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Detection of Chromosomes Bearing the Loci for Blue and Olive Mutations in *Rana nigromaculata*

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(With 12 Text-figures)

INTRODUCTION

In anurans, many genes controlling the body color and pattern have been reported by many investigators. Genes for burnsi (*B*) and kandiyohi (*K*) in *Rana pipiens* (MOORE, 1942; VOLPE, 1955, 1960, 1961) and for expanding the three kinds of dermal chromatophores (*E*) in *Rana nigromaculata* (NISHIOKA and UEDA, 1977a) are dominant, but the other color mutations are usually due to single recessive genes. Most of the color mutants were accidentally discovered in the field, while the others were induced by irradiation of gametes (NISHIOKA, 1977).

The mapping of the genes for color and pattern was first attempted by VOLPE (1970) using diploid gynogenetic offspring of heterozygous mothers in *Rana pipiens*. He has confirmed that the gene for kandiyohi (*K*) is located closely adjacent to the centromere, while the gene for burnsi (*B*) is located toward the end of the chromosome arm. NACE, RICHARDS and ASHER (1970) also mapped the three genes for kandiyohi, burnsi and melanoid (*m*) by using the method of gynogenesis.

On the other hand, NISHIOKA, OHTANI and SUMIDA (1980) have elucidated six chromosomes on which the loci for four enzymes and three blood proteins are situated by comparing the genotypes of these proteins with the constitution of lampbrush chromosomes in oocytes in mature female offspring of female reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda* backcrossed with males of these parental species.

The purpose of the present study is to determine the chromosomes which bear the loci for olive and blue mutations by comparing the genotypes at the loci for these mutations with the constitutions of lampbrush chromosomes in female backcross offspring of reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda*. An additional purpose is to presume the position of the locus for olive mutation on the chromosome by using a female backcross hybrid with a translocation on chromosome No. 3.

MATERIALS AND METHODS

Rana nigromaculata HALLOWELL and *Rana brevipoda* ITO were used as materials. In 1967, three pairs of *Rana nigromaculata* were collected from Hiro near Hiroshima. Two males and two females were brown and the others were green all over the dorsal surface at the immature frog stage. A male and a female *Rana brevipoda* were collected from Konko near Okayama in 1970. They were green all over the dorsal surface at both the immature and mature stages.

Blue and olive mutations were induced by neutron-irradiation of spermatozoa, as described by NISHIOKA (1977). The neutron generator was of Toshiba (d, n) He Reaction Type, producing fast neutrons of 14.1 MeV. The conversion coefficient was 6.7×10^{-9} rads/n/sec/4π and the exposure dose rate was about 10 rads/min. The testes of the three male *Rana nigromaculata* were mashed in distilled water to make sperm suspension. Spermatozoa were exposed to 130 rads of neutrons. Eggs of *Rana nigromaculata* were obtained from the three females after accelerating ovulation by pituitary injection. Artificial insemination was made between these eggs and neutron-irradiated spermatozoa. Embryos raised from fertilized eggs were continuously reared until they became sexually mature frogs. From females of these frogs, offspring were produced by diploid gynogenesis in order to disclose recessive mutations induced by neutron-irradiation.

Tadpoles were fed on boiled spinach or chard. While frogs were fed on mosquitos at the young stage and on flies and bag-worms until 1975, thereafter, they were exclusively fed on crickets.

Lampbrush chromosomes were observed on the preparations which were principally made according to GALL's method (1966). A mixture of 5 parts of 0.075M KCl solution and 1 part of 0.075M NaCl solution was used after 10% formalin had been added to the mixture in concentration of 0.08% formaldehyde to disperse the lampbrush chromosomes more easily (NISHIOKA, OHTANI and SUMIDA, 1980).

Serum proteins, hemoglobin and enzymes extracted from the skeletal muscles and liver were analyzed by the method of starch-gel electrophoresis, as utilized by BREWER (1970) and NISHIOKA, OHTANI and SUMIDA (1980).

The detection of chromosomes bearing the loci for blue and olive dorsal colors was made as follows. A male *Rana nigromaculata* homozygous for blue and olive genes was crossed with a female green *Rana brevipoda* to produce hybrids, (B)BN. Two mature females of these hybrids were backcrossed with the above male *Rana nigromaculata* homozygous for blue and olive genes. On the other hand, gynogenetic diploids were produced from four other mature hybrids. Lampbrush chromosomes were observed in the oocytes of female backcrosses and gynogenetic diploids. By comparing the combination of the alleles at the locus for the dorsal color with the constitution of each of the 13 pairs of lampbrush chromosomes in each of these females, the chromosome bearing the locus was detected.

The following abbreviations are used in the present paper.

N ——— A *Rana nigromaculata* chromosome
B ——— A *Rana brevipoda* chromosome
NN ——— *Rana nigromaculata* or a pair of *Rana nigromaculata* chromosomes
BB ——— *Rana brevipoda* or a pair of *Rana brevipoda* chromosomes
BN ——— A hybrid between a female *Rana brevipoda* and a male *Rana nigromaculata* or a combination of a *Rana brevipoda* chromosome and a *Rana nigromaculata* chromosome

OBSERVATION

I. The origin of a male Rana nigromaculata used in crossing with a female Rana brevipoda

Although the blue and olive mutations in dorsal color were induced in spermatozoa of *Rana nigromaculata* by irradiation with 130 rads of neutrons in 1967, the male frog used in mating with a female *Rana brevipoda* for the purpose of detecting the chromosomes bearing the loci for these mutations was an offspring obtained from a female ancestor, N · SN-130, No. 8 (*XxIiEe*), by passing through several generations. Of these generations, the first, second and third generations produced in 1967, 1970 and 1972, respectively, have been reported by NISHIOKA (1977). As described previously, two bluish-olive mutants (*xxiiEe*) were produced from mating between a bluish-olive female (*xxiiEE*) and a brownish-olive male (*xxiiee*), 24 green frogs (*XxIiEe*) were produced from mating between a bluish-olive female (*xxiiEE*) and a brown male (*XXIIee*), and 16 bluish-olive mutants (*xxiiEE*) were produced from a bluish-olive female (*xxiiEE*) by diploid gynogenesis (cf. NISHIOKA, 1977: Table 25, experiments in 1972).

In 1975, a male of the 24 green frogs was mated with one of the 16 bluish-olive mutants which were produced by diploid gynogenesis and were all females. From this mating, 17 bluish-olive mutants (*xxiiEE* or *xxiiEe*) and 24 greenish-olive mutants (*XxiiEE* or *XxiiEe*) were produced together with 17 blue (*xxIiEE* or *xxIiEe*) and 26 green frogs (*XxIiEE* or *XxIiEe*). In 1976, a male of the bluish-olive mutants produced in 1975 was mated with a female of the bluish-olive mutants produced by diploid gynogenesis in 1972. From this mating, 43 bluish-olive mutants (*xxiiEE* or *xxiiEe*) were obtained. A male (*xxiiEE*) of these bluish-olive mutants was used in crossing with a female *Rana brevipoda*.

II. Color and lampbrush chromosomes of the female offspring from female hybrids

In 1978, hybrids were produced from crossing between a male bluish-olive (*xxiiEE*) *Rana nigromaculata*, 76 NN♂, No. 1, and a female green (*XXIIEE*) *Rana brevipoda*, 75 BB♀, No. 1 (Fig. 1). The hybrids were all wild-type green (*XxIiEE*). As female hybrids almost attained sexual maturity in November of this year, lampbrush chromosomes were observed in oocytes of six females, 78 BN♀, Nos. 1～6. The results indicated that the oocytes always contained

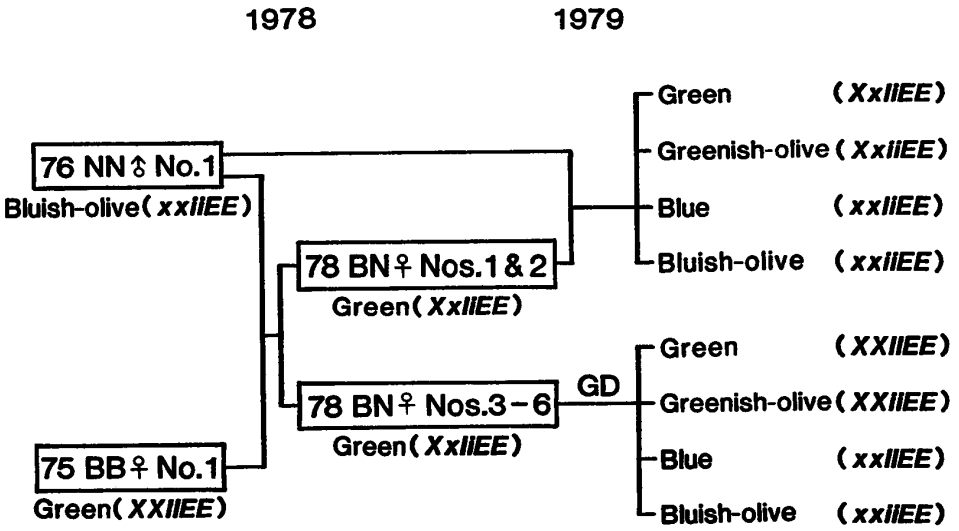

Fig. 1. Coloration of the offspring obtained from hybrids between a bluish-olive female *Rana nigromaculata* and a green male *Rana brevipoda* by backcrossing or diploid gynogenesis.

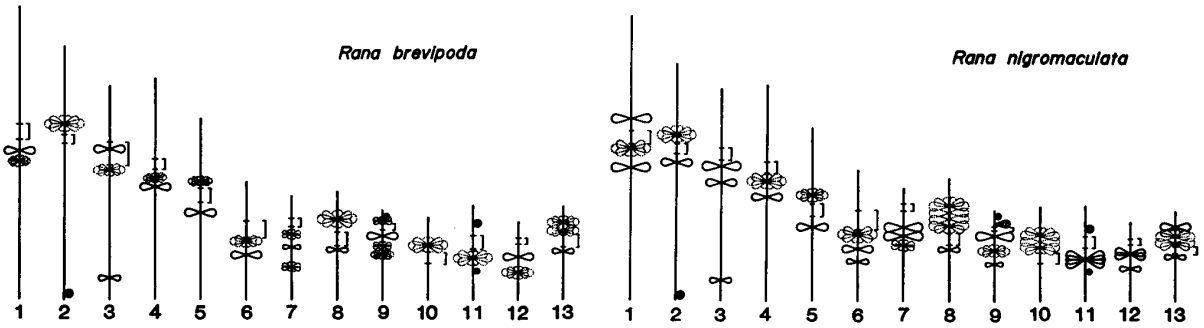

Fig. 2. Diagrams of the thirteen lampbrush chromosomes of *Rana brevipoda* and *Rana nigromaculata*.

Marks drawn with a solid and a dotted line represent a simple and a compound type of giant loops, respectively. A black spot indicates a sphere. A pair of short horizontal bars on each chromosome indicates the segment including the centromere.

13 bivalents, each of which consisted of a *Rana nigromaculata* chromosome and a *Rana brevipoda* chromosome (Figs. 2 and 3). However, a deficiency or an inversion was found in the chromosomes derived from *Rana nigromaculata* in five of the six hybrids. More specifically, a deficiency and an inversion in chromosome No. 9 were found in female hybrids Nos. 1 and 5, a deficiency in chromosome No. 6 was found in female hybrid No. 2, and a deficiency in chromosome No. 6 and a deficiency and an inversion in chromosome No. 9 were found in female hybrids Nos. 3 and 4. Neither deficiency nor inversion was found in the chromosomes of female hybrid No. 6.

