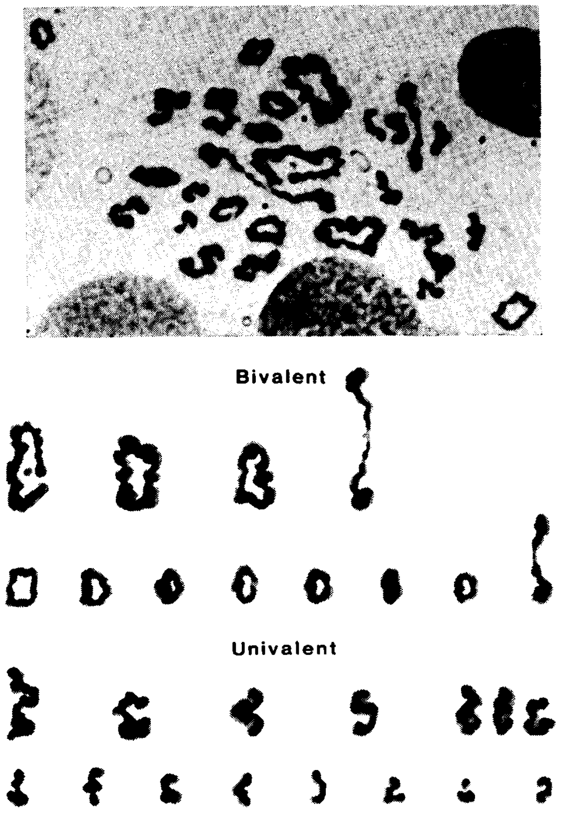

Fig. 9. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 12 bivalents and 15 univalents in an allotriploid male produced from a female *Rana nigromaculata* and a male *Rana brevipoda*. ×880

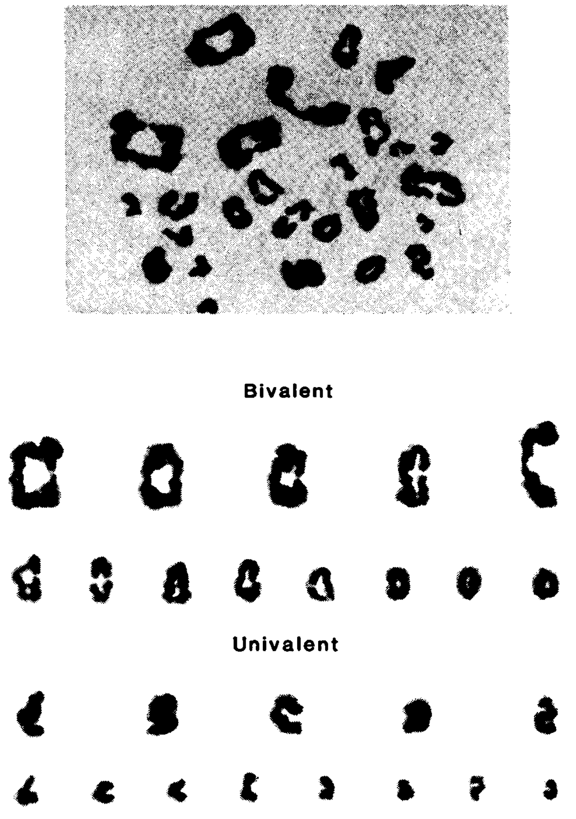

Fig. 10. Spread of a spermatocyte at the first meiosis and the chromosome complement containing 13 bivalents and 13 univalents in an allotriploid male produced from a female *Rana brevipoda* and a male *Rana nigromaculata*. ×880

Meiotic spreads containing no trivalent numbered 101 (39.9%), of which 46 contained 13 bivalents and 13 univalents (Fig. 10), 35 contained 12 bivalents and 15 univalents, 11 contained 11 bivalents and 17 univalents, four contained 10 bivalents and 19 univalents, two contained 9 bivalents and 21 univalents, one contained 8 bivalents and 23 univalents, one contained 7 bivalents and 25 univalents and the remaining contained 5 bivalents and 29 univalents.

