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Detection of Chromosomes Bearing the Loci for Seven Kinds of Proteins in Japanese Pond Frogs

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(With 24 Text-figures)

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INTRODUCTION

Although the inheritance of spontaneous and induced characters in amphibians has frequently been reported by many investigators, there has been no study which has confirmed the chromosomes carrying the genes of these inheritable characters. This is mainly attributable to the small number of such characters in one species as well as to the difficulty involved in marking each chromosome. However, these two are recently being resolved. The number of genes for various characters has rapidly increased with the utilization of electrophoresis in the analysis of chemical substances and the use of irradiation in the induction of mutations. The marking of each chromosome has become possible by making use of lampbrush chromosomes of oocytes.

Rana nigromaculata and *R. brevipoda* are closely allied pond-frog species in Japan. While the males of reciprocal hybrids between them are almost completely sterile, the females are fertile to a large extent. Thus, the lampbrush chromosomes can be observed in oocytes of female hybrids. Moreover, female hybrids have

13 bivalent chromosomes in their oocytes in the same way as the females of their parental species.

Each of the 13 bivalents in the oocytes of *Rana nigromaculata* is distinguishable from that of *Rana brevipoda* principally by difference in the number, site, shape and size of the landmarks of the homologues as well as in the relative length (OHTANI, 1975). When a female hybrid is backcrossed with a male *Rana nigromaculata* or *R. brevipoda,* female offspring have 13 bivalent chromosomes in their oocytes. In this case, the origin of the homologues of each bivalent chromosome can be identified on the basis of the landmarks and relative length.

In order to determine the relation between the genes for seven chemical extracts, that is, hemoglobin, albumin, serum protein C, LDH, MDH, α-GDH and IDH, and the chromosomes, the present authors examined the lampbrush chromosomes of oocytes of female backcross offspring between *Rana nigromaculata* and *R. brevipoda* on the one hand, and analyzed the above substances extracted from the backcross offspring by electrophoresis on the other hand. The results of these studies are described in the present report.

MATERIALS AND METHODS

Eight matings were made by artificial fertilization during the breeding season of 1973 among two female (Nos. 1 and 2) and two male (Nos. 1 and 2) *Rana nigromaculata* HALLOWELL (NN) and two female (Nos. 1 and 2) and two male (Nos. 1 and 2) *Rana brevipoda* ITO (BB). *Rana nigromaculata* were collected from the suburbs of Hiroshima, while *Rana brevipoda* were obtained from the suburbs of Okayama. The symbols of the matings and the abbreviations of the offspring produced are as follows.

Symbol of mating	Abbreviations of offspring
73NN, No. 1♀ × 73NN, No. 1♂	N_1N_1
73NN, No. 2♀ × 73NN, No. 2♂	N_2N_2
73BB, No. 1♀ × 73BB, No. 1♂	B_1B_1
73BB, No. 2♀ × 73BB, No. 2♂	B_2B_2
73NN, No. 1♀ × 73BB, No. 1♂	N_1B_1
73NN, No. 2♀ × 73BB, No. 2♂	N_2B_2
73BB, No. 1♀ × 73NN, No. 1♂	B_1N_1
73BB, No. 2♀ × 73NN, No. 2♂	B_2N_2

As the offspring attained sexual maturity in 1975, three female hybrids between female *Rana nigromaculata* and male *Rana brevipoda* (N_1B_1, Nos. 1 and 2; N_2B_2, No. 3) and three female reciprocal hybrids (B_1N_1, Nos. 1 and 2; B_2N_2, No. 3) were backcrossed with three male *Rana nigromaculata* (N_1N_1, Nos. 1 and 2; N_2N_2, No. 3) and three male *Rana brevipoda* (B_1B_1, Nos. 1 and 2; B_2B_2, No. 3), in order to produce second-generation offspring. Besides these matings, second-generation offspring were also produced from *Rana nigromaculata* and *Rana brevipoda* as controls.

The symbols of these matings and the number of female offspring whose lampbrush chromosomes were analyzed are as follows.

Symbol of mating	Number of mature females analyzed	
N_1N_1, No. 1♀ × N_1N_1, No. 1♂	3	
N_1N_1, No. 1♀ × B_1B_1, No. 1♂	3	
B_1B_1, No. 1♀ × B_1B_1, No. 1♂	3	
B_1B_1, No. 1♀ × N_1N_1, No. 1♂	5	
N_1B_1, No. 1♀ × N_1N_1, No. 1♂	6	
N_1B_1, No. 2♀ × N_1N_1, No. 2♂	31	42
N_2B_2, No. 3♀ × N_2N_2, No. 3♂	5	
N_1B_1, No. 1♀ × B_1B_1, No. 1♂	10	
N_1B_1, No. 2♀ × B_1B_1, No. 2♂	31	45
N_2B_2, No. 3♀ × B_2B_2, No. 3♂	4	
B_1N_1, No. 1♀ × N_1N_1, No. 1♂	6	
B_1N_1, No. 2♀ × N_1N_1, No. 2♂	33	47
B_2N_2, No. 3♀ × N_2N_2, No. 3♂	8	
B_1N_1, No. 1♀ × B_1B_1, No. 1♂	12	48
B_1N_1, No. 2♀ × B_1B_1, No. 2♂	36	

The preparations of mitotic chromosomes were made according to the method of VOLPE and GEBHARDT (1968) from leucocytes of peripheral blood cultured *in vitro*. Lampbrush chromosomes were observed on the preparations which were principally made according to the method of GALL (1966). As the fluid for dispersing lampbrush chromosomes, a mixture of 5 parts of 0.075M KCl solution and 1 part of 0.075M NaCl solution was used after 10% formalin was added to the mixture to make the concentration of formaldehyde 0.08%.

Hemoglobin of erythrocytes, serum protein C, albumin of serum and four kinds of enzymes, LDH, MDH, α-GDH and IDH, extracted from skeletal muscles were obtained from the above female frogs and various kinds of male frogs, and analyzed by electrophoresis. The preparation and analyses of these substances were carried out by the following methods.

Before drawing blood, the frog was injected with 0.1 ml of heparin solution (100,000 units of heparin sodium were dissolved in 50 ml of RINGER's solution for frog use) into the body cavity, anesthetized 5 minutes later and subjected to laparotomy. A blood sample was drawn from the heart into a syringe and centrifuged at 2,300 r.p.m. for 3 minutes at room temperature to separate erythrocytes from serum. The serum was put into an analyzer cup of 2 ml and stored at −70°C in a stocker (Revco) until it was used for analysis. The frog was put into a vinyl bag and stored at −70°C, too. Erythrocytes were washed three times in RINGER's solution for frog use, packed and then stored at −70°C.

In order to make hemolysate preparation, packed erythrocytes were hemolyzed by adding cold deionized water two to three times the volume of the erythrocytes

and centrifuged at 3,000 r.p.m. for 3 minutes. The supernatant was centrifuged again at 10,000 r.p.m. for 20 minutes in a cold centrifuge. One part of the supernatant was added with 0.1～0.2 part of 2% $K_3Fe(CN)_6$ and 0.1～0.2 part of 0.5% KCN to convert hemoglobin into the cyanmethemoglobin principally according to the method of Moss and INGRAM (1968) and immediately subjected to electrophoresis.

A muscular extract containing various enzymes was obtained from a small piece of skeletal muscle removed from the frog stored at −70°C by crushing it in the same quantity of distilled water.

As the buffer solutions for the analysis of hemoglobin, a tris-borate-EDTA buffer of pH 8.6 (0.9M tris, 0.5M boric acid and 0.02M EDTA) was used by diluting to one part in twenty for making starch-gel and to one part in eight for the bridge. Each of the buffer solutions was added with KCN until the concentration of KCN became 0.01%, according to IUCHI and YAMAGAMI (1969). Starch-gel was made to contain starch at a concentration of 12%. Each sample for electrophoresis was absorbed on a small piece of WHATMAN No. 3 filter paper and inserted into a slit cut in the starch-gel. The electrophoretic run was performed at a constant voltage of 12.5V/cm or 14.3V/cm for 6 hours at 2°C. After electrophoresis was completed, the starch-gel was horizontally sliced into two plates. The upper plate was stained with o-dianisidine (Sigma Chemical Co.), while the lower plate was stained with amido black 10B.

For differentiation of albumin and serum protein C from serum, a tris-borate-EDTA buffer of pH 8.0 (2.1 M tris, 2.0 M boric acid and 0.068 M EDTA) was used by diluting it to one part in 100 for making starch-gel and to one part in 10 for the bridge. The electrophoretic run was made at 18.8 V/cm or 21.4 V/cm for 4 hours at 2°C. Upon completion of electrophoresis, the starch-gel was stained with amido black 10B after it was sliced into two plates.

Of the four kinds of enzymes extracted from the skeletal muscle, LDH, MDH and α–GDH were analyzed by making use of a tris-citrate buffer of pH 6.0 (2.23 M tris and 0.86 M citric acid). This buffer was diluted to one part in 10 for the bridge, while 0.88 ml of the buffer was filled to 250 ml and then 30 g of starch was dissolved for making starch-gel. On the other hand, IDH was analyzed by making use of a tris-citrate buffer of pH 7.0 (1.35 M tris and 0.43 M citric acid) which was diluted to one part in ten for the bridge, while 1.67 ml of this buffer was filled to 250 ml and then 30 g of starch was dissolved for making starch-gel. The electrophoretic run for these enzymes was made at 12.5 V/cm or 14.3 V/cm for 4 hours at 2°C. The enzymes were detected by the agar overlay method and the specific staining procedures, according to BREWER (1970).