1. Dorsal color of female backcrosses and gynogenetic diploids

In the breeding season of 1979, two (Nos. 1 and 2) of the six female hybrids

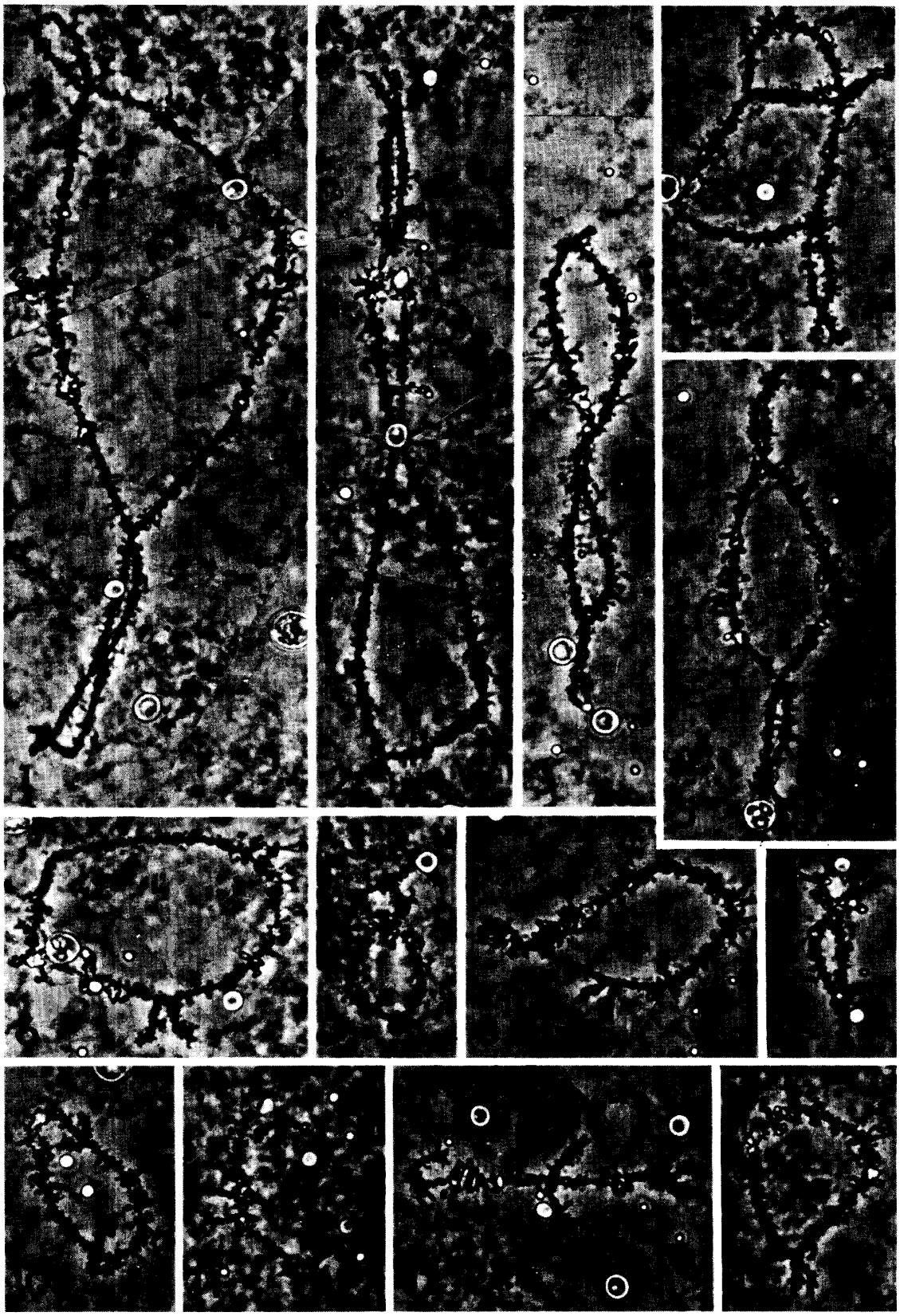

Fig. 3. Microphotographs of the thirteen bivalent (lampbrush) chromosomes in an oocyte of a female hybrid between *Rana brevipoda* and *Rana nigromaculata*, BN, No. 6. ×300

were backcrossed with a male *Rana nigromaculata*, 76 NN♂, No. 1, which was a bluish-olive mutant (*xxiiEE*) and was used in crossing with a female green *Rana brevipoda* in 1978 (Fig. 1). It was found that all the tadpoles completed metamorphosis during the period from 55 to 72 days after insemination. The dorsal

TABLE 1

Dorsal colors of female frogs whose lampbrush chromosomes were analyzed

Parents		Number of mature females				
Female	Male	Total	Green	Greenish-olive	Blue	Bluish-olive
BN, No. 1	NN, No. 1	81	21	23	18	19
BN, No. 2	NN, No. 1	19	5	7	3	4
BN, Nos. 3~6	GD	34	10	8	9	7
Total		134	36	38	30	30

TABLE 2

The constitution of each of the 13 bivalent chromosomes in each of 36 green (wild-type) frogs of 134 mature female offspring produced from six female hybrids by backcrossing or diploid gynogenesis

Parents		Individual no.	Bivalent chromosome number												
Female	Male		1	2	3	4	5	6	7	8	9	10	11	12	13
BN, No. 1	NN, No. 1	1	NN	BN	BN	BN	NN	BN	NN	BN	N^sN^s	NN	NN	NN	NN
		2	NN	BN	BN	BN	BN	NN^d	NN	BN	N^sN	NN	BN	BN	NN
		3	BN	NN	$\frac{B}{N}N$	BN	BN	BN	BN	BN	N^sN^s	NN	NN	NN	BN
		4	BN	BN	BN	BN	BN	NN^d	NN	BN	BN^s	BN	NN	NN	NN
		5	NN	BN	BN	NN	BN	BN^d	NN	BN	BN^s	NN	NN	NN	BN
		6	BN	NN	BN	BN	BN	NN	BN	BN	N^sN	BN	BN	NN	NN
		7	NN	NN	BN	NN	BN	BN	BN	BN	N^sN^s	BN	NN	BN	BN
		8	NN	NN	BN	BN	NN	NN	NN	BN	BN	BN	NN	NN	BN
		9	NN	BN	BN	BN	BN	BN	NN	NN	BN^s	NN	NN	BN	BN
		10	NN	BN	BN	NN	BN	BN^d	BN	BN	N^sN	NN	NN	NN	NN
		11	BN	NN	BN	BN	BN	BN	BN	NN	BN^s	BN	NN	BN	NN
		12	NN	BN	BN	BN	NN	BN	NN	BN	N^sN^s	NN	BN	BN	NN
		13	BN	BN	BN	BN	BN	BN^d	BN	BN	N^sN^s	NN	NN	NN	NN
		14	BN	BN	BN	NN	BN	BN^d	NN	BN	BN	BN	BN	BN	NN
		15	NN	BN	BN	BN	BN	NN^d	NN	BN	BN^s	BN	NN	BN	BN
		16	BN	BN	BN	BN	BN	BN	NN	BN	BN^s	BN	NN	BN	NN
		17	NN	NN	BN	BN	BN	NN	NN	BN	N^sN	NN	BN	NN	NN
		18	BN	BN	BN	NN	BN	BN	BN	BN	N^sN	BN	NN	BN	NN
		19	BN	NN	BN	BN	BN	BN	BN	BN	BN	NN	BN	NN	BN
		20	BN	NN	BN	BN	BN	BN	NN	BN	N^sN^s	BN	NN	BN	BN
		21	NN	NN	BN	NN	NN	BN^d	NN	BN	BN	NN	NN	BN	NN
BN, No. 2	NN, No. 1	1	NN	BN	BN	BN	BN	BN	NN	NN	NN	BN	NN	NN	NN
		2	NN	BN	BN	BN	BN	BN	NN	BN	BN	BN	NN	NN	NN
		3	NN	NN	BN	BN	BN	N^dN^d	NN	BN	NN	NN	NN	NN	NN
		4	BN	NN	BN	BN	BN	N^dN^d	BN	BN	BN	BN	BN	BN	NN
		5	BN	BN	BN	BN	BN	N^dN^d	NN	BN	NN^s	BN	BN	$\frac{B}{N}N$	NN
BN, Nos. 3~6	GD	1	NN	NN	BB	NN	BB	NN	NN	BN	N^sN^s	NN	NN	NN	BB
		2	NN	NN	BB	BB	BN	NN	NN	BB	N^sN^s	BN	BN	NN	BB
		3	BB	BB	BB	BB	NN	BB	BB	BB	BB	BB	BB	BB	NN
		4	BB	NN	BB	BB	BB	N^dN^d	BB	BB	BB	BB	BB	BB	BB
		5	NN	BB	BB	BB	BB	N^dN^d	BB	BB	BN	BB	BB	NN	BB
		6	BB	BB	BB	BB	BB	NN	BB	BB	BB	BB	NN	BB	BN
		7	NN	BB	$\frac{B}{N}B$	BB	BB	BB	NN	NN	BB	BB	NN	NN	BB
		8	BB	NN	BB	NN	BB	BB	BB	BB	BB	BB	NN	NN	NN
		9	$\frac{N}{B}B$	NN	BB	BB	BB	NN	BB	BN	BB	BB	NN	NN	BB
		10	NN	BB	BB	NN	BB	BB	NN	BB	BB	BB	BB	NN	NN

TABLE 3

The constitution of each of the 13 bivalent chromosomes in each of 38 greenish-olive mutants of 134 mature offspring produced from six female hybrids by backcrossing or diploid gynogenesis

Parents		Individual	Bivalent chromosome number												
Female	Male	no.	1	2	3	4	5	6	7	8	9	10	11	12	13
BN, No. 1	NN, No. 1	1	BN	BN	NN	BN	NN	BN	NN	BN	N^sN	NN	NN	NN	BN
		2	NN	NN	NN	NN	BN	NN	BN	BN	N^sN	NN	NN	BN	BN
		3	BN	BN	NN	BN	BN	NN	BN	BN	N^sN^s	NN	NN	NN	NN
		4	BN	NN	NN	BN	BN	NN^d	BN	NN	N^sN	NN	NN	BN	NN
		5	BN	BN	NN	BN	BN	NN	NN	BN	N^sN^s	NN	NN	NN	BN
		6	NN	BN	NN	NN	BN	NN	BN	NN	N^sN	BN	NN	$\frac{N}{B}N$	NN
		7	NN	NN	NN	NN	BN	NN	BN	BN	N^sN^s	NN	BN	NN	BN
		8	NN	BN	NN	NN	BN	BN	BN	BN	BN^s	BN	BN	BN	NN
		9	BN	BN	NN	NN	BN	NN	NN	BN	BN^s	NN	NN	BN	BN
		10	NN	NN	NN	NN	NN	NN	NN	BN	BN^s	BN	NN	NN	NN
		11	BN	NN	NN	NN	NN	NN^d	NN	NN	N^sN^s	BN	BN	NN	NN
		12	BN	NN	NN	BN	NN	BN	NN	BN	BN^s	BN	NN	NN	NN
		13	NN	BN	NN	NN	BN	NN	NN	BN	N^sN	NN	NN	BN	NN
		14	NN	BN	NN	BN	BN	NN^d	NN	BN	BN	NN	BN	BN	BN
		15	NN	NN	NN	NN	BN	NN^d	BN	BN	BN^s	NN	BN	BN	NN
		16	BN	BN	NN	BN	BN	BN	NN	BN	BN^s	BN	NN	NN	NN
		17	NN	BN	NN	BN	BN	BN	NN	BN	N^sN^s	BN	NN	NN	NN
		18	NN	NN	NN	NN	NN	BN^d	NN	BN	BN^s	BN	NN	NN	NN
		19	NN	BN	NN	NN	BN	NN^d	BN	BN	BN^s	BN	BN	NN	NN
		20	NN	NN	NN	NN	BN	NN^d	BN	BN	BN^s	NN	NN	BN	NN
		21	BN	BN	NN	BN	NN	BN	NN	BN	N^sN	NN	NN	NN	BN
		22	NN	NN	NN	BN	NN	NN^d	NN	BN	BN	BN	NN	NN	BN
		23	BN	BN	NN	BN	NN	NN^d	NN	NN	BN^s	NN	NN	NN	BN
BN, No. 2	NN, No. 1	1	BN	BN	NN	BN	BN	N^dN^d	BN	BN	BN	BN	NN	NN	NN
		2	NN	NN	NN	BN	BN	N^dN	BN	BN	NN^s	BN	BN	NN	NN
		3	BN	BN	NN	BN	BN	N^dN^d	BN	BN	NN	BN	NN	NN	NN
		4	BN	BN	NN	NN	BN	N^dN^d	NN	BN	BN	BN	NN	NN	NN
		5	NN	NN	NN	BN	NN	N^dN	NN	BN	BN^s	NN	BN	NN	NN
		6	NN	BN	NN	BN	NN	N^dN	NN	BN	NN	BN	BN	BN	BN
		7	BN	BN	NN	BN	BN	N^dN^d	BN	BN	BN^s	NN	NN	BN	NN
BN, Nos. 3~6	GD	1	NN	NN	NN	NN	BB	NN	BN	BB	BB	BB	BN	NN	NN
		2	BB	NN	NN	NN	NN	N^dN^d	BN	BN	BB	BB	NN	NN	BB
		3	BB	NN	NN	BB	BB	BB	NN	NN	NN	BB	BB	BN	BB
		4	BB	BB	NN	BB	NN	N^dN^d	BB	BN	BN	NN	BB	BB	BB
		5	NN	BB	NN	BB	BB	N^dN^d	NN	BB	BB	BB	BB	BB	BB
		6	NN	NN	NN	BB	BB	BB	NN	BB	N^sN^s	BB	BB	NN	NN
		7	BB	NN	NN	BB	BB	BB	BB	BB	BB	BB	NN	NN	BB
		8	BB	BB	NN	BB	BB	BN	NN	NN	BN	BB	BB	BB	NN