Meiotic spreads containing 1 trivalent numbered 87 (34.4%), of which 50 included 12 bivalents and 12 univalents, 26 included 11 bivalents and 14 univalents, nine included 10 bivalents and 16 univalents and the remaining two included 9 bivalents and 18 univalents in addition. The spreads containing 1 trivalent were divided into two groups. The single trivalent was a small chromosome in 73 spreads, while it was a large chromosome in the others.

Meiotic spreads containing 2 trivalents numbered 46 (18.2%), of which 29 included 11 bivalents and 11 univalents, 14 included 10 bivalents and 13 univalents and the remaining three included 9 bivalents and 15 univalents. The spreads containing 2 trivalents were divided into three groups. The 2 trivalents were 2 small chromosomes in 27 spreads, 1 large and 1 small chromosome in 18 spreads and 2 large chromosomes in the remaining spread.

Meiotic spreads containing 3 trivalents numbered 16 (6.3%), of which 10 included 10 bivalents and 10 univalents, three included 9 bivalents and 12 univalents, one included 8 bivalents and 14 univalents, one included 7 bivalents and 16 univalents and the remainder included 5 bivalents and 20 univalents. The spreads containing 3 trivalents were divided into three groups. The 3 trivalents consisted of 1 large and 2 small chromosomes in seven spreads, 3 small chromosomes in seven other spreads and 2 large and 1 small chromosome in the remaining two spreads.

Meiotic spreads containing four trivalents numbered three (1.2%), of which two contained 9 bivalents and 9 univalents in addition, while the other contained 8 bivalents and 11 univalents. The 4 trivalents consisted of 1 large and 3 small chromosomes in two spreads and 4 small chromosomes in the other spread.

As a total of 239 trivalents were found in 253 meiotic spreads from seven male allotriploids, the mean number of trivalents per spread was calculated to be 0.94. In these meiotic spreads, there were 180 triplets whose homologous chromosomes remained as univalents without forming trivalents or bivalents. This number of triplets corresponded to 5.5% of 3289 triplets contained in the spreads. Of the 239 trivalents, 24 (10.0%) were of ring and rod-shaped, while the other 215 (90.0%) were long rod-shaped. Of a total of 2870 bivalents found in the 253 spreads, 2096 (73.0%) were ring-shaped and the other 774 (27.0%) were rod-shaped (Table 2).

DISCUSSION

1. Autotriploids

Böök (1940, 1945) has observed the first meiotic metaphase in a male triploid newt, *Triturus vulgaris* (= *Triton taeniatus*), which he happened to collect from the field. This report is the first on the meiosis in triploid amphibians. He described the existence of typical trivalents, bivalents and a various number of univalents. In 23 completely analyzed spermatocytes, there were 3～9, 5.9 on the average, trivalents, 3～10, 6.5 on the average, bivalents and 3～8, 5.3 on the average, univalents, while male diploids of this species usually formed 12 bivalents at the first meiotic metaphase. The trivalents were divided into three types in shape, Y-shaped, P-shaped and V-shaped. These types occurred from differences in conjugating way of the three homologous chromosomes of each trivalent. He (1945) analyzed the equilibrium position of the V-shaped trivalents at metaphase. At metaphase of the second meiotic division, chromosome numbers varying from 4 to 21 were counted.