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 *R. nigromaculata* chromosomes
BB ..... *Rana brevipoda* or a pair of *R. brevipoda* chromosomes

NB ..... A hybrid between a female *Rana nigromaculata* and a male *R. brevipoda*, or a combination of a *R. nigromaculata* chromosome and a *R. brevipoda* chromosome

BN ..... A hybrid between a female *Rana brevipoda* and a male *R. nigromaculata*, or a combination of a *R. brevipoda* chromsome and a *R. nigromaculata* chromosome

OBSERVATION

I. Comparison between mitotic and lampbrush chromosomes

NISHIOKA (1972) reported on the karyotypes of *Rana nigromaculata* and *Rana brevipoda*, which were clarified by observing 100 metaphase spreads prepared from the tail-tips of tadpoles by the squash method with water pretreatment and were, thereafter, confirmed by examining the chromosomes of reciprocal hybrids between the two species and auto- and allotriploids. In 1975, she made karyotype analyses of 50 metaphase spreads prepared from cultured blood cells of 12 frogs consisting of three male and three female *Rana nigromaculata* and three male and three female *Rana brevipoda* by the air dry method. She also observed the chromosomes of reciprocal hybrids and auto- and allotriploids of these two species in the same manner. The results showed that the karyotypes of the two

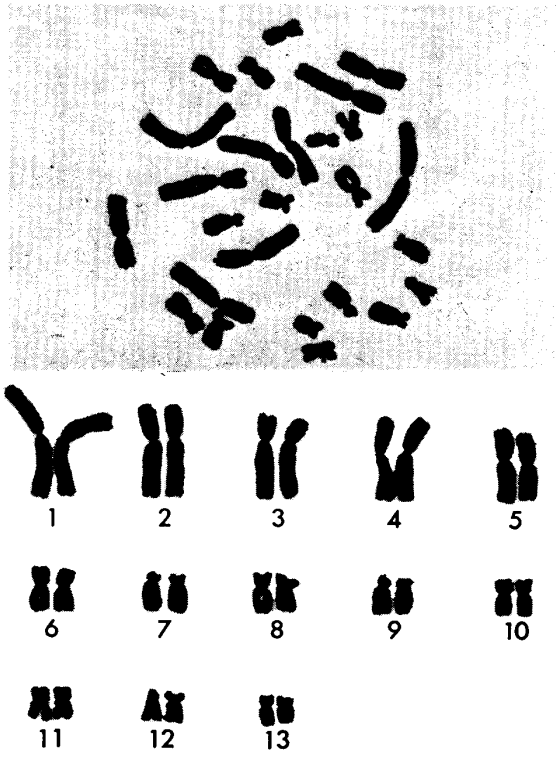

Fig. 1. Metaphase plate and the karyotype of a cultured blood cell from *Rana nigromaculata*. ×1400

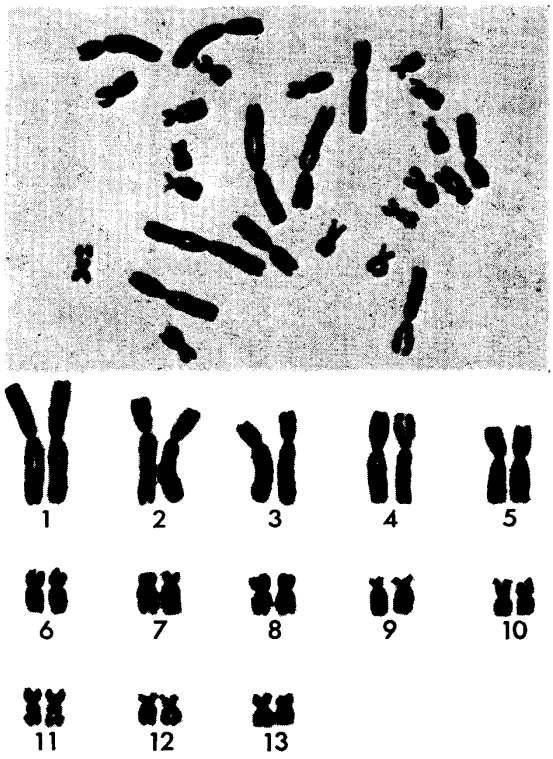

Fig. 2. Metaphase plate and the karyotype of a cultured blood cell from *Rana brevipoda*. ×1400

species observed in the preparations of cultured blood cells almost completely coincided with those reported previously by her (Figs. 1 and 2). The 13 pairs of homologues were numbered from 1 to 13 in order of length. They consisted of 5 pairs of large chromosomes and 8 pairs of small ones. There were slight differences between the karyotypes of the two species as shown diagramatically in Fig. 3.

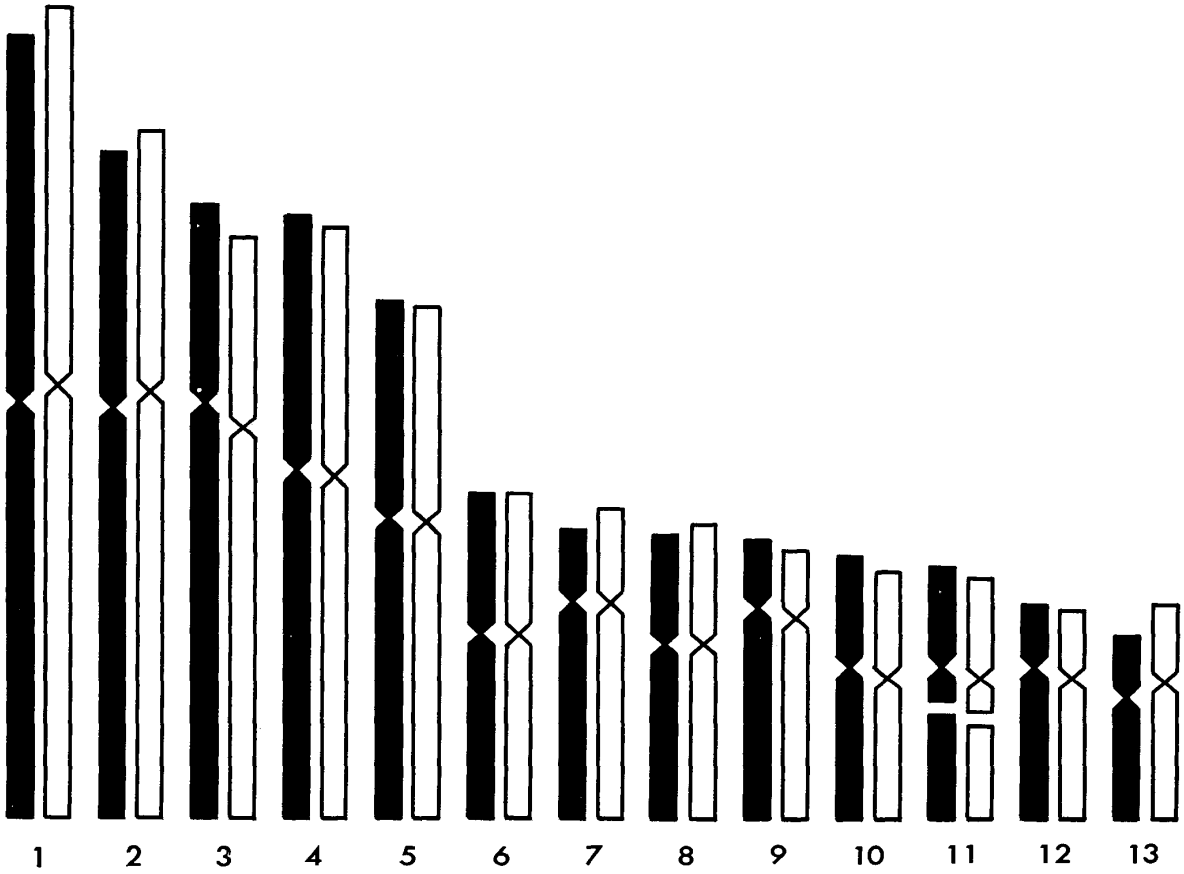

Fig. 3. Composite ideogram showing differences in relative chromosome length and centromere postion between *Rana nigromaculata* and *Rana brevipoda*.

Black and white bars represent *nigromaculata* and *brevipoda* chromosomes, respectively. Constrictions indicate the centromeres. A gap in chromosome No. 11 indicates the secondary constriction.

OHTANI (1975) reported on the lampbrush chromosomes in oocytes of female *Rana nigromaculata*, *Rana brevipoda* and reciprocal hybrids between the two species. He could distinguish each lampbrush chromosome from the others by its relative length and the kind and position of landmarks. He arranged the 13 pairs of lampbrush chromosomes in order of length and numbered from 1 to 13. In 1980, he discovered for the first time the site of centromere in each homologue. It was situated near a landmark and was recognized as a minute granule in the middle of a short segment covered with numerous loops which were distinctly larger than those of the other parts of the chromosome. In this short segment, a chiasma was scarcely formed. Thus, the 13 pairs of lampbrush chromosomes were arranged from No. 1 to No. 13 as shown in Fig. 4 in correspondence to those

of mitotic chromosomes mainly on the basis of the site of centromere in each chromosome as well as relative chromosome length and similarity of landmarks in analogous chromosomes between the two species. This arrangement of lampbrush chromosomes fairly differed from that reported previously by OHTANI (1975). While the order of chromosomes 1, 2, 5 and 6 was not changed, that of the others was changed as follows; 3 from 4, 4 from 3, 7 from 8, 8 from 7, 9 from 11, 10 from 12, 11 from 9, 12 from 13 and 13 from 10. Such changes in order