color of the backcrosses was examined one month after metamorphosis. Of 292 young frogs produced from a female hybrid, BN♀, No. 1, by backcrossing with the male *Rana nigromaculata*, 76 NN♂, No. 1, 71 were green (wild-type), 91 were greenish-olive, 64 were blue and the remaining 66 were bluish-olive. Of 225 young frogs produced from the other female hybrid, BN♀, No. 2, by backcrossing with the same male, 64 were green (wild-type), 59 were greenish-olive, 49 were blue and the remaining 53 were bluish-olive. In total, 135 (26.1%), 150 (29.0%), 113 (21.9%) and 119 (23.0%) were green (*XxIiEE*), greenish-olive (*XxiiEE*), blue (*xxIiEE*) and bluish-olive (*xxiiEE*), respectively. These numbers seemed to show that the four kinds of frogs appeared in nearly the same frequency.

From the remaining four female hybrids, 78 BN♀, Nos. 3~6, offspring were produced by diploid gynogenesis. When examined one month after meta-

TABLE 4

The constitution of each of the 13 bivalent chromosomes in each of 30 blue mutants of 134 mature female offspring produced from six female hybrids by backcrossing or diploid gynogenesis

Parents		Individual	Bivalent chromosome number												
Female	Male	no.	1	2	3	4	5	6	7	8	9	10	11	12	13
BN, No. 1	NN, No. 1	1	BN	BN	BN	BN	NN	NN	NN	NN	N^sN	NN	NN	NN	BN
		2	NN	BN	BN	BN	BN	NN^d	NN	NN	N^sN	NN	NN	NN	NN
		3	NN	BN	BN	NN	NN	BN^d	NN	NN	BN	BN	NN	NN	BN
		4	NN	NN	BN	BN	NN	NN	NN	NN	BN^s	NN	NN	BN	BN
		5	NN	NN	BN	NN	NN	BN	NN	NN	N^sN	BN	BN	BN	NN
		6	BN	NN	BN	BN	BN	NN^d	NN	NN	BN^s	BN	NN	NN	NN
		7	NN	BN	BN	NN	NN	BN	BN	BN	BN	BN	NN	NN	BN
		8	NN	BN	BN	BN	NN	NN	BN	NN	BN^s	NN	NN	NN	BN
		9	BN	NN	BN	NN	BN	BN^d	BN	NN	N^sN	NN	BN	NN	BN
		10	BN	BN	BN	BN	BN	BN^d	NN	NN	N^sN^s	BN	BN	BN	NN
		11	BN	NN	BN	BN	NN	BN	NN	NN	N^sN	NN	NN	BN	NN
		12	NN	BN	BN	BN	BN	BN^d	NN	NN	BN	NN	BN	BN	BN
		13	BN	NN	BN	BN	BN	NN^d	BN	BN	N^sN	NN	BN	NN	NN
		14	BN	BN	BN	NN	BN	BN	BN	NN	BN	BN	NN	BN	NN
		15	BN	BN	BN	NN	NN	BN	NN	BN	BN	BN	NN	BN	NN
		16	BN	BN	BN	NN	NN	BN	BN	NN	N^sN^s	BN	BN	NN	BN
		17	BN	BN	BN	BN	NN	BN^d	NN	NN	BN^s	NN	BN	NN	BN
		18	NN	NN	BN	NN	BN	NN	NN	NN	BN	BN	BN	BN	BN
BN, No. 2	NN, No. 1	1	BN	BN	BN	BN	BN	N^dN	NN	NN	NN	BN	BN	NN	NN
		2	BN	BN	BN	BN	BN	N^dN^d	$\frac{N}{B}N$	NN	BN	NN	NN	BN	BN
		3	BN	BN	BN	BN	BN	BN^d	NN	NN	BN	BN	NN	BN	BN
BN, Nos. 3~6	GD	1	NN	NN	BB	BB	NN	BN	BB	NN	N^sN^s	BB	BB	NN	BB
		2	NN	NN	BB	BB	NN	NN	BB	NN	N^sN^s	NN	NN	NN	BB
		3	NN	BB	BB	BB	NN	BB	NN	NN	N^sN^s	BB	BB	BB	NN
		4	BB	NN	BB	BB	BB	N^dN^d	NN	NN	N^sN^s	BB	BB	BN	BB
		5	NN	BB	BB	BB	BB	NN	BB	BB	N^sN^s	BB	NN	NN	BB
		6	BB	NN	BB	BB	BB	NN	BN	NN	BB	BB	NN	NN	BB
		7	NN	NN	BB	BB	NN	N^dN^d	BB	NN	BB	BB	NN	NN	BB
		8	NN	NN	BB	NN	NN	BB	BB	NN	BB	BB	BB	BB	BB
		9	NN	BB	BB	NN	NN	N^dN^d	BN	NN	N^sN^s	BB	NN	BN	NN

morphosis, it was found that 50 (32.1%) of 156 young frogs were green (*XXIIEE*), 47 (30.1%) were greenish-olive (*XXiiEE*), 29 (18.6%) were blue (*xxIIEE*) and the remaining 30 (19.2%) were bluish-olive (*xxiiEE*).

All the young frogs produced from backcrossing of female hybrid BN♀, No. 1 with male *Rana nigromaculata* NN♂, No. 1 and from female hybrids BN♀, Nos. 3~6 by diploid gynogenesis as well as 100 young frogs, including 25 green, 25 greenish-olive, 25 blue and 25 bluish-olive, produced from backcrossing of female hybrid BN♀, No. 2 with male *Rana nigromaculata* NN♂, No. 1 were continuously reared until attaining sexual maturity. From November 2, 1979 to June 19, 1980, lampbrush chromosomes were examined in 81 of 92 females produced from backcrossing, BN♀, No. 1×NN♂, No. 1, 19 of 21 females produced from BN♀, No. 2×NN♂, No. 1 and 34 of 156 females produced from female hybrids BN♀, Nos. 3~6 by diploid gynogenesis, 134 females in total. The dorsal colors of these 134 female frogs whose lampbrush chromosomes were observed are presented in Table 1. The results of examining the constitutions of lampbrush chromosomes in four kinds of female frogs which were green (wild-type), greenish-olive, blue and bluish-olive in dorsal color are presented in Tables 2~5.

TABLE 5

The constitution of each of the 13 bivalent chromosomes in each of 30 bluish-olive mutants of 134 mature offspring produced from six female hybrids by backcrossing or diploid gynogenesis

Parents		Indi-vidual	Bivalent chromosome number												
Female	Male	no.	1	2	3	4	5	6	7	8	9	10	11	12	13
BN, No. 1	NN, No. 1	1	BN	BN	NN	NN	NN	NN	BN	BN	N^sN	BN	NN	NN	NN
		2	BN	NN	NN	BN	BN	NN	NN	NN	N^sN	BN	NN	BN	NN
		3	BN	BN	NN	BN	BN	NN	NN	NN	BN	NN	NN	BN	NN
		4	BN	NN	NN	BN	NN	BN	BN	NN	BN	BN	BN	NN	BN
		5	BN	BN	NN	BN	BN	NN	BN	NN	BN	NN	NN	BN	NN
		6	BN	BN	NN	NN	BN	NN^d	BN	NN	N^sN	BN	NN	NN	NN
		7	BN	BN	NN	BN	NN	BN	BN	BN	BN	BN	NN	BN	BN
		8	NN	BN	NN	BN	BN	NN^d	BN	NN	BN	NN	BN	BN	NN
		9	NN	NN	NN	BN	BN	BN	BN	NN	BN^s	NN	BN	BN	BN
		10	NN	BN	NN	BN	NN	NN^d	NN	NN	N^sN	BN	BN	BN	BN
		11	NN	BN	NN	BN	BN	BN	BN	NN	BN	BN	BN	BN	NN
		12	NN	BN	NN	BN	BN	$\frac{B}{N}N$	BN	NN	N^sN	NN	NN	BN	BN
		13	BN	NN	NN	BN	BN	NN	NN	NN	BN	NN	BN	NN	BN
		14	BN	NN	NN	BN	BN	NN^d	NN	BN	BN	NN	BN	BN	NN
		15	NN	NN	NN	BN	BN	BN^d	BN	NN	BN^s	NN	BN	NN	BN
		16	NN	NN	NN	NN	BN	NN	BN	BN	BN^s	NN	NN	NN	NN
		17	BN	NN	NN	BN	NN	NN	BN	NN	BN^s	BN	NN	BN	NN
		18	BN	NN	NN	NN	NN	NN	NN	NN	BN^s	BN	BN	NN	NN
		19	BN	NN	NN	NN	BN	NN	NN	NN	BN^s	NN	BN	NN	BN
BN, No. 2	NN, No. 1	1	BN	NN	NN	NN	BN	BN	NN	NN	BN	BN	NN	NN	BN
		2	NN	BN	NN	BN	BN	N^dN^d	NN	NN	NN	NN	NN	NN	NN
		3	BN	NN	NN	BN	BN	N^dN	NN	NN	NN^s	NN	NN	NN	BN
		4	NN	NN	NN	NN	NN	N^dN	NN	NN	NN^s	BN	NN	BN	NN
BN, Nos. 3~6	GD	1	NN	BN	NN	BB	NN	BB	NN	NN	BB	BB	NN	BN	BB
		2	BB	NN	NN	BB	BB	NN	NN	NN	BB	BB	BB	BB	NN
		3	NN	BB	NN	BB	BB	NN	NN	NN	BB	BB	BB	BB	BB
		4	NN	NN	NN	NN	BB	NN	NN	NN	N^sN^s	BB	BB	BN	BB
		5	NN	BB	NN	NN	NN	BB	BB	BB	N^sN^s	BB	NN	NN	NN
		6	BB	NN	NN	BB	BB	BB	NN	NN	BB	BB	NN	BB	BB
		7	NN	NN	NN	BB	BB	NN	BB	BB	BB	BB	BB	BN	NN

2. Deficiency in *Rana nigromaculata* chromosome No. 6 of female backcrosses

Female hybrid BN♀, No. 1 had no deficiency in chromosome No. 6 derived from *Rana nigromaculata*, while there was a deficiency in one of the homologous chromosomes of bivalent No. 6 in the paternal *Rana nigromaculata*, NN♂, No. 1, which was a bluish-olive mutant. Of 81 female backcrosses produced from mating of female hybrid BN♀, No. 1 with male *Rana nigromaculata* NN♂, No. 1, 39 had bivalent chromosome No. 6 constituted of B and N, that is a *Rana brevipoda* chromosome and a *Rana nigromaculata* chromosome. If a *Rana nigromaculata* chromosome having a deficiency is called N^d, bivalent chromosome No. 6 in 12 females was BN^d, while it was BN in the other 27. Of the female backcrosses, the other 42 had bivalent chromosome No. 6 constituted of a pair of *Rana nigromaculata* chromosomes. In 18 of them, bivalent chromosome No. 6 was NN^d, while it was NN in the other 24 (Tables 2~5; Figs. 4 and 5).