Since FANKHAUSER (1934) discovered two triploid embryos in European newts, *Triturus helveticus* (= *Triton palmatus*), many triploids have been found in various urodelan species, such as *Notophthalmus viridescens*, (= *Triturus viridescens*), *Cynops pyrrhogaster* (= *Triturus pyrrhogaster*), *Eurycea bislineata* and *Ambystoma mexicanum* (=axolotl), (cf. FANKHAUSER, 1945). Numerous urodelan autotriploids have also been produced by artificial means in *Notophthalmus viridescens* (cf. FANKHAUSER, 1945), *Cynops pyrrhogaster* (FANKHAUSER, CROTTA and PERROT, 1942; FANKHAUSER, 1945; KAWAMURA, 1951a), *Taricha granulosa similans* (= *Triturus similans*) (COSTELLO, 1942; COSTELLO and HOLMQUIST, 1957), *Ambystoma mexicanum* (FANKHAUSER and HUMPHREY, 1942), *Triturus vulgaris* (BÖÖK, 1945), *Triturus vulgaris, Triturus alpestris, Triturus helveticus* (= *Triton palmatus*), *Triturus cristatus*, (FISCHBERG, 1945, 1947, 1948) and *Pleurodeles waltl* (BEETSCHEN, 1960; FERRIER and JAYLET, 1978). Of these urodelan autotriploids produced artificially, *Triturus alpestris* (FISCHBERG, 1945, 1948), *Ambystoma mexicanum* (HUMPHREY and FANKHAUSER, 1949; FANKHAUSER and HUMPHREY, 1950), *Cynops pyrrhogaster* (KAWAMURA, 1951a) and *Pleurodeles waltl* (BEETSCHEN, 1960) were reared until sexual maturity. Although it was found that spermatozoa were distinctly fewer in the males of these autotriploids than those in the control diploids, meiotic chromosomes were not observed except that trivalents, bivalents and univalents were found by BEETSCHEN (1960). However, production of various aneuploid offspring together with a few diploid ones from triploid males by mating with diploid females was reported in *Cynops pyrrhogaster* (KAWAMURA, 1951a) and *Ambystoma mexicanum* (FANKHAUSER and HUMPHREY, 1954).

In anurans, the first discovery of autotriploids was made by HERTWIG and HERTWIG (1920) in *Rana esculenta*. The controls of their hybridization experiments were unexpectedly triploids. WICKBOM (1945) observed the chromosomes of three triploid males found in field-caught *Rana esculenta*.

Many triploids were parthenogenetically produced in *Rana nigromaculata* by KAWAMURA (1939). Some parthenogenetic triploids produced in *Rana nigromaculata* and *Rana japonica* by KAWAMURA (1940) completed metamorphosis. In the testes of adult triploids obtained from *Rana nigromaculata* eggs by refrigerating after insemination, spermatozoa were much fewer than those of the control diploids (KAWAMURA, 1941a, b). Some univalents, bivalents and trivalents appeared in the first meiotic metaphase. These chromosomes were very irregular in distribution between the poles at the anaphase. Most of the primary and secondary spermatocytes degenerated, while only a few of them became spermatids of unequal size and eventually to spermatozoa.

Triploid *Rana pipiens* embryos were produced in a large number by heating the eggs (Briggs, 1947). Triploid tadpoles autopsied during metamorphosis usually appeared to be males, and it was evident that sex reversal of genetic females occurred in the triploids (Humphrey, Briggs and Fankhauser, 1950). Meiosis was not observed in male triploid *Rana pipiens.* Triploid *Rana japonica* produced by Kawamura and Tokunaga (1952) were similar to the above triploid *Rana pipiens* in that young triploid frogs were all males. They were also similar to triploid *Rana nigromaculata* (Kawamura, 1941a, b) in that the testes of mature triploid males contained many degenerating spermatocytes and a few spermatozoa. Kawamura (1951a) has reported that the embryos and tadpoles produced from triploid male *Rana nigromaculata* by mating with diploid females were intermediate between 2n and 3n in chromosome number. Nishioka (1971) has also described briefly the reproductive capacity of autotriploids in *Rana nigromaculata* and *Rana brevipoda.* All these autotriploids were very abnormal in spermatogenesis and scarcely contained normally shaped spermatozoa in their testes. Although a few eggs of normal diploid females were fertilized with sperm of male autotriploids, all the fertilized eggs died before attaining the hatching stage.