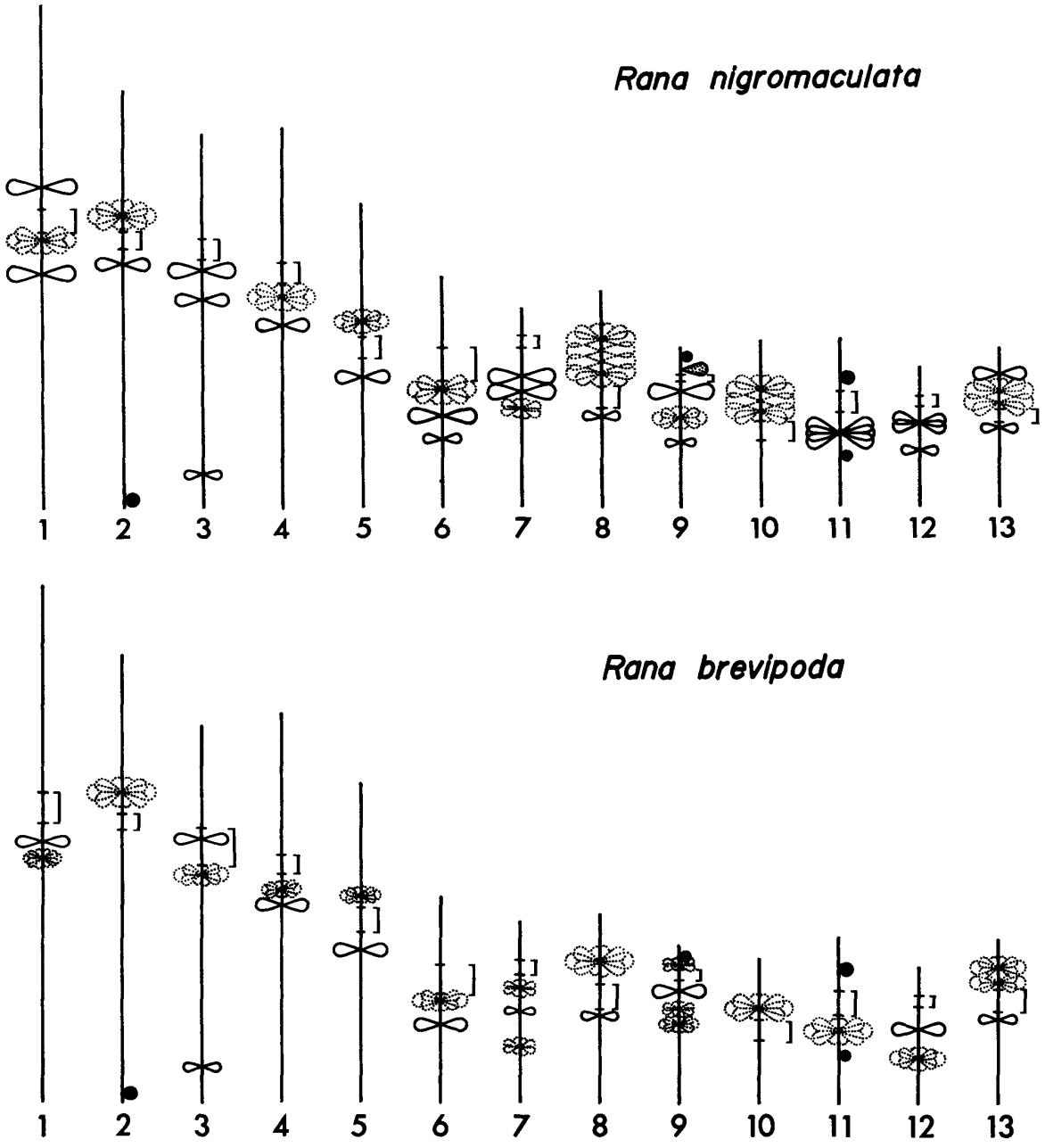

Fig. 4. Maps of the thirteen lampbrush chromosomes of *Rana nigromaculata* and *Rana brevipoda*.

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.

may be attributable to an excessive elongation of lampbrush chromosomes at the portion bearing compound giant loops or a secondary constriction.

II. Lactate dehydrogenase (LDH)

Electrophoretic analyses of LDH were made in 30 *Rana nigromaculata* and 30 *R. brevipoda.* These frogs included individuals collected from the field in 1973 and their offspring. The electrophoretic patterns consisted of five bands that moved toward the anode, that is, they were expressed by two genes, *A* and *B* (Fig. 5a, c). While gene *A* of *Rana nigromaculata* was the same as that of *R. brevipoda* in expression, gene *B* of the former was faster than that of the latter in mobility of bands.

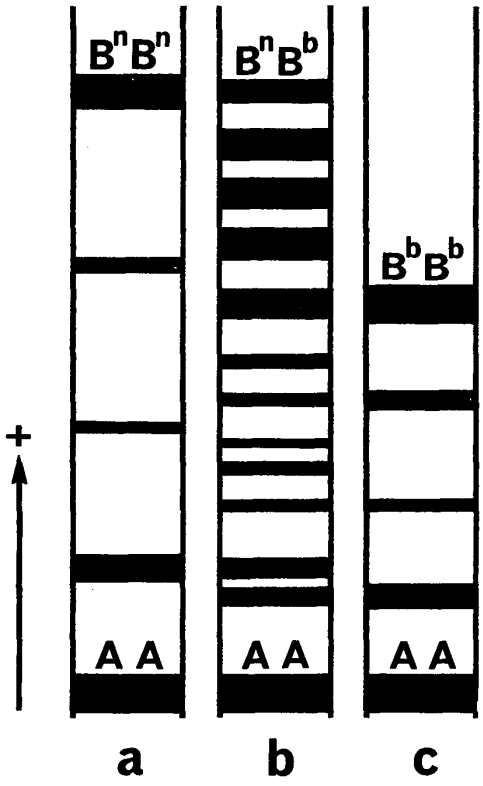

Fig. 5. Electrophoretic patterns of LDH.
a. *Rana nigromaculata* type b. Hybrid type c. *Rana brevipoda* type

Electrophoretic patterns of LDH were examined in 40 mature male and female reciprocal hybrids between *Rana nigromaculata* and *R. brevipoda.* It was observed that the hybrids revealed characteristic hybrid bands (Fig. 5b). Next, electrophoretic patterns of LDH were examined in 80 mature males and females produced by backcrosses between female reciprocal hybrids and males of the parental species. Of the 40 frogs obtained by backcrossing with male *Rana nigromaculata,* 20 were of *Rana nigromaculata* type and 20 were of hybrid type. Of 40 backcross hybrid offspring obtained from matings with male *Rana brevipoda,* 19 were of *Rana brevipoda* type and 21 were of hybrid type. Thus, it was evident

that gene *B* of *Rana nigromaculata* was allelic and codominant in expression with gene *B* of *Rana brevipoda*. Gene *B* of *Rana nigromaculata* and *R. brevipoda* were called B^n and B^b, respectively.

In order to identify the chromosome bearing gene B^n or B^b, the constitution of each of the 13 bivalent chromosomes in oocytes and the electrophoretic pattern of LDH were examined in each of 182 mature female frogs produced from backcrosses between female reciprocal hybrids and males of the parental species (Table 1).

TABLE 1

Number of frogs whose genotypes of LDH-B agreed in constitution with each of the 13 lampbrush (bivalent) chromosomes in their oocytes

Parents		No. of	Bivalent chromosome no.												
Female	Male	frogs	1	2	3	4	5	6	7	8	9	10	11	12	13
N_1B_1, No. 1	N_1N_1, No. 1	6	4	3	4	6	3	4	3	3	2	3	2	4	1
N_1B_1, No. 2	N_1N_1, No. 2	31	18	13	16	25	12	17	20	11	12	13	14	15	15
N_2B_2, No. 3	N_2N_2, No. 3	5	4	3	2	5	0	1	2	4	3	0	3	2	2
NB	NN	42	26	19	22	36	15	22	25	18	17	16	19	21	18
N_1B_1, No. 1	B_1B_1, No. 1	10	6	5	3	8	4	6	9	7	5	7	3	7	8
N_1B_1, No. 2	B_1B_1, No. 2	31	21	18	17	28	14	21	17	20	18	18	17	14	15
N_2B_2, No. 3	B_2B_2, No. 3	4	3	3	0	4	2	3	3	3	2	1	2	2	1
NB	BB	45	30	26	20	40	20	30	29	30	25	26	22	23	24
B_1N_1, No. 1	N_1N_1, No. 1	6	3	5	5	5	3	2	1	1	2	3	3	3	3
B_1N_1, No. 2	N_1N_1, No. 2	33	20	16	20	31	23	15	14	20	18	16	21	19	15
B_2N_2, No. 3	N_2N_2, No. 3	8	6	4	4	8	7	4	4	6	7	5	6	4	5
BN	NN	47	29	25	29	44	33	21	19	27	27	24	30	26	23
B_1N_1, No. 1	B_1B_1, No. 1	12	5	4	7	11	8	4	3	5	4	6	5	7	5
B_1N_1, No. 2	B_1B_1, No. 2	36	20	20	16	34	20	19	20	14	21	21	13	20	19
BN	BB	48	25	24	23	45	28	23	23	19	25	27	18	27	24
Total		182	110	94	94	165	96	96	96	94	94	93	89	97	89
		(%)	(60.4)	(51.6)	(51.6)	(90.7)	(52.7)	(52.7)	(52.7)	(51.6)	(51.6)	(51.1)	(48.9)	(53.3)	(48.9)

1. Backcross offspring produced from NB♀ × NN♂

Six mature females produced from a mating, N_1B_1, No. 1♀ × N_1N_1, No. 1♂, and five mature females produced from a mating, N_2B_2, No. 3♀ × N_2N_2, No. 3♂, were examined in terms of electrophoretic pattern of LDH. It was found that four were of *Rana nigromaculata* type (B^nB^n) and seven of hybrid type (B^nB^b). The genotype for the LDH pattern in each female completely agreed in constitution with bivalent chromosome No. 4 in the oocytes of this female. Of 31 mature females produced from a mating, N_1B_1, No. 2♀ × N_1N_1, No. 2♂, 14 were of *Rana nigromaculata* type (B^nB^n) and 17 were of hybrid type (B^nB^b) in LDH pattern. Ten of the former and 15 of the latter frogs had bivalent chromosome No. 4 which agreed in constitution with the genotypes, respectively, while the constitution of bivalent chromosome No. 4 did not agree with the genotypes in the remaining four and two frogs, respectively (Figs. 6 and 7).