Female hybrid BN♀, No. 2 had a deficiency in chromosome No. 6 derived from male *Rana nigromaculata* NN♂, No. 1. Of 19 female backcrosses produced from mating of female hybrid BN♀, No. 2 with male *Rana nigromaculata* NN♂,

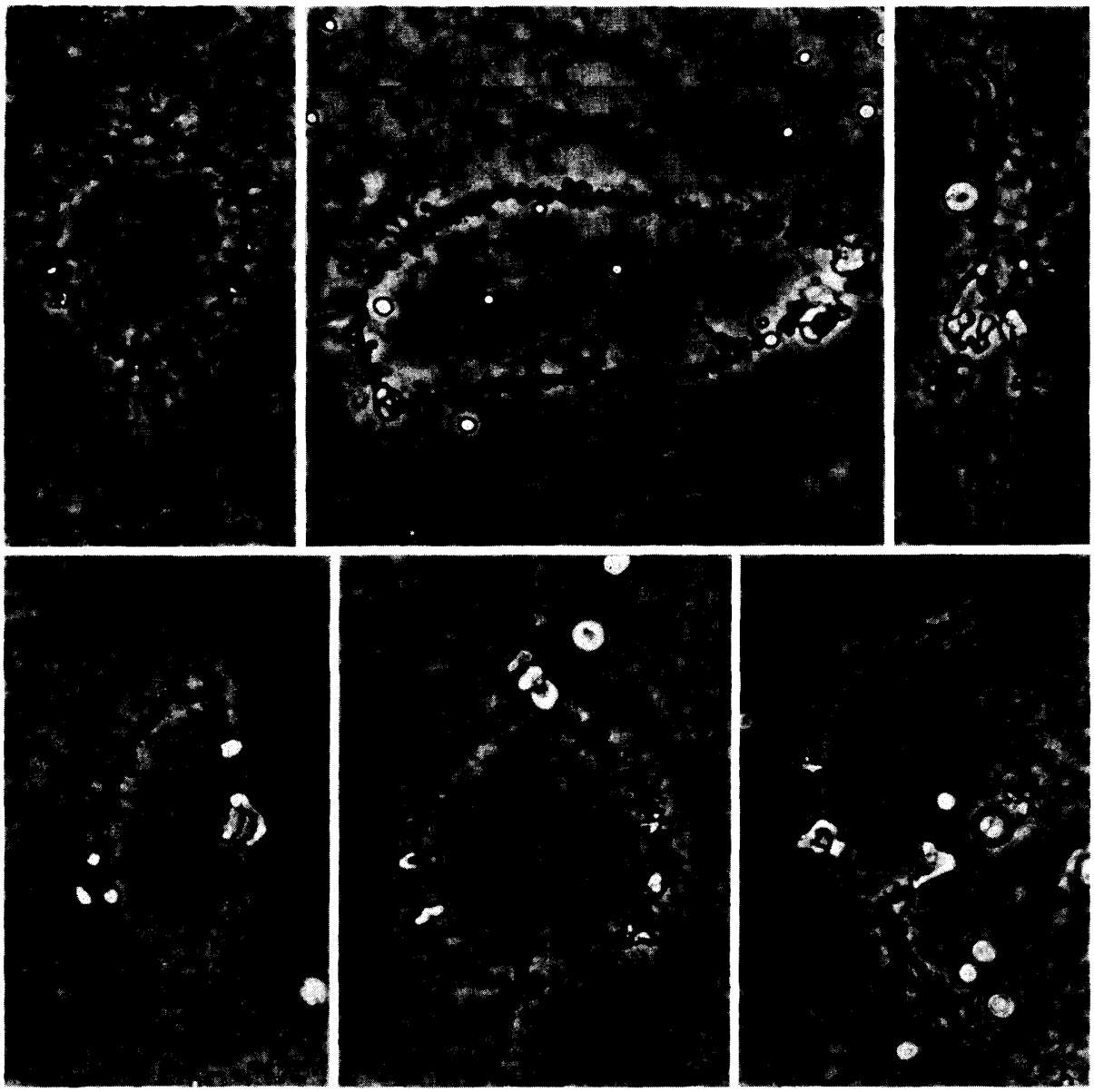

Fig. 4. Microphotographs of bivalent (lampbrush) chromosome No. 6 in oocytes of female offspring produced from female hybrids by backcrossing or diploid gynogenesis. ×500

a. A pair of *Rana brevipoda* chromosomes in bluish-olive mutant female No. 1 produced from BN♀, Nos. 3~6, by diploid gynogenesis.
b. A pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 12 produced from BN♀, No. 1×NN♂, No. 1.
c. A pair of *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 6 produced from BN♀, No. 1×NN♂, No. 1.
d. A pair of a *Rana brevipoda* chromosome and a *Rana nigromaculata* chromosome with a deficiency in wild-type female No. 5 produced from BN♀, No. 1×NN♂, No. 1.
e. A pair of *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 14 produced from BN♀, No. 1×NN♂, No. 1. One of the two chromosomes has a deficiency.
f. A pair of *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 1 produced from BN♀, No. 2×NN♂, No. 1. Each of the two chromosomes has a deficiency.

An arrow indicates deficiency of a simple giant loop.

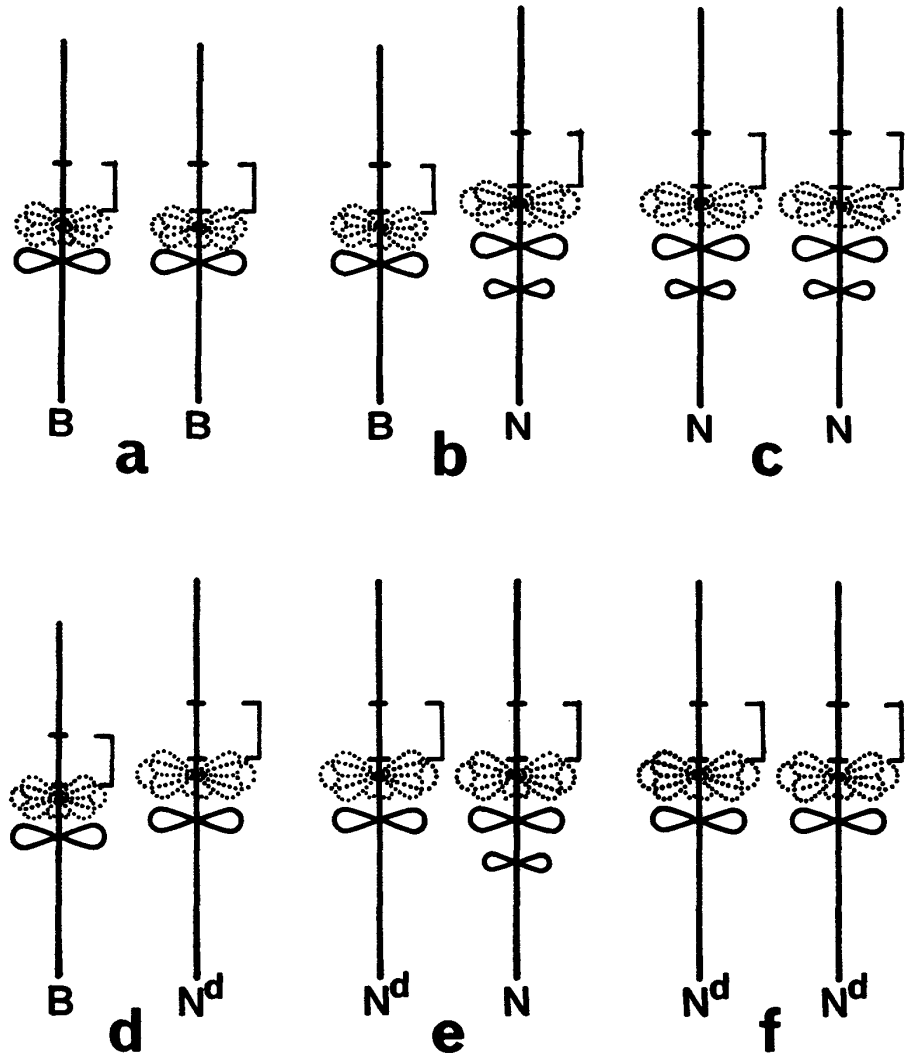

Fig. 5. Diagrams showing the constitution of bivalent chromosome No. 6 in oocytes of female offspring produced from female hybrids by backcrossing or diploid gynogenesis.

a. A pair of *Rana brevipoda* chromosomes in bluish-olive mutant female No. 1 produced from BN♀, Nos. 3~6, by diploid gynogenesis.

b. A pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 12 produced from BN♀, No. 1×NN♂, No. 1.

c. A pair of *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 6 produced from BN♀, No. 1×NN♂, No. 1.

d. A pair of a *Rana brevipoda* chromosome and a *Rana nigromaculata* chromosome with a deficiency in wild-type female No. 5 produced from BN♀, No. 1×NN♂, No. 1.

e. A pair of *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 14 produced from BN♀, No. 1×NN♂, No. 1. One of the two chromosomes has a deficiency.

f. A pair of *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 1 produced from BN♀, No. 2×NN♂, No. 1. Each of the two chromosomes has a deficiency.

A pair of short horizontal bars on each chromosome indicates the segment including the centromere.

No. 1, four had bivalent chromosome No. 6 constituted of B and N. While bivalent chromosome No. 6 in one of them was BN^d in constitution, it was BN in the other three. In nine of the other 15 female backcrosses, bivalent chromosome No. 6 was N^dN^d in constitution, while it was N^dN in the other six (Tables 2~5).

Two (Nos. 3 and 4) of female hybrids BN♀, Nos. 3~6 had a deficiency in chromosome No. 6 derived from *Rana nigromaculata*. Bivalent chromosome No. 6 was examined in 34 mature females produced from the four female hybrids (BN♀, Nos. 3~6) by diploid gynogenesis. The results showed that no deficiency was found in 12 of 20 females which were NN in constitution of bivalent chromosome No. 6, while bivalent chromosome No. 6 in the other eight females was N^dN^d, that is, each homologous chromosome had a deficiency. Bivalent chromosome No. 6 of two other females was BN, in which no deficiency was found. In the remaining 12 females, bivalent chromosome No. 6 was BB in constitution (Tables 2~5).

3. Inversion and deficiency in *Rana nigromaculata* chromosome No. 9 of female backcrosses

Female hybrid BN♀, No. 1 had an inversion and a deficiency in chromosome No. 9 derived from *Rana nigromaculata*. The constitution of bivalent chromosome No. 9 was examined in 81 female backcrosses produced from mating of female hybrid BN♀, No. 1 with the paternal *Rana nigromaculata* which had an inversion and a deficiency in chromosome No. 9. It was found that bivalent chromosome No. 9 was constituted of a pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes in 46 of them. If a *Rana nigromaculata* chromosome having an inversion and a deficiency is called N^s, bivalent chromosome No. 9 was BN^s in 26 females, while it was BN in the other 20. Bivalent chromosome No. 9 was constituted of a pair of *Rana nigromaculata* chromosomes in 35 other female backcrosses. In 13 of them, bivalent chromosome No. 9 was N^sN^s, while it was N^sN in the other 22 (Tables 2~5; Figs. 6 and 7).