The behavior of the three homologous chromosomes of each triplet in the first meiotic metaphase of triploid male amphibians was analyzed in detail for the first time in the present study. The frequency of trivalent formation in male triploid *Rana nigromaculata* and *Rana brevipoda* was fairly similar to that in a male *Triturus vulgaris* (Böök, 1945). Although the first meiotic spreads in the autotriploids of the two anuran species were divided into 13 kinds on the basis of the number of trivalents, the most numerous spreads in *Rana nigromaculata* contained 8 or 9 trivalents and those in *Rana brevipoda* contained 7 trivalents. Four kinds of meiotic spreads containing 6, 7, 8 and 9 trivalents occupied more than 70% of the total number of meiotic spreads analyzed. The mean number of trivalents per cell was 8.01 in *Rana nigromaculata,* while it was 7.49 in *Rana brevipoda.* Each kind of first meiotic metaphases furthermore was mostly divided into several groups of different combinations of large and small trivalents. These findings seem to indicate that there were no triplets which were especially prone to form trivalents in primary spermatocytes and that trivalent formation was attributed to chance. One of the three homologous chromosomes of each triplet seemed to be able to participate in trivalent formation when meiotic pairing did not quickly occur between the other two. When complete meiotic paring occurred between two of the three homologous chromosomes in each of some triplets, the other chromosome seemed to remain as a univalent.

It was found that the three homologous chromosomes of each triplet remained as univalents without forming trivalents or bivalents in 0.3% of the triplets contained in the meiotic spreads of the triploid *Rana nigromaculata,* while those did so in 2.0% of the triplets contained in the meiotic spreads of the triploid *Rana brevipoda.* Although it is undetermined whether this phenomenon is attributable to precocious separation of diads in metaphase or not, the finding that the frequency of univalents was remarkably higher in triploid *Rana brevipoda* than that in triploid *Rana nigromaculata* is very interesting. A similar difference in univalent formation between triploid males of two species was also observed between diploid males of the same two species (OKUMOTO, 1980). Thus, the ease in producing univalents seems to be attributable to the character specific to each species.

2. Allotriploids

The first production of allotriploid amphibians was briefly reported by KAWAMURA in 1943. He obtained allotriploids from *Rana japonica* eggs fertilized with sperm of *Rana ornativentris* as well as *Cynops p. pyrrhogaster* eggs fertilized with sperm of *Cynops pyrrhogaster ensicauda* (=*Cynops ensicauda*) by refrigeration of the eggs. FISCHBERG (1948) also produced allotriploids from *Triturus helveticus* eggs fertilized with sperm of *Triturus alpestris* by the same method. The allotriploids were compared with diploid hybrids of the two species in color and pattern at the larval stage. KAWAMURA (1952b) reared four reciprocal allotriploids between *Cynops p. pyrrhogaster* and *Cynops p. ensicauda* for several years and found that all of them were females with ill-developed ovaries. In contrast, 19 allotriploids produced by KAWAMURA (1952a) from *Rana japonica* eggs fertilized with sperm of *Rana ornativentris* by refrigeration of the eggs were all males as diploid hybrids between these two species were. The testes of mature allotriploids contained bivalent and univalent chromosomes which were arranged somewhat regularly in the equatorial plate of the first meiotic metaphase. In this respect, allotriploids distinctly differed from autotriploids which contained some irregularly arranged trivalents besides bivalents and univalents. No normal spermatozoa were produced from allotriploid males.

BEÇAK and BEÇAK (1970) has reported that in artificial allotriploids ($3n=33$) produced from females of a tetraploid species ($4n=44$), *Odontophrynus americanus*, mated with males of a diploid species ($2n=22$), *Odontophrynus cultripes*, most of the spermatocytes showed a high frequency of bivalents and univalents and a few trivalents in the first meiotic metaphase, while the remaining had 11 bivalents and 11 univalents or only 33 univalents. The gametes produced were aneuploids in the range of 12 to 21 chromosomes or hyperdiploids and hypotriploids in the range of 23 to 32 chromosomes, besides euploids with 11, 22, or 33 chromosomes. NISHIOKA (1971) has reported on the reproductive capacity of four kinds of male allotriploids between *Rana nigromaculata* and *Rana brevipoda*. These four kinds consisted of different combinations of cytoplasm and genomes of the species. Although they were generally abnormal in spermatogenesis, two kinds of allotriploids were slightly fertile and produced many abnormal embryos by mating with normal diploid females. These offspring were intermediate between 2n and 3n in chromosome number as those of male autotriploid *Rana nigromaculata* produced by KAWAMURA (1951b).