In summation, 18 and 24 of a total of 42 females from NB♀ × NN♂, were B^nB^n and B^nB^b in genotype for LDH pattern, respectively. The genotype of

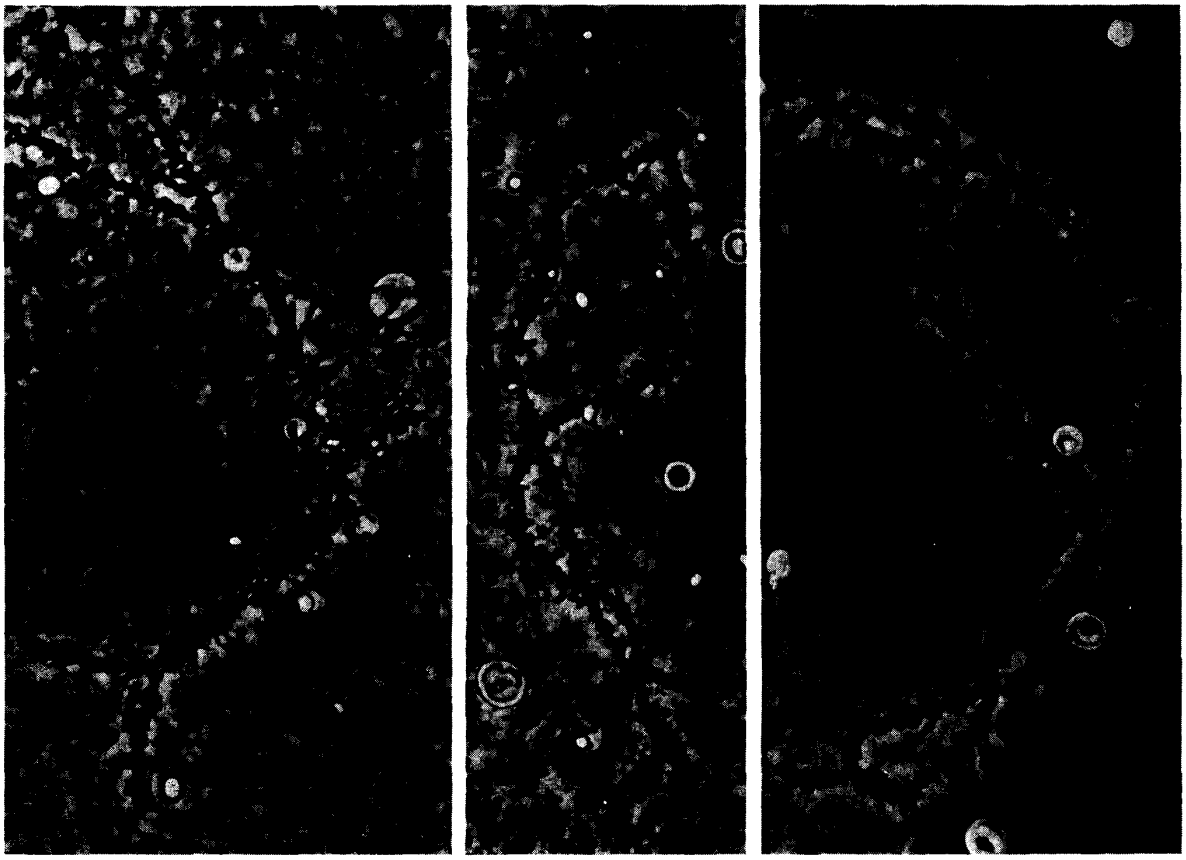

Fig. 6. Microphotographs of lampbrush (bivalent) chromosome No. 4 in oocytes of three female backcross offspring between *Rana nigromaculata* and *R. brevipoda*. ×500

a. A pair of *Rana nigromaculata* chromosomes in a female, (N_1B_1, No. 1♀ × N_1N_1, No. 1♂) No. 2

b. A pair of *Rana nigromaculata* and *R. brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 1

c. A pair of *Rana brevipoda* chromosomes in a female, (B_1N_1, No. 2♀ × B_1B_1, No. 2♂) No. 3

each female agreed in constitution with bivalent chromosome No. 4 in 14 and 22 of 36 females, respectively. In contrast, the genotype for the LDH pattern of each female agreed in constitution with each of the other 12 bivalent chromosomes (Nos. 1~3, 5~13) in 15~26 females (Tables 1, 2 and 15).

2. Backcross offspring produced from NB♀ × BB♂

Of ten mature females produced from a mating, N_1B_1, No. 1♀ × B_1B_1, No. 1♂, three were of *Rana brevipoda* type (B^bB^b) and seven of hybrid type (B^nB^b) in LDH pattern. The genotypes for these LDH patterns agreed in constitution with bivalent chromosome No. 4 in two of the former and six of the latter females. Of 31 mature females produced from a mating, N_1B_1, No. 2♀ × B_1B_1, No. 2♂, 13 were of *Rana brevipoda* type (B^bB^b) and 18 of hybrid type (B^nB^b) in LDH pattern. The genotypes for these LDH patterns agreed in constitution with bivalent chromosome No. 4 in 11 and 17 females, respectively. Of four mature females produced from N_2B_2, No. 3♀ × B_2B_2, No. 3♂, two were of *Rana brevipoda* type (B^bB^b) and two of hybrid type (B^nB^b) in LDH pattern.

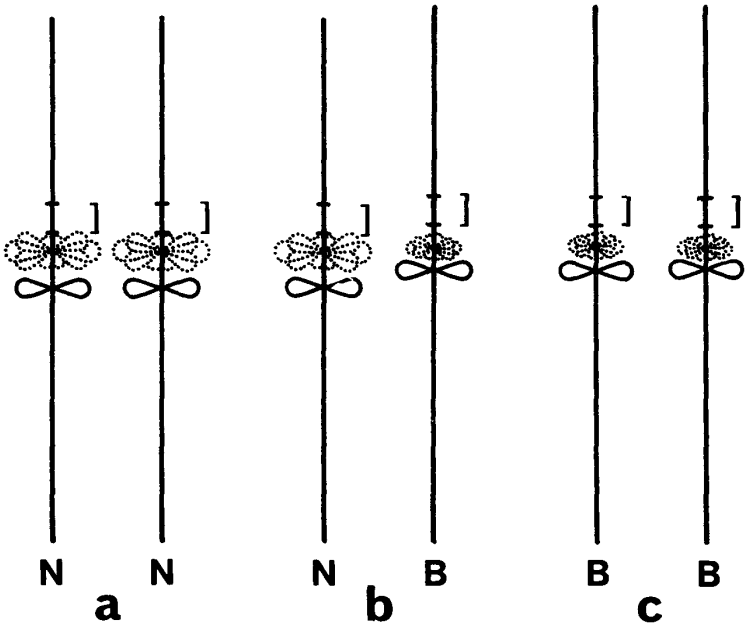

Fig. 7. Diagrams showing the constitution of bivalent chromosome No. 4 in the oocytes of three female backcross offspring between *Rana nigromaculata* and *R. brevipoda*.

a. A pair of *Rana nigromaculata* chromosomes in a female, (N_1B_1, No. 1♀ ×N_1N_1, No. 1♂) No. 2

b. A pair of *Rana nigromaculata* and *R. brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ ×N_1N_1, No. 2♂) No. 1

c. A pair of *Rana brevipoda* chromosomes in a female, (B_1N_1, No. 2♀ × B_1B_1, No. 2♂) No. 3

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

The genotypes for these LDH patterns agreed in constitution with bivalent chromosome No. 4 in all the females.

In summation, 18 and 27 of a total of 45 females from NB♀ ×BB♂ were B^bB^b and B^nB^b in genotype for LDH pattern, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 4 in 15 and 25 females, respectively, while it did not agree with the latter in the remaining five females. In contrast, the genotype of each female agreed in constitution with each of the other 12 bivalent chromosomes (Nos. 1~3, 5~13) in 20~30 females (Tables 1, 2 and 16).

3. Backcross offspring produced from BN♀ ×NN♂

Of six mature females produced from a mating, B_1N_1, No. 1♀ ×N_1N_1, No. 1♂, two were of *Rana nigromaculata* type (B^nB^n) and four of hybrid type (B^nB^b) in LDH pattern. Of 33 mature females produced from B_1N_1, No. 2♀ ×N_1N_1, No. 2♂, 18 were of *Rana nigromaculata* type (B^nB^n) and 15 of hybrid type (B^nB^b) in LDH pattern. Two and 16 females having genotype B^nB^n and 3 and 15 females having genotype B^nB^b in the above two mating groups, respectively,

TABLE 2
Relationship between the constitution of bivalent chromosome No. 4 and the genotypes of LDH-B

Kind	Bivalent chromosome		LDH-B			No. of frogs whose chromosomes were compared with the genotypes	
	Constitution	No. of frogs	Phenotype	Genotype	No. of frogs	Agreed	Disagreed
NB♀×NN♂	NN	16	*nigrom.*	B^nB^n	18	14	2
	NB	26	hybrid	B^nB^b	24	22	4
	Total	42			42	36	6
NB♀×BB♂	BB	17	*brevip.*	B^bB^b	18	15	2
	NB	28	hybrid	B^nB^b	27	25	3
	Total	45			45	40	5
BN♀×NN♂	NN	22	*nigrom.*	B^nB^n	23	21	1
	NB	25	hybrid	B^nB^b	24	23	2
	Total	47			47	44	3
BN♀×BB♂	BB	28	*brevip.*	B^bB^b	25	25	3
	NB	20	hybrid	B^nB^b	23	20	0
	Total	48			48	45	3
	NN	38	*nigrom.*	B^nB^n	41	35	3
	NB	99	hybrid	B^nB^b	98	90	9
	BB	45	*brevip.*	B^bB^b	43	40	5
	Total	182			182	165	17

had bivalent chromosome No. 4 which agreed in constitution with the genotype for LDH pattern. Of eight mature females produced from a mating, B_2N_2, No. 3♀×N_2N_2, No. 3♂, three were of *Rana nigromaculata* type (B^nB^n) and five of hybrid type (B^nB^b) in LDH pattern. All these females had bivalent chromosome No. 4 which agreed in constitution with the genotype for LDH pattern.

In summation, 23 and 24 of a total of 47 females from BN♀×NN♂ were B^nB^n and B^nB^b in genotype for LDH pattern, respectively. These genotypes agreed in constitution with bivalent chromosome No. 4 in 21 and 23 females, respectively, while they did not agree with the latter in the remaining three females. In contrast, the genotype for the LDH pattern of each female agreed in constitution with each of the other 12 bivalent chromosomes in 19~33 females (Tables 1, 2 and 17).

4. Backcross offspring produced from BN♀×BB♂

Of 12 mature females produced from a mating, B_1N_1, No. 1♀×B_1B_1, No. 1♂, nine were of *Rana brevipoda* type (B^bB^b) and three of hybrid type (B^nB^b) in LDH pattern. Nine and two of these females had bivalent chromosome No. 4 which agreed in constitution with the genotype for LDH pattern. Of 36 mature females produced from a mating, B_1N_1, No. 2♀×B_1B_1, No. 2♂, 16 were of *Rana brevipoda* type (B^bB^b) and 20 of hybrid type (B^nB^b) in LDH pattern. These

genotypes agreed in constitution with bivalent chromosome No. 4 in 16 and 18 females, respectively, while they did not agree with the latter in only two females.

In summation, 25 and 23 of a total of 48 mature females were B^bB^b and B^nB^b in genotype for LDH pattern, respectively. These genotypes agreed in constitution with bivalent chromosome No. 4 in 25 and 20 females, respectively. In contrast, the genotypes for the LDH patterns agreed in constitution with each of the other 12 bivalent chromosomes (Nos. 1~3, 5~13) in 18~28 females, while they differed from the latter in 20~30 females (Tables 1, 2 and 18).