Female hybrid BN♀, No. 2 had chromosome No. 9 which had neither inversion nor deficiency. The constitution of bivalent chromosome No. 9 was examined in 19 female backcrosses produced from female hybrid BN♀, No. 2 mated with the male *Rana nigromaculata* (76 NN♂, No. 1) having an inversion and a deficiency in chromosome No. 9. The results showed that bivalent chromosome No. 9 was BN^s in two of nine female backcrosses whose bivalent chromosome No. 9 was constituted of a pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes, while it was BN in the other seven. Of 10 female backcrosses whose bivalent chromosome No. 9 was constituted of a pair of *Rana nigromaculata* chromosomes, four was NN^s, while the other six was NN in constitution (Tables. 2~5).

Of female hybrids BN♀, Nos. 3~6, three (Nos. 3~5) had an inversion and a deficiency in chromosome No. 9 derived from *Rana nigromaculata*. The other female hybrid (No. 6) had neither inversion nor deficiency in *Rana nigromaculata* chromosome No. 9. Lampbrush chromosomes were observed in 34 mature

females produced from the four hybrids (Nos. 3~6) by diploid gynogenesis. The results showed that bivalent chromosome No. 9 was constituted of a pair of *Rana nigromaculata* chromosomes in 12 females. In 11 of the latter, it was N^sN^s in constitution, while it was NN in the remainder. Bivalent chromosome No. 9 was constituted of a pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes in

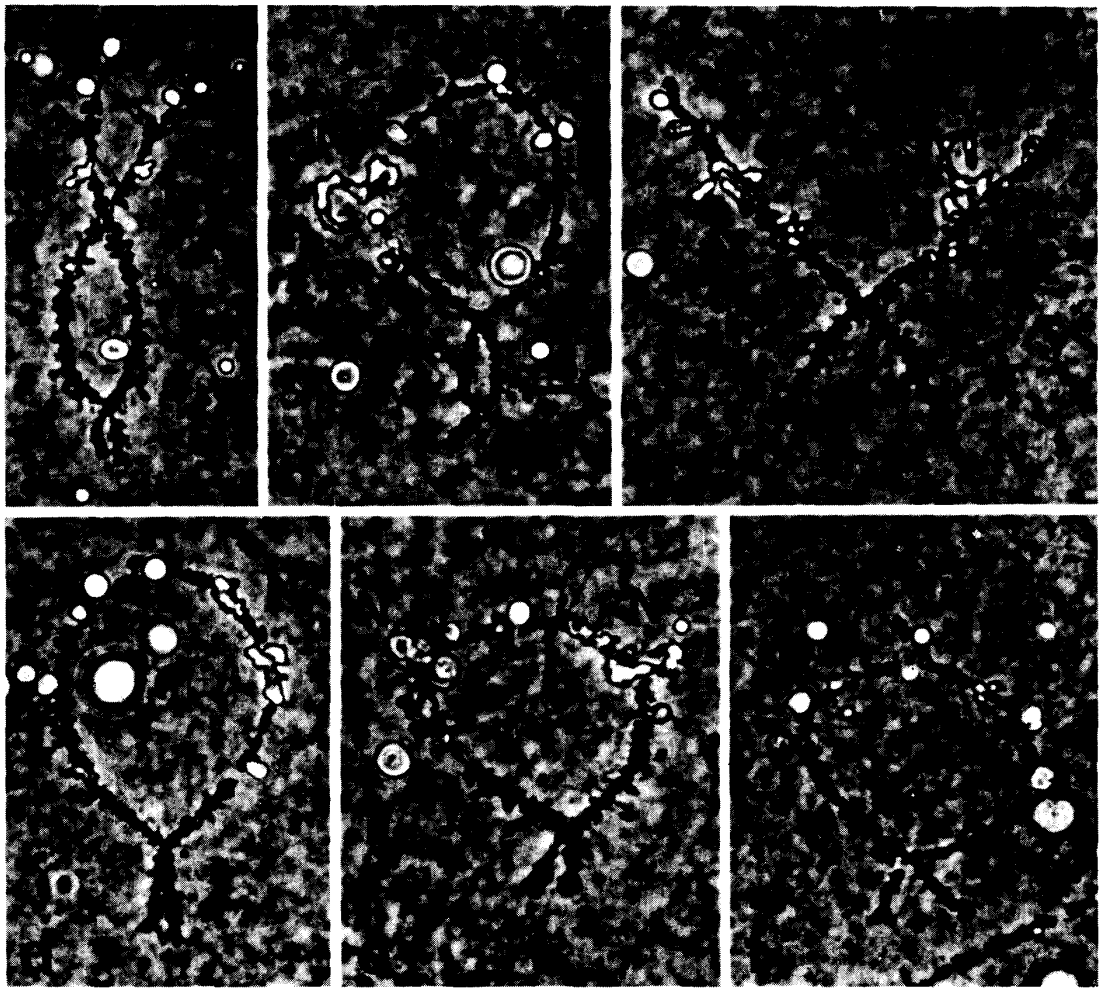

Fig. 6. Microphotographs of bivalent (lampbrush) chromosome No. 9 in oocytes of female offspring produced from female hybrids by backcrossing or diploid gynogenesis. ×500

a. A pair of *Rana brevipoda* chromosomes in blue mutant female No. 6 produced from BN♀, Nos. 3~6, by diploid gynogenesis.
b. A pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes in wild-type female No. 2 produced from BN♀, No. 2×NN♂, No. 1.
c. A pair of *Rana nigromaculata* chromosomes in bluish-olive mutant female No. 2 produced from BN♀, No. 2×NN♂, No. 1.
d. A pair of a *Rana brevipoda* chromosome and a *Rana nigromaculata* chromosome with an inversion and a deficiency in greenish-olive mutant female No. 19 produced from BN♀, No. 1×NN♂, No. 1.
e. A pair of *Rana nigromaculata* chromosomes in wild-type female No. 6 produced from BN♀, No. 1×NN♂, No. 1. One of the two chromosomes has an inversion and a deficiency.
f. A pair of *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 7 produced from BN♀, No. 1×NN♂, No. 1. Each of the two chromosomes has an inversion and a deficiency.

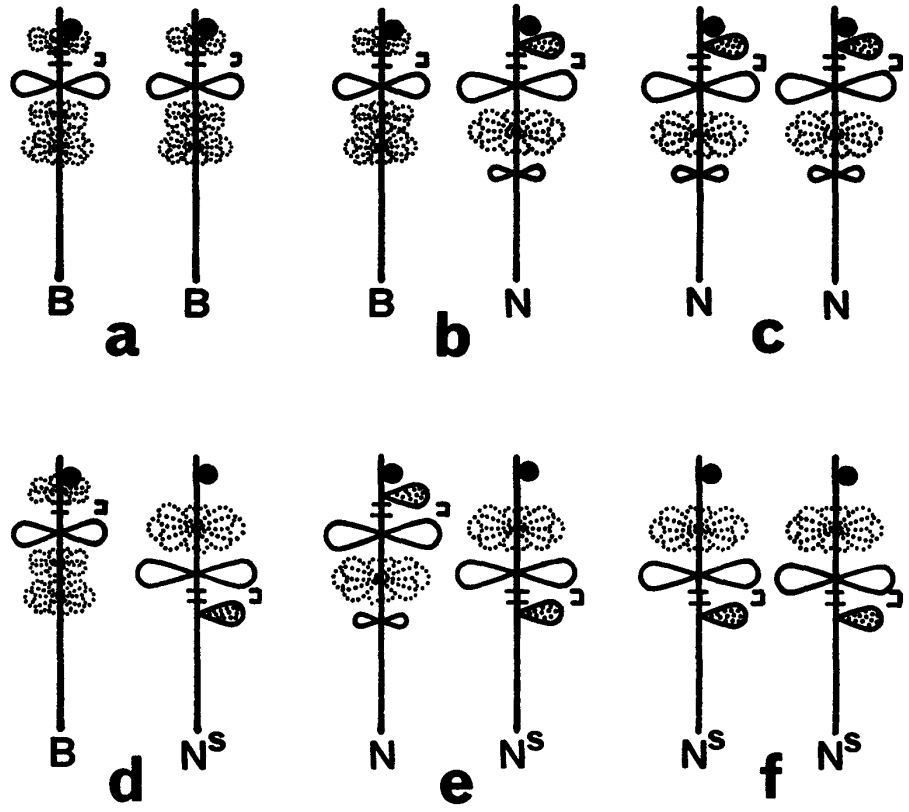

Fig. 7. Diagrams showing the constitution of bivalent chromosome No. 9 in oocytes of female offspring produced from female hybrids by backcrossing or diploid gynogenesis.

a. A pair of *Rana brevipoda* chromosomes in blue mutant female No. 6 produced from BN♀, Nos. 3~6, by diploid gynogenesis.

b. A pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes in wild-type female No. 2 produced from BN♀, No. 2×NN♂, No. 1.

c. A pair of *Rana nigromaculata* chromosomes in bluish-olive mutant female No. 2 produced from BN♀, No. 2×NN♂, No. 1.

d. A pair of a *Rana brevipoda* chromosome and a *Rana nigromaculata* chromosome with an inversion and a deficiency in greenish-olive mutant female No. 19 produced from BN♀, No. 1×NN♂, No. 1.

e. A pair of *Rana nigromaculata* chromosomes in wild-type female No. 6 produced from BN♀, No. 1×NN♂, No. 1. One of the two chromosomes has an inversion and a deficiency.

f. A pair of *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 7 produced from BN♀, No. 1×NN♂, No. 1. Each of the two chromosomes has an inversion and a deficiency.

A pair of short horizontal bars on each chromosome indicates the segment including the centromere.

three females. Neither inversion nor deficiency was found in chromosome No. 9 derived from *Rana nigromaculata*. Bivalent chromosome No. 9 was constituted of a pair of *Rana brevipoda* chromosomes in the remaining 19 females (Tables 2~5).

III. Chromosome bearing the locus for an olive or a blue mutant gene

The recessive olive mutant gene (*i*) of greenish- and bluish-olive frogs as well as the recessive blue mutant gene (*x*) was situated on a *Rana nigromaculata* chromosome (N), while the dominant gene (*I* or *X*) was situated on a *Rana brevipoda* chromosome (B). Thus, in the backcrosses produced from female hybrids (*XxIi* in genotype) by mating with the paternal *Rana nigromaculata* (*xxii* in genotype) as well as in the offspring produced from female hybrids (*XxIi* in genotype) by diploid gynogenesis, the bivalent chromosomes bearing the recessive genes in phenotypically olive or blue frogs should be a pair of *Rana nigromaculata* chromosomes. In contrast, the bivalent chromosomes in phenotypically green (wild-type) frogs should be a pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes or a pair of *Rana brevipoda* chromosomes.

A total of 134 offspring produced from the six female hybrids (BN♀, Nos. 1～6) by backcrossing with the paternal *Rana nigromaculata* or by diploid gynogenesis were divided in phenotypic color into four groups, green (wild-type), greenish-olive, blue and bluish-olive. The constitution of each of the 13 bivalent chromosomes in each of the females belonging to each group is presented in Tables 2～5. In each of the four groups, the number of females which had a pair of *Rana nigromaculata* chromosomes (NN), a pair of *Rana brevipoda* chromosomes (BB) or a pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes (BN) in constitution of each of the 13 bivalent chromosomes is presented in Table 6.