As natural triploids in amphibians, UZZELL (1964) has reported a wide distribution of two allotriploid urodelan species together with their two original diploid species in eastern Canada and northeastern United States. While the diploid species, *Ambystoma jeffersonianum* and *Ambystoma laterale*, are bisexual, the allotriploid species, *Ambystoma tremblayi* and *Ambystoma platineum*, consist almost exclusively of females which reproduce gynogenetically. Günther (1970) assumed seven triploids found among 24 *Rana esculenta* collected from East Germany to be allotriploids between *Rana ridibunda* and *Rana lessonae*, as the hybrid nature of *Rana esculenta* had been repeatedly confirmed by Berger (1967, 1968) and many other investigators. Thereafter, Günther (1975b) noticed that triploids sometimes occupied more than 80% of individuals in *Rana esculenta* populations distributed in East Germany. Male triploids generally showed 39 chromosomes arranged multifariously in the first meiotic metaphase. However, many triploids contained 13 bivalents and 13 univalents in numerous first meiotic metaphases, while some other triploids revealed aneuploid numbers of univalents and bivalents and also heterologous trivalent and multivalent chromosomes. It was remarkable that diploid and haploid number of chromosomes were counted in some of the first and second meiotic metaphases, respectively, in all the male triploid *Rana esculenta*

In the present study, it was found that the chromosomes of the first meiotic spreads in male allotriploids between *Rana nigromaculata* and *Rana brevipoda* were very similar to those between female tetraploid *Odontophrynus americanus* and male diploid *Odontophrynus cultripes* (Beçak and Beçak, 1970) in that only a few or no trivalents were formed in the first meiosis. They differed from triploid *Rana esculenta* (Günther, 1975b) which were assumed to be allotriploids between *Rana ridibunda* and *Rana lessonae* in that there were no first meiotic spreads containing diploid number of chromosomes. In contrast to autotriploids of *Rana nigromaculata* or *Rana brevipoda*, meiotic spreads containing no, one or two trivalents occupied about 80% of the total number of meiotic spreads analyzed in reciprocal allotriploids between the two species. Ohtani (1978) also observed that the lampbrush chromosomes in most of the oocytes obtained from a female allotriploid between a female *Rana brevipoda* and a male *Rana nigromaculata* consisted of a mixture of one to five trivalents, 8～12 bivalents and 8～12 univalents. The remaining oocytes contained 13 bivalents and 13 univalents, having no trivalents. In the present study, it was found that the mean number of trivalents per cell was 1.58 in male allotriploids between female *Rana nigromaculata* and male *Rana brevipoda* and 0.94 in the reciprocal allotriploids. The paucity of trivalents in these allotriploids seems to support the assumption stated above in the autotriploids that one of the three homologous chromosomes of each triplet could participate in trivalent formation when meiotic pairing did not quickly occur between the other two. In allotriploids, two homologous chromosomes derived from the mother must have been prone to conjugate quickly with each other owing to their affinity and to result in leaving the remaining chromosome derived from the father as a univalent.

It was remarkable that the three homologous chromosomes of each triplet remained as univalents without forming trivalents or bivalents in 5.5% of the triplets contained in the first meiotic spreads of male allotriploids between female

Rana brevipoda and male *Rana nigromaculata*, while those did so in 2.2% of the triplets contained in the reciprocal allotriploids. This finding is similar to that reported by the present author (1980) in reciporcal diploid hybrids of the two species. The mean number of univalents per nucleus in male diploid *Rana nigromaculata* and *Rana brevipoda* was 0.10 and 0.51, respectively, while it was 13.51 in male diploid hybrids between female *Rana nigromaculata* and male *Rana brevipoda* and 14.13 in the reciprocal hybrids. The frequencies of univalents in the first meiotic spreads of spermatocytes of diploid and triploid hybrids between female *Rana brevipoda* and male *Rana nigromaculata* as well as diploid *Rana brevipoda* were evidently higher than those in diploid and triploid hybrids of the reciprocal combination as well as *Rana nigromaculata*. This seems to indicate that the ease in producing univalents is controlled by a factor or factors inherited by cytoplasm.