5. Summary of the experiments with LDH

Electrophoretic patterns of LDH were examined in 182 mature female backcross offspring. Then, the constitution of the genotype (B^nB^n, B^nB^b or B^bB^b) for the LDH pattern of each female was collated with that of homologues (NN, NB or BB) in each of the 13 bivalent chromosomes in her oocytes. It agreed with the constitution of bivalent chromosome No. 4 in 165 (90.7%) of the analyzed females, while it agreed with the constitution of each of the other 12 bivalent chromosomes (Nos. 1~3, 5~13) in 89 (48.9%)~110 (60.4%), that is, in about half the number of females (Table 1). Thus, chromosome No. 4 was assumed to bear the gene for LDH-B.

The constitution of bivalent chromosome No. 4 was NN in 38, BB in 45 and NB in 99 out of the 182 mature females. Each of these constitutions agreed with that of the genotype for LDH pattern in 35, 40 and 90 females, respectively, while it differed from the latter in 17 females (Table 2).

III. Malate dehydrogenase (MDH)

Malate dehydrogenase (MDH) was analyzed by electrophoresis in 30 *Rana nigromaculata* and 30 *R. brevipoda*. These frogs included individuals collected from the field in 1973 and their offspring. The electrophoretic patterns of MDH in

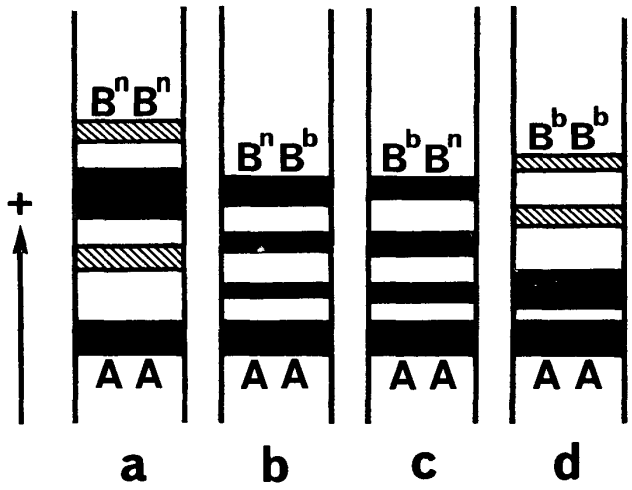

Fig. 8. Electrophoretic patterns of MDH.

a. *Rana nigromaculata* type b. Hybrid(NB) type
c. Hybrid (BN) type d. *Rana brevipoda* type

the two species consisted of four bands, that is, two major bands and two subbands, which moved toward the anode (Fig. 8a, d). Each of the two major bands was expressed by one gene, *A* or *B*. While gene *A* of *Rana nigromaculata* was the same as that of *R. brevipoda,* gene *B* of the former was distinctly faster in mobility than that of the latter. Gene *B* of *Rana nigromaculata* and *R. brevipoda* were called B^n and B^b, respectively.

Electrophoretic patterns of MDH were examined in 20 male and 20 female reciprocal hybrids between *Rana nigromaculata* and *R. brevipoda.* As a result, these hybrids showed MDH patterns characteristic of hybrids (Fig. 8b, c). Next, electrophoretic patterns of MDH were examined in 40 mature males and females produced from backcrosses between female reciprocal hybrids and male *Rana nigromaculata.* It was found that 18 frogs were of the *Rana nigromaculata* type (B^nB^n), while 22 were of the hybrid type (B^nB^b). Electrophoretic patterns of MDH were also examined in 40 mature males and females produced from backcrosses between female reciprocal hybrids and male *Rana brevipoda.* It was observed that 21 frogs were of the *Rana brevipoda* type (B^bB^b), while 19 were of the hybrid type (B^nB^b). These results indicated that B^n and B^b were codominant alleles. Then, in order to identify the chromosome bearing gene B^n or B^b for MDH, the constitution of each of the 13 bivalent chromosomes in oocytes as well as the electrophoretic pattern of MDH were examined in each of 182 mature females produced from backcrosses between female reciprocal hybrids and male *Rana nigromaculata* or *R. brevipoda* (Table 3).

1. Backcross offspring produced from NB♀ × NN♂

Of six mature females produced from a mating, N_1B_1, No. 1♀ × N_1N_1, No. 1♂, four were of the *Rana nigromaculata* type (B^nB^n) and two of the hybrid type (B^nB^b) in MDH pattern. Except one of the former females, bivalent chromosome No. 3 (Figs. 9 and 10) agreed in constitution with the genotype for MDH pattern. Of 31 mature females produced from a mating, N_1B_1, No. 2♀ × N_1N_1, No. 2♂, 9 were of the *Rana nigromaculata* type (B^nB^n) and 22 of the hybrid type (B^nB^b) in MDH pattern. In eight and 19 of these females, the genotype of each female agreed in constitution with bivalent chromosome No. 3. However, in one of the 19 females which were of the hybrid type in MDH pattern, one homologue of bivalent chromosome No. 3 had a translocation from a *Rana brevipoda* homologue into a *R. nigromoaculata* homologue (Figs. 9d and 10d), while the other homologue was that of *Rana nigromaculata.* The remaining one and three females had bivalent chromosome No. 3 which differed in constitution from the genotype, respectively. Of five mature females produced from a mating, N_2B_2, No. 3♀ × N_2N_2, No. 3♂, two were of the *Rana nigromaculata* type (B^nB^n) and three of the hybrid type (B^nB^b). In all these females, these genotypes for MDH pattern agreed in constitution with bivalent chromosome No. 3.

In summation, 15 and 27 of a total of 42 females were B^nB^n and B^nB^b in genotype for MDH pattern, respectively. These genotypes agreed in constitution with bivalent chromosome No. 3 in 13 and 24 females including one with a

TABLE 3
Number of frogs whose genotypes of MDH-B agreed in constitution with each of the 13 lampbrush (bivalent) chromosomes in their oocytes

Parents		No. of	Bivalent chromosome no.												
Female	Male	frogs	1	2	3	4	5	6	7	8	9	10	11	12	13
N_1B_1, No. 1	N_1N_1, No. 1	6	3	4	5	3	2	5	2	2	3	4	3	3	4
N_1B_1, No. 2	N_1N_1, No. 2	31	13	12	27	16	21	18	17	10	17	12	16	16	20
N_2B_2, No. 3	N_2N_2, No. 3	5	1	4	5	2	3	2	3	1	2	3	2	5	3
NB	NN	42	17	20	37	21	26	25	22	13	22	19	21	24	27
N_1B_1, No. 1	B_1B_1, No. 1	10	5	4	10	5	7	3	5	6	6	4	6	6	5
N_1B_1, No. 2	B_1B_1, No. 2	31	17	14	29	16	18	19	13	10	20	14	19	20	19
N_2B_2, No. 3	B_2B_2, No. 3	4	0	2	3	1	3	2	0	2	3	4	1	3	2
NB	BB	45	22	20	42	22	28	24	18	18	29	22	26	29	26
B_1N_1, No. 1	N_1N_1, No. 1	6	4	4	6	4	4	1	2	2	3	2	2	2	4
B_1N_1, No. 2	N_1N_1, No. 2	33	18	16	28	17	15	11	16	18	14	20	15	21	13
B_2N_2, No. 3	N_2N_2, No. 3	8	5	7	7	3	4	6	5	3	5	4	3	5	4
BN	NN	47	27	27	41	24	23	18	23	23	22	26	20	28	21
B_1N_1, No. 1	B_1B_1, No. 1	12	4	6	9	5	8	6	5	7	6	4	9	5	5
B_1N_1, No. 2	B_1B_1, No. 2	36	22	19	31	17	21	18	14	21	14	20	19	21	18
BN	BB	48	26	25	40	22	29	24	19	28	20	24	28	26	23
Total		182	92	92	160	89	106	91	82	82	93	91	95	107	97
		(%)	(50.5)	(50.5)	(87.9)	(48.9)	(58.2)	(50.0)	(45.1)	(45.1)	(51.1)	(50.0)	(52.2)	(58.8)	(53.3)

translocation, respectively. In contrast, the genotype of each female agreed in constitution with each of the other 12 bivalent chromosomes in 13~27 females, while it differed from the latter in 15~29 females (Tables 3, 4 and 15).

2. Backcross offspring produced from NB♀ × BB♂

In each of ten mature females produced from a mating, N_1B_1, No. 1♀ × B_1B_1, No. 1♂, the genotype for MDH pattern agreed in constitution with bivalent chromosome No. 3. Of these females, six were of the *Rana brevipoda* type (B^bB^b) and four of the hybrid type (B^nB^b) in MDH pattern. Of 31 mature females produced from a mating, N_1B_1, No. 2♀ × B_1B_1, No. 2♂, 17 were of the *Rana brevipoda* type (B^bB^b) and 14 of the hybrid type (B^nB^b). The genotype of each female agreed in constitution with bivalent chromosome No. 3 in 16 and 13 of these females, respectively. Of four mature females produced from a mating, N_2B_2, No. 3♀ × B_2B_2, No. 3♂, three were of the *Rana brevipoda* type (B^bB^b) and one was of the hybrid type (B^nB^b) in MDH pattern. Except one of the former three females, these genotypes agreed in constitution with bivalent chromosome No. 3 (Tables 4 and 16).

In summation, 26 and 19 of a total of 45 females produced from NB♀ × BB♂ were B^bB^b and B^nB^b in genotype for MDH pattern, respectively. These genotypes agreed in constitution with bivalent chromosome No. 3 in 24 and 18 females, respectively. In contrast, the genotype of each female agreed in constitution with each of the other 12 bivalent chromosomes (Nos. 1, 2, 4~13) in 18~29 females, while it differed from the latter in 16~27 females (Tables 3, 4 and 16).