TABLE 6

Number of females which were NN, BN or BB in constitution of each of the 13 bivalent chromosomes in their oocytes in four kinds of mature female offspring produced from six female hybrids by backcrossing or diploid gynogenesis

Phenotype and Genotype	No. of frogs	Constitution of bivalent chromosomes	Bivalent chromosome number												
			1	2	3	4	5	6	7	8	9	10	11	12	13
Green (wild-type)		NN	19	16	0	9	5	15	21	4	16	13	23	21	21
XXIIEE	36	BN	12	15	26	20	23	17	9	25	13	15	9	12	9
		BB	5	5	10	7	8	4	6	7	7	8	4	3	6
Greenish-olive		NN	19	17	38	15	12	27	21	6	16	16	23	24	23
XXiiEE	38	BN	14	18	0	17	20	8	15	28	18	15	10	11	10
		BB	5	3	0	6	6	3	2	4	4	7	5	3	5
Blue		NN	15	13	0	10	16	15	17	26	15	11	17	16	11
xxIIEE	30	BN	13	14	21	13	11	13	8	3	12	11	9	12	12
		BB	2	3	9	7	3	2	5	1	3	8	4	2	7
Bluish-olive		NN	14	17	30	9	9	20	16	24	10	12	16	12	16
xxiiEE	30	BN	14	11	0	16	16	7	12	4	15	11	10	15	10
		BB	2	2	0	5	5	3	2	2	5	7	4	3	4

1. Olive mutant gene

Of the 134 female offspring of female hybrids BN♀, Nos. 1～6, 68 olive mutants

including 38 greenish-olive and 30 bluish-olive were all NN in constitution of bivalent chromosome No. 3. In each of the other 12 bivalent chromosomes, 21～47 of the 68 olive mutants were NN in constitution. None of 66 female offspring including 36 green (wild-type) and 30 blue was NN in constitution of bivalent chromosome No. 3, while all of them were BN or BB. In each of the other 12 bivalent chromosomes, 19～40 of the 66 green or blue females were NN in constitution. Thus, the olive mutant gene is assumed to be located in chromosome No. 3 (Table 6; Figs. 8 and 9).

On the other hand, the number of frogs whose alleles at the locus for the olive mutant gene agreed in constitution with each of the 13 bivalent chromosomes in

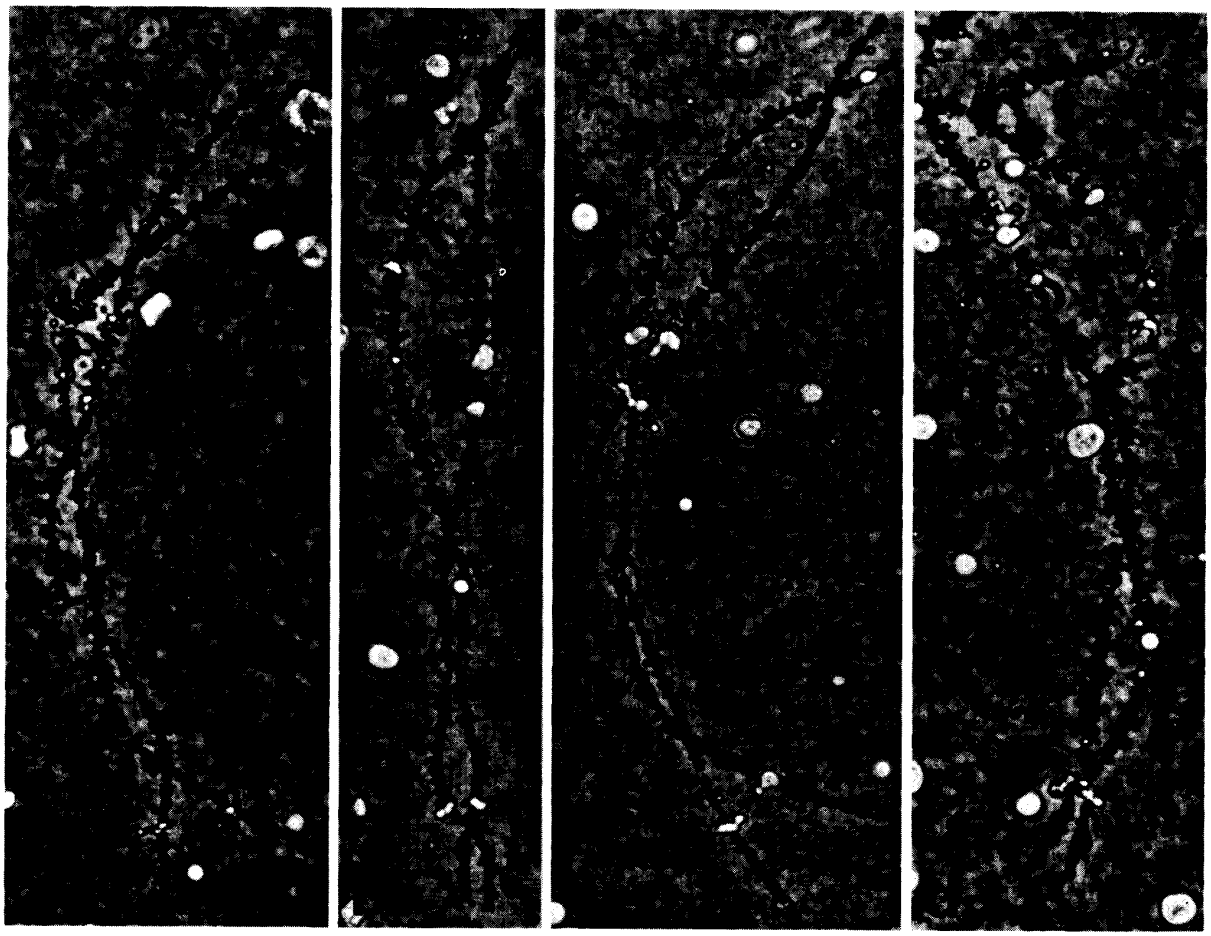

Fig. 8. Microphotographs of bivalent (lampbrush) chromosome No. 3 in oocytes of female offspring produced from female hybrids by backcrossing or diploid gynogenesis. ×350

a. A pair of *Rana brevipoda* chromosomes in blue mutant female No. 6 produced from BN♀, Nos. 3～6, by diploid gynogenesis.

b. A pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes in wild-type female No. 2 produced from BN♀, No. 1×NN♂, No. 1.

c. A pair of *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 1 produced from BN♀, No. 2×NN♂, No. 1.

d. A pair of *Rana nigromaculata* chromosomes in wild-type female No. 3 produced from BN♀, No. 1×NN♂, No. 1. One of the two chromosomes has a translocation from a *Rana brevipoda* chromosome.

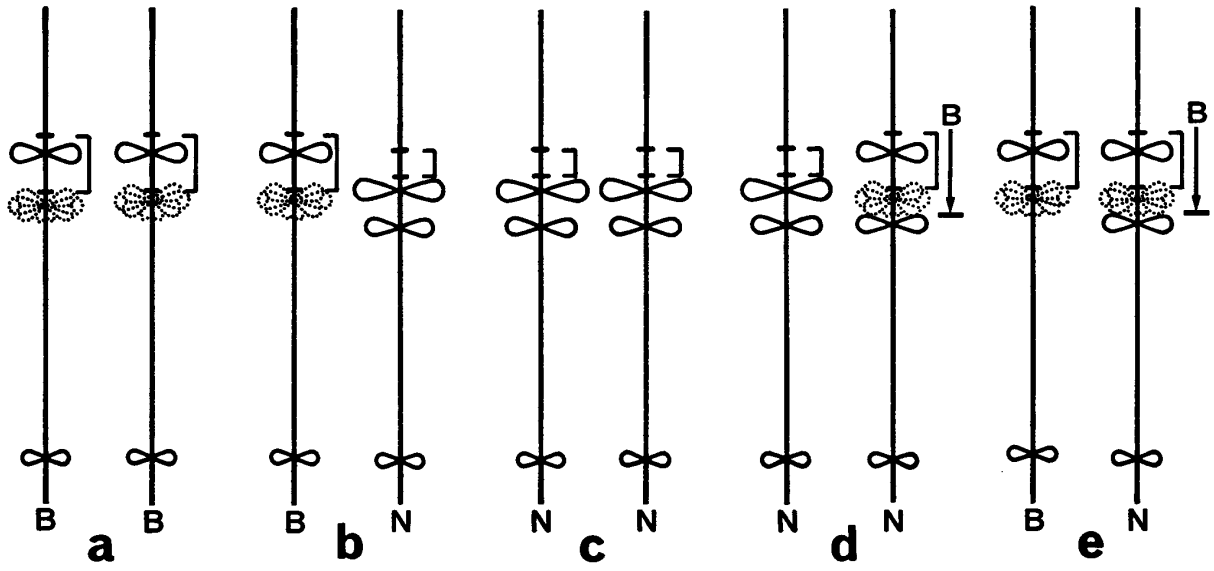

Fig. 9. Diagrams showing the constitution of bivalent chromosome No. 3 in oocytes of female offspring produced from female hybrids by backcrossing or diploid gynogenesis.

a. A pair of *Rana brevipoda* chromosomes in blue mutant female No. 6 produced from BN♀, Nos. 3~6, by diploid gynogenesis.

b. A pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes in wild-type female No. 2 produced from BN♀, No. 1×NN♂, No. 1.

c. A pair of *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 1 produced from BN♀, No. 2×NN♂, No. 1.

d. A pair of *Rana nigromaculata* chromosomes in wild-type female No. 3 produced from BN♀, No. 1×NN♂, No. 1. One of the two chromosomes has a translocation from a *Rana brevipoda* chromosome.

e. A pair of a *Rana brevipoda* chromosome and a *Rana nigromaculata* chromosome with a translocation from a *Rana brevipoda* chromosome in wild-type female No. 7 produced from BN♀, Nos. 3~6, by diploid gynogenesis.

A pair of short horizontal bars on each chromosome indicates the segment including the centromere.

TABLE 7

Number of females whose constitution of alleles at the locus for the olive mutant gene agreed with that of each of the 13 bivalent chromosomes in 134 female offspring produced from female hybrids by backcrossing or diploid gynogenesis

Parents		No. of	Bivalent chromosome number												
Female	Male	frogs	1	2	3	4	5	6	7	8	9	10	11	12	13
BN, No. 1	NN, No. 1	81	40	43	**81**	42	39	56	35	41	36	42	40	42	43
BN, No. 2	NN, No. 1	19	10	11	**19**	11	11	13	9	8	10	10	11	12	10
BN, Nos. 3~6	GD	34	15	17	**34**	18	16	15	22	17	15	18	14	12	20
Total		134	65	71	**134**	71	66	84	66	66	61	70	65	66	73
		(%)	(48.5)	(53.0)	**(100)**	(53.0)	(49.3)	(62.7)	(49.3)	(49.3)	(45.5)	(52.2)	(48.5)	(49.3)	(54.5)

their oocytes was counted in the 134 female offspring of the six female hybrids. It was found that the constitution of alleles agreed with that of bivalent chromosome No. 3 in all the females, while it agreed with that of each of the other 12

bivalent chromosomes in 61 (45.5%)~84 (62.7%) females (Table 7).

Of the 134 female offspring, 68 were NN, 46 were BN and 18 were BB in constitution of bivalent chromosome No. 3. One of the remaining two was NN having a translocation from B and the other was BN having a translocation from B (Figs. 8d, 9d and 9e). The constitution of bivalent chromosome No. 3 in each of these female offspring completely agreed with that of the alleles at the locus of the olive mutant gene (Table 8).