SUMMARY

1. In order to clarify the behavior of chromosomes in the first meiotic division of spermatocytes in auto- and allotriploids, the testes of male triploid *Rana nigromaculata*, triploid *Rana brevipoda* and reciprocal allotriploids between these two species were observed by the squash method after water pretreatment.

2. The first meiotic spreads in triploid *Rana nigromaculata* and *Rana brevipoda* contained trivalents, bivalents and univalents. They were divided into 13 kinds on the basis of the numbers of trivalents. The most numerous spreads of triploid *Rana nigromaculata* contained 8 or 9 trivalents, while those of triploid *Rana brevipoda* contained 7 trivalents. Four kinds of meiotic spreads containing 6, 7, 8 and 9 trivalents occupied more than 70% of the total number of meiotic spreads analyzed in the two species. The mean number of trivalents per cell was 8.01 in triploid *Rana nigromaculata* and 7.49 in triploid *Rana brevipoda*.

3. Each kind of first meiotic spreads was mostly subdivided into several groups on the basis of different combinations of large and small trivalents. The number of bivalents was equal to that of univalents in more than 90% of the total number of meiotic spreads analyzed in autotriploids of the two species, while univalents were more numerous than bivalents by three or more in the remaining.

4. While the three homologous chromosomes of each triplet remained as univalents without forming trivalents or bivalents in 0.3% of the triplets contained in the meiotic spreads of the triploid *Rana nigromaculata*, those did so in 2.0% of the triplets contained in the meiotic spreads of the triploid *Rana brevipoda*.

5. The chromosomes of the first meiotic spreads in male allotriploids between *Rana nigromaculata* and *Rana brevipoda* mainly consisted of bivalents and univalents, while trivalent formation was rather rare in contrast to that in triploid *Rana nigromaculata* or *Rana brevipoda*. The first meiotic spreads were divided into seven kinds on the basis of the numbers of trivalents which varied from none to 6. While those containing one trivalent were most numerous in allotriploids between female *Rana nigromaculata* and male *Rana brevipoda*, those containing no trivalent were so in the reciprocal allotriploids. Three kinds of meiotic spreads containing

0, 1 and 2 trivalents occupied about 80% of the total number of meiotic spreads analyzed in the two kinds of allotriploids. The mean number of trivalents per cell was 1.58 in allotriploids between female *Rana nigromaculata* and male *Rana brevipoda*, while 0.94 in the reciprocal allotriploids.

6. Each kind of first meiotic spreads was subdivided into a few groups on the basis of different combinations of large and small trivalents. The number of bivalents was equal to that of univalents in 70% of the total number of meiotic spreads analyzed in the two kinds of allotriploids, while univalents were more numerous than bivalents by three or more in the remaining.

7. While the three homologous chromosomes of each triplet remained as univalents without forming trivalents or bivalents in 2.2% of the triplets contained in the meiotic spreads of the allotriploids between female *Rana nigromaculata* and male *Rana brevipoda*, those did so in 5.5% of the triplets contained in the meiotic spreads of the reciprocal allotriploids.

8. There were two kinds of trivalents in shape occurring from conjugating manner of the three homologous chromosomes. In one kind of trivalents, two homologous chromosomes were conjugated by their two ends and the remaining was conjugated with them by its one end, while in the other kind the three homologous chromosomes were united in file. The latter kind of trivalents was far more numerous than the former in the two kinds of autotriploids, as well as in the two kinds of allotriploids.

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