3. Backcross offspring produced from BN♀ × NN♂

In each of six mature females produced from a mating, B_1N_1, No. 1♀ × N_1N_1, No. 1♂, the genotype for MDH pattern agreed in constitution with

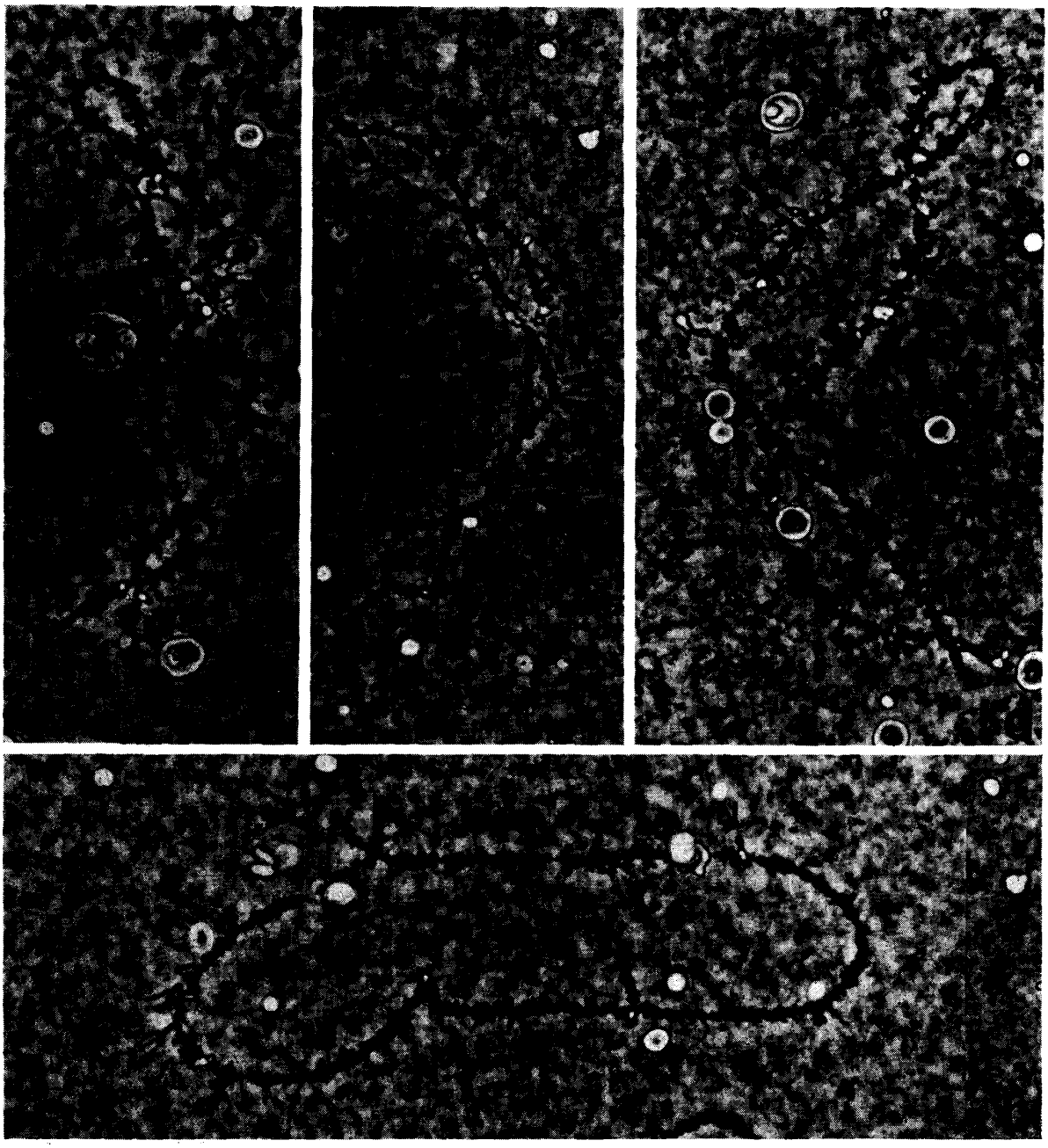

Fig. 9. Microphotographs of lampbrush (bivalent) chromosome No. 3 in oocytes of four female backcross offspring between *Rana nigromaculata* and *R. brevipoda*. × 500

a. A pair of *Rana nigromaculata* chromosomes in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 30
b. A pair of *Rana nigromaculata* and *R. brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ × B_1B_1, No. 2♂) No. 24
c. A pair of *Rana brevipoda* chromosomes in a female, (B_1N_1, No. 2♀ × B_1B_1, No. 2♂) No. 2
d. A pair of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome with a translocation from a *R. brevipoda* chromosome in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 31

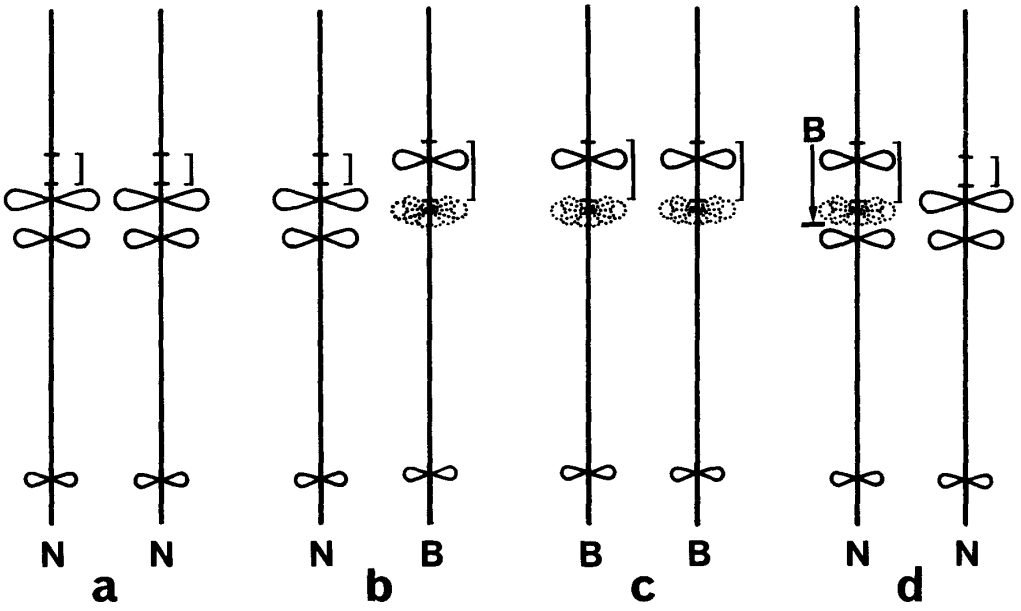

Fig. 10. Diagrams showing the constitution of bivalent chromosome No. 3 in the oocytes of four female backcross offspring between *Rana nigromaculata* and *R. brevipoda*.

a. A pair of *Rana nigromaculata* chromosomes in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 30
b. A pair of *Rana nigromaculata* and *R. brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ × B_1B_1, No. 2♂) No. 24
c. A pair of *Rana brevipoda* chromosomes in a female, (B_1N_1, No. 2♀ × B_1B_1, No. 2♂) No. 2
d. A pair of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome with a translocation from a *R. brevipoda* chromosome in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 31

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

bivalent chromosome No. 3. One of these females was of the *Rana nigromaculata* type (B^nB^n) in MDH pattern, while the other five were of the hybrid type (B^nB^b). Of 33 mature females produced from a mating, B_1N_1, No. 2♀ × N_1N_1, No. 2♂, 16 were of the *Rana nigromaculata* type (B^nB^n) and 17 of hybrid type (B^nB^b). These genotypes agreed in constitution with bivalent chromosome No. 3 in 13 and 15 females, respectively. Of eight mature females produced from a mating, B_2N_2, No. 3♀ × N_2N_2, No. 3♂, six were of the *Rana nigromaculata* type (B^nB^n) and two of the hybrid type (B^nB^b). Except for one of the former six females, each female had bivalent chromosome No. 3 which agreed in constitution with the genotype for MDH pattern (Table 3).

In summation, 23 and 24 of a total of 47 females were B^nB^n and B^nB^b in genotype for MDH pattern, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 3 in 19 and 22 females, respectively. In contrast, the genotype of each female agreed in constitution with each of the other 12 bivalent chromosomes (Nos. 1, 2, 4~13) in 18~28 females, while it differed from the latter in 19~29 females (Tables 3, 4 and 17).

TABLE 4
Relationship between the constitution of bivalent chromosome No. 3 and the genotypes of MDH-B

Kind	Bivalent chromosome		MDH-B			No. of frogs whose chromosomes were compared with the genotypes	
	Constitution	No. of frogs	Phenotype	Genotype	No. of frogs	Agreed	Disagreed
NB♀ × NN♂	NN	16	*nigrom.*	B^nB^n	15	13	3
	NB	25	hybrid	B^nB^b	26	23	2
	$N\frac{B}{N}$	1	hybrid	B^nB^b	1	1	0
	Total	42			42	37	5
NB♀ × BB♂	BB	25	*brevip.*	B^bB^b	26	24	1
	NB	20	hybrid	B^nB^b	19	18	2
	Total	45			45	42	3
BN♀ × NN♂	NN	21	*nigrom.*	B^nB^n	23	19	2
	NB	26	hybrid	B^nB^b	24	22	4
	Total	47			47	41	6
BN♀ × BB♂	BB	22	*brevip.*	B^bB^b	24	19	3
	NB	26	hybrid	B^nB^b	24	21	5
	Total	48			48	40	8
	NN	37	*nigrom.*	B^nB^n	38	32	5
	NB	97	hybrid	B^nB^b	93	84	13
	BB	47	*brevip.*	B^bB^b	50	43	4
	$N\frac{B}{N}$	1	hybrid	B^nB^b	1	1	0
	Total	182			182	160	22

4. Backcross offspring produced from BN♀ × BB♂

Of 12 mature females produced from a mating, B_1N_1, No. 1♀ × B_1B_1, No. 1♂, seven were of the *Rana brevipoda* type (B^bB^b) and five of the hybrid type (B^nB^b) in MDH pattern. These genotypes agreed in constitution with bivalent chromosome No. 3 in five and four females, respectively. Of 36 mature females produced from a mating, B_1N_1, No. 2♀ × B_1B_1, No. 2♂, 17 were of the *Rana brevipoda* type (B^bB^b) and 19 of the hybrid type (B^nB^b) in MDH pattern. These genotypes agreed in constitution with bivalent chromosome No. 3 in 14 and 17 females, respectively.

In summation, 24 and 24 of a total of 48 females produced from BN♀ × BB♂ were B^bB^b and B^nB^b in genotype for MDH pattern, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 3 in 19 and 21 females, respectively, while it differed from the latter in the remaining eight females. In contrast, the genotype of each female agreed in constitution with each of the other 12 bivalent chromosomes (Nos. 1, 2, 4~13) in 19~29 females, while it differed from the latter in 19~29 females (Tables 3, 4 and 18).