TABLE 8

Relationship between the constitution of bivalent chromosome No. 3 and that of alleles at the locus for the olive mutant gene in 134 female offspring produced from female hybrids by backcrossing or diploid gynogenesis

Kind	Bivalent chromosome		Olive mutation			No. of frogs whose chromosomes were compared with the genotypes	
	Constitution	No. of frogs	Pheno-type	Geno-type	No. of frogs	Agree	Disagree
BN♀×NN♂	NN	53	Olive	*ii*	53	53	0
	BN	46	Wild	*Ii*	46	46	0
	$\frac{B}{N}$N	1	Wild	*Ii*	1	1	0
	Total	100			100	100	0
BN♀ GD	NN	15	Olive	*ii*	15	15	0
	BB	18	Wild	*II*	18	18	0
	$\frac{B}{N}$B	1	Wild	*II*	1	1	0
	Total	34			34	34	0
	NN	68	Olive	*ii*	68	68	0
	BN	46	Wild	*Ii*	46	46	0
	BB	18	Wild	*II*	18	18	0
	$\frac{B}{N}$N	1	Wild	*Ii*	1	1	0
	$\frac{B}{N}$B	1	Wild	*II*	1	1	0
	Total	134			134	134 ($\chi^2=134$, $P<0.00001$)	0

2. Blue mutant gene

Of the 134 female offspring of female hybrids BN♀, Nos. 1~6, 60 were blue mutants. In 50 of these 60 blue mutants, bivalent chromosome No. 8 was NN in constitution, while it was BN in seven and BB in the remaining three blue mutants. In each of the other 12 bivalent chromosomes, 19~35 of the 60 blue mutants were NN in constitution. Of 74 green (wild-type) or greenish-olive frogs, 10 were NN in constitution of bivalent chromosome No. 8, while 64 were BN or BB. In each of the other 12 bivalent chromosomes, 17~46 of the 74 frogs were NN in constitution. Thus, it was assumed that the blue mutant gene was located on chromosome No. 8 (Table 6; Figs. 10 and 11).

On the other hand, the number of frogs whose alleles at the locus for the blue mutant gene agreed in constitution with each of the 13 bivalent chromosomes

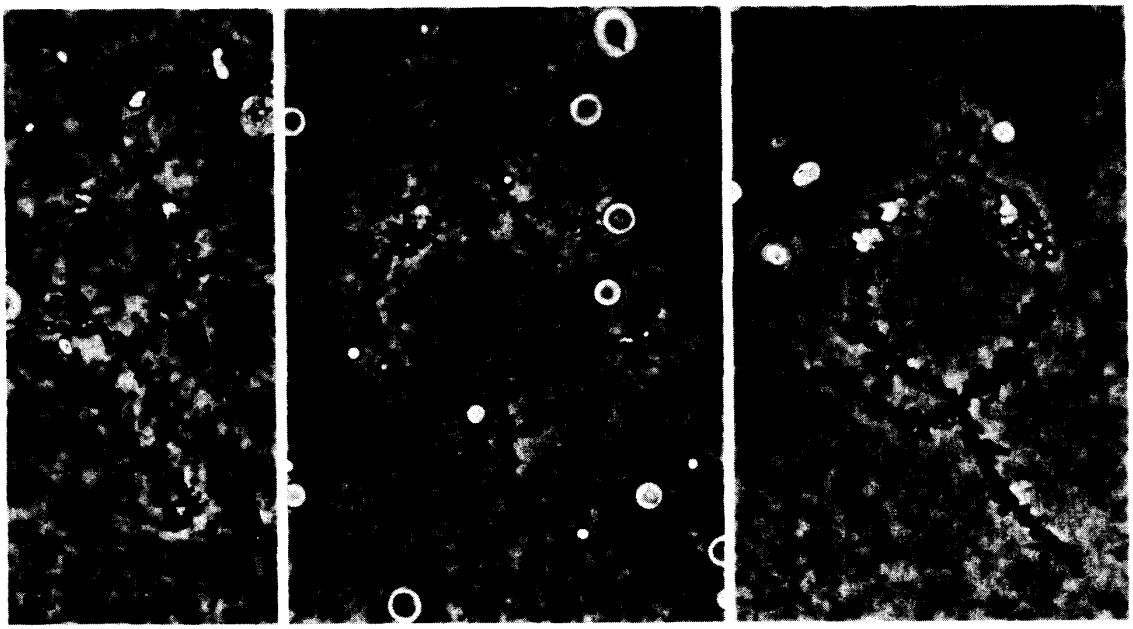

Fig. 10. Microphotographs of bivalent (lampbrush) chromosome No. 8 in oocytes of female offspring produced from female hybrids by backcrossing or diploid gynogenesis. ×500

a. A pair of *Rana brevipoda* chromosomes in wild-type female No. 6 produced from BN♀, Nos. 3～6, by diploid gynogenesis.

b. A pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 1 produced from BN♀, No. 1×NN♂, No. 1.

c. A pair of *Rana nigromaculata* chromosomes in blue mutant female No. 6 produced from BN♀, Nos. 3～6, by diploid gynogenesis.

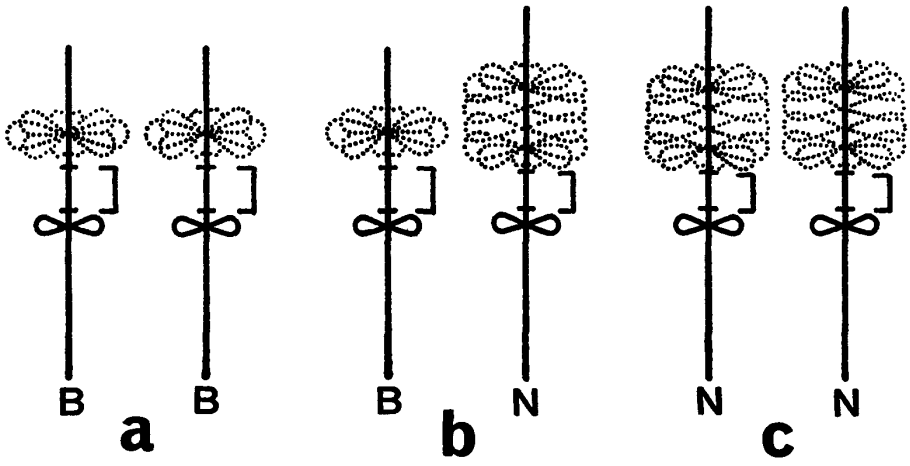

Fig. 11. Diagrams showing the constitution of bivalent chromosome No. 8 in oocytes of female offspring produced from female hybrids by backcrossing or diploid gynogenesis.

a. A pair of *Rana brevipoda* chromosomes in wild-type female No. 6 produced from BN♀, Nos. 3～6, by diploid gynogenesis.

b. A pair of *Rana brevipoda* and *Rana nigromaculata* chromosomes in greenish-olive mutant female No. 1 produced from BN♀, No. 1×NN♂, No. 1.

c. A pair of *Rana nigromaculata* chromosomes in blue mutant female No. 6 produced from BN♀, Nos. 3～6, by diploid gynogenesis.

A pair of short horizontal bars on each chromosome indicates the segment including the centromere.

in their oocytes was counted in the 134 female offspring produced from the six female hybrids (BN♀, Nos. 1~6). As presented in Table 9, the constitution of alleles agreed with that of bivalent chromosome No. 8 in 114 (85.1%) of them ($\chi^2=65.9$, $P<0.00001$), while it agreed with that of each of the other 12 bivalent chromosomes in 57 (42.5%)~82 (61.2%).

TABLE 9

Number of females whose constitution of alleles at the locus for the blue mutant gene agreed with that of each of the 13 bivalent chromosomes in 134 female offspring produced from female hybrids by backcrossing or diploid gynogenesis

Parents		No. of	Bivalent chromosome number												
Female	Male	frogs	1	2	3	4	5	6	7	8	9	10	11	12	13
BN, No. 1	NN, No. 1	81	35	42	40	39	48	43	36	**68**	35	39	31	38	36
BN, No. 2	NN, No. 1	19	8	11	9	13	11	7	12	**18**	10	12	11	8	4
BN, Nos. 3~6	GD	34	22	18	17	17	23	18	17	**28**	22	17	19	13	17
Total		134	65	71	66	69	82	68	65	**114**	67	68	61	59	57
		(%)	(48.5)	(53.0)	(49.3)	(51.5)	(61.2)	(50.7)	(48.5)	**(85.1)**	(50.0)	(50.7)	(45.5)	(44.0)	(42.5)

Of the 134 female offspring analyzed, 60 were NN, 60 were BN and the remaining 14 were BB in constitution of bivalent chromosome No. 8 (Table 10). In 50, 53 and 11, respectively, of these numbers of female offspring, the constitution of bivalent chromosome No. 8 agreed with that of the alleles at the locus of the blue mutant gene, while it did not agree with the latter in 10, 7 and 3 female offspring, respectively (Table 10).

TABLE 10

Relationship between the constitution of bivalent chromosome No. 8 and that of alleles at the locus for the blue mutant gene in 134 female offspring produced from female hybrids by backcrossing or diploid gynogenesis

Kind	Bivalent chromosome		Blue mutation			No. of frogs whose chromosomes were compared with the genotypes	
	Consti-tution	No. of frogs	Pheno-type	Geno-type	No. of frogs	Agree	Disagree
BN♀×NN♂	NN	44	Blue	*xx*	44	37	7
	BN	56	Wild	*Xx*	56	49	7
	Total	100			100	86	14
BN♀ GD	NN	16	Blue	*xx*	16	13	3
	BN	4	Wild	*Xx*	4	4	0
	BB	14	Wild	*XX*	14	11	3
	Total	34			34	28	6
	NN	60	Blue	*xx*	60	50	10
	BN	60	Wild	*Xx*	60	53	7
	BB	14	Wild	*XX*	14	11	3
	Total	134			134	114 ($\chi^2=65.9$, $P<0.0001$)	20

IV. Linkage of the locus for the olive mutation with those for two enzymes

The results of the above examinations showed that the olive and blue mutant genes are located on chromosomes Nos. 3 and 8, respectively. On the other hand, NISHIOKA, OHTANI and SUMIDA (1980) has reported that the locus for MDH-B is situated on chromosome No. 3, together with the findings that the loci for Ab, α-GDH, LDH-B, IDH-B and Hb, and protein C are located on chromosomes Nos. 1, 2, 4, 6 and 9, respectively.

Recently, the electrophoretic patterns of Ab, protein C and Hb obtained from blood, IDH-B, LDH-B, MDH-B and α-GDH extracted from skeletal muscles, and six other enzymes, ME-A, ME-B, ADH-A, Pep-A, Pep-B and Pep-C, were examined in 100 female offspring of female hybrids (BN) backcrossed with male *Rana nigromaculata* (NN). The genotype of each of these proteins was compared in constitution with each of the 13 bivalent (lampbrush) chromosomes in the oocytes of the 100 females in order to determine the chromosome on which the locus is situated. As shown in Table 11, the results added bivalent chromosome No. 5 bearing the locus for Pep-A to the six stated above.