5. Summary of the experiments with MDH

Electrophoretic patterns of MDH were examined in 182 mature female backcross offspring. Then, the genotype for the MDH pattern of each female was collated with the constitution of each of the 13 bivalent chromosomes in her oocytes. The results showed that the genotype of each female agreed in constitution with bivalent chromosome No. 3 in 160 (87.9%) of the analyzed females. In contrast, the genotype agreed in constitution with each of the other 12 bivalent chromosomes (Nos. 1, 2, 4~13) in 82 (45.1%)~107 (58.8%), that is, in about half the number of females. Thus, chromosome No. 3 was assumed to bear the gene for MDH-B (Table 3).

The homologues of bivalent chromosome No. 3 were NN in 37, BB in 47, NB in 97 and NN with a translocation from a *Rana brevipoda* chromosome in one of the 182 analyzed females. Each of these constitutions agreed with that of the genotype for MDH pattern in 32 (B^nB^n), 43 (B^bB^b), 84 (B^nB^b) and 1 (B^nB^b) female, while it differed from the latter in a total of 22 females (Table 4).

IV. α-Glycerophosphate dehydrogenase (α-GDH)

Electrophoretic patterns of α-GDH were examined in 30 *Rana nigromaculata* and 30 *R. brevipoda*. These frogs were collected from the field in 1973 and their offspring. The electrophoretic pattern of each species consisted of five bands (Fig. 11a, c). The α-GDH of *Rana brevipoda* was faster in mobility than that of *R. nigromaculata*. Then, the electrophoretic patterns of α-GDH were examined in 40 male and female reciprocal hybrids of these two species. The results showed that the patterns of these hybrids consisted of the sum of the parental bands, although both the slowest band of *Rana nigromaculata* and the fastest one of *R. brevipoda* did not appear (Fig. 11b).

Electrophoretic patterns of α-GDH were also examined in 80 male and female backcross offspring including 20 from NB♀×NN♂, 20 from BN♀×NN♂,

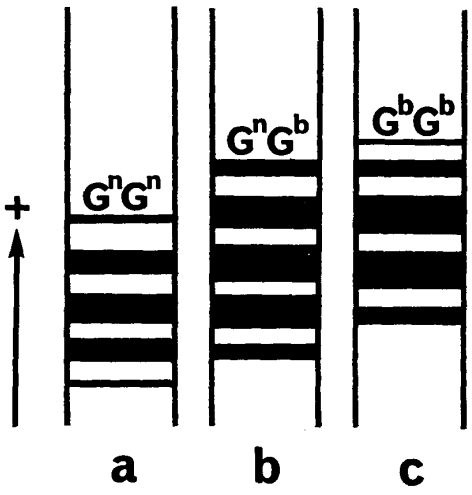

Fig. 11. Electrophoretic patterns of α-GDH.

a. *Rana nigromaculata* type b. Hybrid type c. *Rana brevipoda* type

20 from NB♀×BB♂ and 20 from BN♀×BB♂. The results indicated that 20 were of the *Rana nigromaculata* type, 40 of the hybrid type and 20 of the *R. brevipoda* type. Thus, it was evident that the α-GDH patterns of *Rana nigromaculata* and *R. brevipoda* were expressed by two codominant alleles, G^n and G^b.

Electrophoretic pattern of α-GDH as well as the constitution of homologues of each of the 13 bivalent chromosomes were examined in 182 female mature backcross offspring in order to identify the chromosome bearing the gene for α-GDH (Table 5).

TABLE 5

Number of frogs whose genotypes of α-GDH agreed in constitution with each of the 13 lampbrush (bivalent) chromosomes in their oocytes

Parents		No. of	Bivalent chromosome no.												
Female	Male	frogs	1	2	3	4	5	6	7	8	9	10	11	12	13
N_1B_1, No. 1	N_1N_1, No. 1	6	1	6	3	3	2	4	4	4	5	4	3	3	2
N_1B_1, No. 2	N_1N_1, No. 2	31	19	21	13	17	12	11	16	17	20	13	17	11	11
N_2B_2, No. 3	N_2N_2, No. 3	5	3	4	3	4	1	2	3	3	2	1	2	3	3
NB	NN	42	23	31	19	24	15	17	23	24	27	18	22	17	16
N_1B_1, No. 1	B_1B_1, No. 1	10	7	10	4	7	7	3	4	2	4	6	4	2	5
N_1B_1, No. 2	B_1B_1, No. 2	31	17	22	21	20	16	17	13	16	16	16	19	16	13
N_2B_2, No. 3	B_2B_2, No. 3	4	2	4	1	3	3	4	2	4	1	2	3	3	2
NB	BB	45	26	36	26	30	26	24	19	22	21	24	26	21	20
B_1N_1, No. 1	N_1N_1, No. 1	6	4	4	6	4	4	1	2	2	3	2	2	2	4
B_1N_1, No. 2	N_1N_1, No. 2	33	14	22	22	17	15	21	18	14	12	17	19	19	13
B_2N_2, No. 3	N_2N_2, No. 3	8	7	5	7	5	6	6	7	3	7	6	5	3	6
BN	NN	47	25	31	35	26	25	28	27	19	22	25	26	24	23
B_1N_1, No. 1	B_1B_1, No. 1	12	9	8	3	5	4	6	7	7	6	8	3	5	7
B_1N_1, No. 2	B_1B_1, No. 2	36	18	24	16	22	18	18	20	18	25	13	17	18	17
BN	BB	48	27	32	19	27	22	24	27	25	31	21	20	23	24
Total		182	101	130	99	107	88	93	96	90	101	88	94	85	83
		(%)	(55.5)	(71.4)	(54.4)	(58.8)	(48.4)	(51.1)	(52.7)	(49.5)	(55.5)	(48.4)	(51.6)	(46.7)	(45.6)

1. Backcross offspring produced from NB♀×NN♂

Of six mature females produced from a mating, N_1B_1, No. 1♀×N_1N_1, No. 1♂, four were of the *Rana nigromaculata* type (G^nG^n) and two of the hybrid type (G^nG^b) in α-GDH pattern. These genotypes agreed in constitution with bivalent chromosome No. 2 in all these six females. Of 31 mature females produced from a mating, N_1B_1, No. 2♀×N_1N_1, No. 2♂, 24 were of the *Rana nigromaculata* type (G^nG^n) and seven of the hybrid type (G^nG^b) in α-GDH pattern. These genotypes agreed in constitution with bivalent chromosome No. 2 (Figs. 12 and 13) in 15 and 6 females, respectively. Of five mature females produced from a mating, N_2B_2, No. 3♀×N_2N_2, No. 3♂, two were of the *Rana nigromaculata* type (G^nG^n) and three of the hybrid type (G^nG^b) in α-GDH pattern. Except one of the females with the *Rana nigromaculata* type, these genotypes agreed in constitution with bivalent chromosome No. 2 (Table 6).

In summation, 30 and 12 of a total of 42 mature females were G^nG^n and G^nG^b in genotype for α-GDH pattern, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 2 in 20 and 11 females,

respectively, while it differed from the latter in the remaining 11 females. In contrast, the genotype of each female agreed in constitution with each of the other 12 bivalent chromosome (Nos. 1, 3~13) in 27 females at most, usually in about half the number of females (Tables 5, 6 and 15).

2. Backcross offspring produced from NB♀ × BB♂

Of ten mature females produced from a mating, N_1B_1, No. 1♀ × B_1B_1, No. 1♂, six were of the *Rana brevipoda* type (G^bG^b) and four of the hybrid type (G^nG^b) in α-GDH pattern. These genotypes agreed in constitution with bivalent chromosome No. 2 in all the females. Of 31 mature females produced from a mating, N_1B_1, No. 2♀ × B_1B_1, No. 2♂, 13 were of the *Rana brevipoda* type (G^bG^b) and 18 of the hybrid type (G^nG^b). These genotypes agreed in

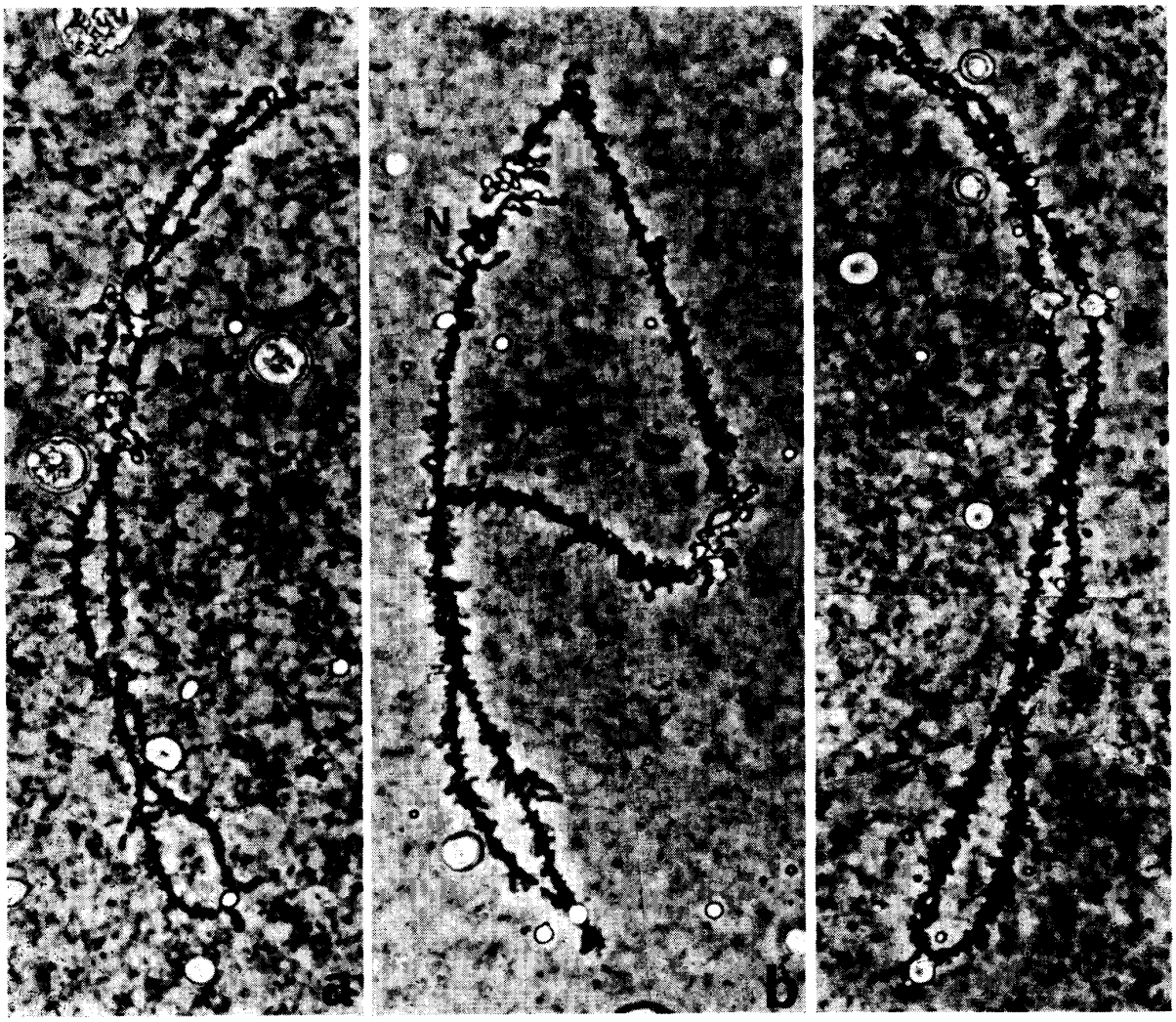

Fig. 12. Microphotographs of lampbrush (bivalent) chromosome No. 2 in oocytes of three female backcross offspring between *Rana nigromaculata* and *R. brevipoda*. × 500