TABLE 11

Percentages of 100 female frogs (BN♀ × NN♂) whose genotypes in each locus agreed in constitution with each of the 13 bivalent chromosomes in their oocytes

Locus	Bivalent chromosome number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
ii gene	50	54	**100**	53	50	69	44	59	46	52	50	54	53
xx gene	43	53	49	52	59	50	48	**86**	45	51	41	46	40
Ab	**93**	51	51	60	53	48	51	44	49	55	43	47	50
Hb	53	57	69	46	41	**98**	50	50	53	57	51	53	58
Prot-C	47	51	50	48	53	52	45	52	**93**	57	51	59	52
IDH-B	54	56	70	49	44	**96**	53	51	52	56	52	54	57
LDH-B	62	58	52	**91**	60	46	44	47	52	42	44	50	51
α-GDH	53	**79**	52	60	63	49	43	56	49	53	35	52	44
MDH-B	51	49	**87**	58	51	64	39	54	53	47	45	47	52
ME-A	48	**70**	49	55	60	52	48	57	44	48	32	53	41
ME-B	51	55	**88**	50	45	64	43	46	45	55	49	49	48
ADH-A	**90**	54	50	55	56	47	52	49	50	54	46	48	47
Pep-A	58	58	58	53	**70**	52	62	53	52	48	44	54	43
Pep-B	57	57	57	**74**	47	54	42	40	47	45	51	62	50
Pep-C	50	**96**	55	59	56	56	46	51	52	50	44	52	49

The genotypes of Ab and ADH-A agreed in constitution with bivalent chromosome No. 1 in 93% and 90%, respectively, and the genotypes of α-GDH, ME-A and Pep-C agreed with bivalent chromosome No. 2 in 79%, 70% and 96%, respectively. The genotypes of MDH-B and ME-B agreed with bivalent chromosome No. 3 in 87% and 88%, respectively, the recombination rates being 13% and 12%, respectively. The two loci for these enzymes constitute a linkage group together with the locus for the olive mutation on bivalent chromosome

No. 3. The genotypes of LDH–B and Pep–B agreed with bivalent chromosome No. 4 in 91% and 74%, respectively, that of Pep–A agreed with bivalent chromosome No. 5 in 70%, those of Hb and IDH–B agreed with bivalent chromosome No. 6 in 98% and 96%, respectively, and that of protein C agreed with bivalent chromosome No. 9 in 93%.

As the locus for the olive mutation agreed in constitution with bivalent chromosome No. 3 in 100%, this locus is assumed to be situated near the centromere. In one (No. 3) of wild-type female offspring of female hybrid BN♀, No. 1 backcrossed with male *Rana nigromaculata* NN♂, No. 1, bivalent chromosome No. 3 consisted of a pair of *Rana nigromaculata* chromosomes, one of which had a translocated portion of a *Rana brevipoda* chromosome in the short arm $\left(\frac{B}{N}\right)$ (Tables 2 and 8; Figs. 8d and 9d). It is assumed that the locus for the olive mutation is situated near the centromere on the short arm of *Rana nigromaculata* chromosome No. 3 (Fig. 12). The genotype (*Ii*) agreed in constitution with the BN portion of the short arm where the translocation occurred. In this female, the genotype of ME–B agreed in constitution with the NN portion of the bivalent chromosome, while that of MDH–B agreed with the BN portion. Thus, it is assumed that the locus for MDH–B is situated on the short arm of bivalent chromosome No. 3 at the site of 13% in recombination rate from the centromere, while the locus for ME–B is situated on the long arm at the site of 12% in recombination rate from the centromere.

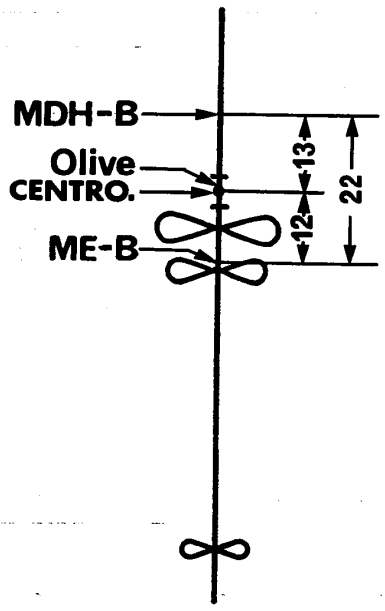

Fig. 12. Diagram showing the locus for the olive gene on the short arm of *Rana nigromaculata* chromosome No. 3.

DISCUSSION

1. Color mutations and linkage

Various color mutations have been reported by Moore (1942), Volpe (1955,

1956, 1960, 1961), ANDERSON and VOLPE (1958), VOLPE and DASGUPTA (1962), BROWDER (1968, 1972), RICHARDS, TARTOF and NACE (1969) and SMITH-GILL, RICHARDS and NACE (1972) in *Rana pipiens*, by EALES (1933) and SMALLCOMBE (1949) in *Rana temporaria*, by TOKUNAGA (1949), MORIYA (1952) and NISHIOKA and UEDA (1977a, 1985a) in *Rana nigromaculata*, by DAITO (1968) and NISHIOKA and UEDA (1977b, 1985c) in *Hyla arborea japonica*, by HOPERSKAYA (1975) in *Xenopus laevis*, by FROST, ELLINGER and MURPHY (1982) in *Bombina orientalis* and by NISHIOKA and UEDA (1985b) in *Rhacophorus schlegelii*.

NISHIOKA (1977) has reported that nine kinds of color mutations, blue, grayish-brown, greenish-olive, bluish-olive, yellowish-olive, brownish-olive, albino, gray-eyed and black-eyed, were produced by irradiation of eggs or spermatozoa of *Rana nigromaculata* with X-rays or neutrons. Of these color mutations, albino, gray-eyed and black-eyed are due to single recessive genes, *m*, *g* and *b*, respectively. Of the other six mutations, blue, greenish-olive and bluish-olive are controlled by recessive genes, *xx*, *ii* and *xxii*, respectively, in addition to the presence of a single dominant gene *E*, while grayish-brown, yellowish-olive and brownish-olive are controlled by recessive genes, *xx*, *ii* and *xxii*, respectively, in addition to the presence of single recessive genes *ee*. NISHIOKA and UEDA (1977a) have confirmed that all the three kinds of dermal chromatophores, xanthophores, iridophores and melanophores, are expanded by gene *E*, while they are contracted by genes *ee*. In the presence of *xx*, no carotenoid vesicles are produced in each xanthophore, while in the presence of genes *ii*, the reflecting platelets become remarkably fewer, smaller and thinner in each iridophore.

The fact that the albinism in amphibians at least is not so single as generally considered has been found in *Hyla arborea japonica* (NISHIOKA and UEDA, 1977b) and in *Rana nigromaculata* (NISHIOKA and UEDA, 1985a). The albinos of *Hyla arborea japonica* are sorted into three groups which have different loci on autosomes, while those of *Rana nigromaculata* are divided into five groups having different loci on autosomes.

Linkage of two mutant genes has been first reported by HUMPHREY (1959) in *Ambystoma mexicanum*. According to him, the mutant genes for fluid imbalance (*f*) and abnormalities of the gills (*g*) have loci close together on a chromosome. VOLPE (1970) and NACE, RICHARDS and ASHER (1970) have found that diploid gynogenesis is useful for detecting linkage relationship. They have mapped the loci for the two dominant mutations, kandiyohi (*K*) and burnsi (*B*), on an autosome in *Rana pipiens*. WRIGHT, RICHARDS and NACE (1980), WRIGHT and RICHARDS (1982) and WRIGHT, RICHARDS, FROST, CAMOZZI and KUNZ (1983) have established six linkage groups from tests for linkage or independent assortment in many locus pairs found by electrophoretic analyses of numerous enzymes and a few blood proteins in the offspring of naturally occurring heterozygotes of *Rana pipiens*. WRIGHT, RICHARDS, FROST, CAMOZZI and KUNZ (1983) have reported four new linkage groups from tests in backcrosses of male hybrids between *Rana palustris* and *Rana pipiens* mated with female *Rana pipiens*. ELINSON (1981, 1983) and WRIGHT and RICHARDS (1983) have found a sex-linked locus for an enzyme in

Rana catesbeiana or *Rana clamitans* and two sex-linked loci for enzymes in *Rana pipiens*, respectively.

2. Detection of chromosomes bearing color mutant genes

While numerous genes for color mutations have been described hitherto, there has been no report which refers to the chromosomes on which the loci are situated. The present study could elucidate for the first time the chromosomes on which the genes for color mutations are located. It was confirmed that the two genes for olive and blue mutations are borne on chromosomes Nos. 3 and 8, respectively.

The detection of chromosomes bearing these loci became possible by using female offspring of female hybrids between a female *Rana brevipoda* and a male *Rana nigromaculata* homozygous for blue and olive genes. The offspring were produced from these female hybrids by backcrossing with the above male *Rana nigromaculata* or by diploid gynogenesis. The combination of the alleles at the loci for the color mutations was compared with the constitution of each of the 13 bivalent chromosomes in each of the females to detect the chromosomes corresponding to the alleles.

The use of the lampbrush chromosomes of backcrosses obtained from reciprocal hybrids between *Rana nigromaculata* and *Rana brevipoda* for the purpose of detecting the chromosomes bearing the loci for seven kinds of proteins has been reported by NISHIOKA, OHTANI and SUMIDA (1980). This kind of experiments was somewhat extended in the present study and the position of the genes for six additional enzymes was determined. The results of these studies showed that chromosome No. 3 bears the gene for olive mutation together with genes for MDH–B and ME–B. The positions of these three loci on chromosome No. 3 were estimated by agreement or recombination frequencies between the constitutions of genotypes and those of bivalent chromosomes as well as by examining a wild-type female backcross hybrid, BN♀×NN♂, in which bivalent chromosome No. 3 consists of a pair of *Rana nigromaculata* chromosomes including a translocated portion of a *Rana brevipoda* chromosome in the short arm.

Up to the present, the present authors have elucidated that one to three loci are situated in eight of 13 chromosome pairs as follows: loci for Ab and ADH–A on chromosome No. 1, α–GDH, ME–A and Pep–C on No. 2, olive color, MDH–B and ME–B on No. 3, LDH–B and Pep–B on No. 4, Pep–A on No. 5, Hb and IDH–B on No. 6, blue color on No. 8, and Protein C on No. 9. The genes situated on the remaining five chromosome pairs, Nos. 7, 10, 11, 12 and 13, will be gradually elucidated hereafter by the method of comparing the constitution between the genotypes and bivalent chromosomes. It is the present authors' desire to establish more complete chromosome maps in the *Rana nigromaculata* group. Such chromosome maps will be useful in considering the inheritance and evolution of anurans.

SUMMARY

1. The loci for the olive and blue mutations were compared in constitution with the 13 bivalent (lampbrush) chromosomes of oocytes in mature females produced from female hybrids between female *Rana brevipoda* and a male *Rana nigromaculata* by backcrossing with a male *Rana nigromaculata* or by diploid gynogenesis in order to determine the chromosomes which bear these loci.

2. The olive and blue mutations were induced in *Rana nigromaculata* chromosomes by irradiation of spermatozoa with 130 rads of neutrons in 1967. The male *Rana nigromaculata* used to obtain the above female hybrids was a bluish-olive mutant (*xxiiEE*) produced in 1976 from a mating between a female and a male bluish-olive mutants. The female hybrids were all green (*XxIiEE*). A deficiency or an inversion was found in the chromosomes derived from *Rana nigromaculata* in five of six female hybrids.

3. Two of the six female hybrids (*XxIiEE*) were backcrossed with the male *Rana nigromaculata* (*xxiiEE*). Offspring were also produced from the other four female hybrids by diploid gynogenesis. Lampbrush chromosomes were examined in 100 of 113 mature females produced from backcrossing and in 34 of 156 mature females produced by diploid gynogenesis.

4. Of the 134 female offspring in total, 68 olive mutants including 38 greenish-olive and 30 bluish-olive were all NN in the constitution of bivalent chromosome No. 3. On the other hand, the constitution of alleles agreed with that of bivalent chromosome No. 3 in all the females. It is evident that the olive mutant gene is located on *Rana nigromaculata* chromosome No. 3.

5. Of the 134 female offspring, 60 were blue mutants. In 50 of these blue mutants, bivalent chromosome No. 8 was NN in constitution. On the other hand, the constitution of alleles agreed with that of bivalent chromosome No. 8 in 114 (85.1%) of them. It is very probable that the blue mutant gene is located on *Rana nigromaculata* chromosome No. 8.

6. The olive mutant gene constitutes a linkage group with genes for MDH–B and ME–B. It is assumed that the olive mutant gene is situated near the centromere on the short arm of *Rana nigromaculata* chromosome No. 3, while the latter two genes are situated on the short and long arms, respectively, at the site of 13% and 12% in recombination rate from the centromere.

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