a. A pair of *Rana nigromaculata* chromosomes in a female, (B_1N_1, No. 2♀ × N_1N_1, No. 2♂) No. 18
b. A pair of *Rana nigromaculata* and *R. brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 2
c. A pair of *Rana brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ × B_1B_1, No. 2♂) No. 31

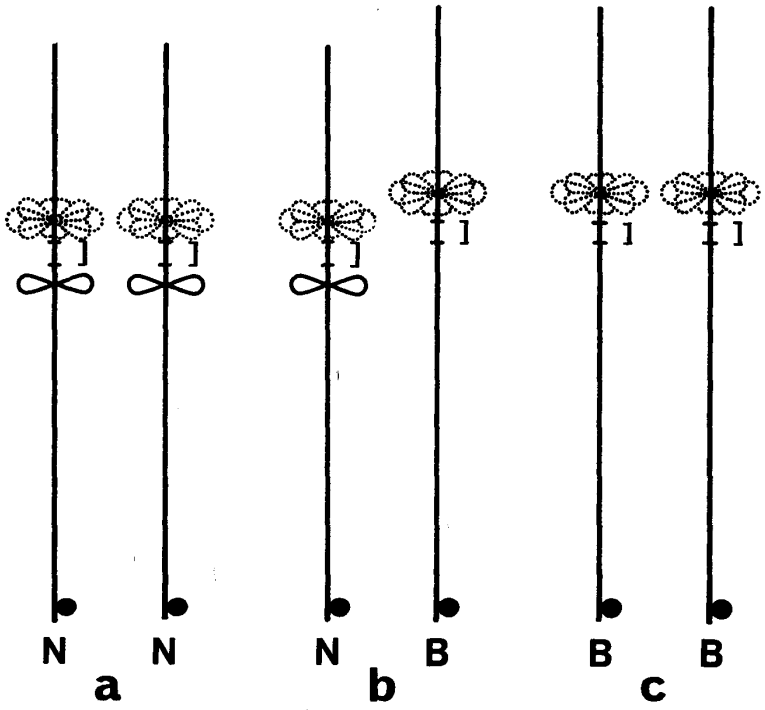

Fig. 13. Diagrams showing the constitution of bivalent chromosome No. 2 in the oocytes of three female backcross offspring between *Rana nigromaculata* and *R. brevipoda*.

a. A pair of *Rana nigromaculata* chromosomes in a female, (B_1N_1, No. 2♀ × N_1N_1, No. 2♂) No. 18
b. A pair of *Rana nigromaculata* and *R. brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 2
c. A pair of *Rana brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ × B_1B_1, No. 2♂) No. 31

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

constitution with bivalent chromosome No. 2 in 11 and 11 females, respectively, while they differed from the latter in two and seven females, respectively. Of four mature females produced from a mating, N_2B_2, No. 3♀ × B_2B_2, No. 3♂, three were of the *Rana brevipoda* type (G^bG^b) and one was of the hybrid type (G^nG^b). These genotypes agreed in constitution with bivalent chromosome No. 2 in all the females (Table 6).

In summation, 22 and 23 of a total of 45 mature females were G^bG^b and G^nG^b in genotype for α-GDH pattern, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 2 in 20 and 16 females, respectively, 36 females in total. In contrast, it agreed in constitution with each of the other 12 bivalent chromosomes (Nos. 1, 3~13) in 30 females at most, usually half the number of females (Tables 5, 6 and 16).

3. Backcross offspring produced from BN♀ × NN♂

One and five of six mature females produced from a mating, B_1N_1, No. 1♀ ×

N_1N_1, No. 1♂, were of the *Rana nigromaculata* type (G^nG^n) and the hybrid type (G^nG^b) in α–GDH pattern, respectively. These genotypes agreed in constitution with bivalent chromosome No. 2 in one and three females, respectively. Of 33 mature females produced from a mating, B_1N_1, No. 2♀ × N_1N_1, No. 2♂, 12 were of the *Rana nigromaculata* type (G^nG^n) and 21 of the hybrid type (G^nG^b). These genotypes agreed in constitution with bivalent chromosome No. 2 in 8 and 14 females, respectively. Of eight mature females produced from a mating, B_2N_2, No. 3♀ × N_2N_2, No. 3♂, four were of the *Rana nigromaculata* type (G^nG^n) and four of the hybrid type (G^nG^b) in α–GDH pattern. These genotypes agreed in constitution with bivalent chromosome No. 2 in four and one female, respectively.

TABLE 6

Relationship between the constitution of bivalent chromosome No. 2 and the genotypes of α–GDH

Kind	Bivalent chromosome		α–GDH			No. of frogs whose chromosomes were compared with the genotypes	
	Constitution	No. of frogs	Phenotype	Genotype	No. of frogs	Agreed	Disagreed
NB♀ × NN♂	NN	21	*nigrom.*	G^nG^n	30	20	1
	NB	21	hybrid	G^nG^b	12	11	10
	Total	42			42	31	11
NB♀ × BB♂	BB	27	*brevip.*	G^bG^b	22	20	7
	NB	18	hybrid	G^nG^b	23	16	2
	Total	45			45	36	9
BN♀ × NN♂	NN	25	*nigrom.*	G^nG^n	17	13	12
	NB	22	hybrid	G^nG^b	30	18	4
	Total	47			47	31	16
BN♀ × BB♂	BB	17	*brevip.*	G^bG^b	23	12	5
	NB	31	hybrid	G^nG^b	25	20	11
	Total	48			48	32	16
	NN	46	*nigrom.*	G^nG^n	47	33	13
	NB	92	hybrid	G^nG^b	90	65	27
	BB	44	*brevip.*	G^bG^b	45	32	12
	Total	182			182	130	52

In summation, 17 and 30 of a total of 47 mature females were G^nG^n and G^nG^b in genotype for α–GDH pattern, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 2 in 13 and 18 females, respectively. When the genotype of each female was collated with the constitution of each of the other 12 bivalent chromosomes, it agreed with the latter in 19~28 females except the case of bivalent chromosome No. 3 (Tables 5, 6 and 17). In this exceptional case, the genotype agreed in constitution with the homologues of this chromosome in 35 females. However, as the agreement of the genotypes with

bivalent chromosome No. 3 was found in 19 out of 42 females from NB♀ × NN♂, in 26 out of 45 females from NB♀ × BB♂ and in 19 out of 48 females from BN♀ × BB♂, chromosome No. 3 was not assumed to bear the gene for α–GDH.

4. Backcross offspring produced from BN♀ × BB♂

Five and seven of 12 mature females produced from a mating, B_1N_1, No. 1♀ × B_1B_1, No. 1♂, were of the *Rana brevipoda* type (G^bG^b) and the hybrid type (G^nG^b) in α–GDH pattern, respectively. The genotypes for these α–GDH patterns agreed in constitution with bivalent chromosome No. 2 in two and six females, respectively. Of 36 mature females produced from a mating, B_1N_1, No. 2♀ × B_1B_1, No. 2♂, 18 were of the *Rana brevipoda* type (G^bG^b) and 18 of the hybrid type (G^nG^b). The genotypes agreed in constitution with bivalent chromosome No. 2 in 10 and 14 females, respectively.

In summation, 23 and 25 of a total of 48 females produced from BN♀ × BB♂ were G^bG^b and G^nG^b in genotype for α–GDH pattern, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 2 in 12 and 20 females, respectively, while it differed from the latter in 5 and 11 females, respectively. In contrast, the genotype of each female agreed in constitution with each of the other 12 bivalent chromosomes in 19～27 females, except that it agreed with bivalent chromosome No. 9 in 31 females (Tables 5, 6 and 18).

5. Summary of the experiments with α–GDH

Electrophoretic patterns of α–GDH were examined in 182 mature female backcross offspring. Then, the constitution of the genotype for the α–GDH pattern of each female was collated with that of each of the 13 bivalent chromosomes in her oocytes. The results indicated that the genotype agreed in constitution with bivalent chromosome No. 2 in 130 (71.4%) females. In contrast, the genotype for α–GDH pattern agreed in constitituion with each of the other 12 bivalent chromosomes in 83 (45.6%)～107 (58.8%) females, that is, about half the number of females. Thus, the gene for α–GDH was assumed to be borne on chromosome No. 2. (Table 5)

The constitution of bivalent chromosome No. 2 was NN in 46, BB in 44 and NB in 92 of a total of 182 females. These chromosome constitutions agreed with the genotypes for α–GDH pattern in 33, 32 and 65 females, respectively, while they differed from the latter in 13, 12 and 27 females, respectively (Table 6).

V. Isocitrate dehydrogenase (IDH)

Electrophoretic analyses of IDH were made in 30 *Rana nigromaculata* and 30 *Rana brevipoda*. These frogs of each species included two pairs collected from the field and 26 offspring produced by these pairs. The electrophoretic pattern of IDH of each species consisted of four bands that moved toward the anode. One of these bands ran extremely faster than a group of three other bands. While the two species were the same in mobility of this group of bands, they differed from each other in mobility of the fast band; the band of *Rana nigromaculata* was

remarkably faster than that of *Rana brevipoda* (Fig. 14a, c).

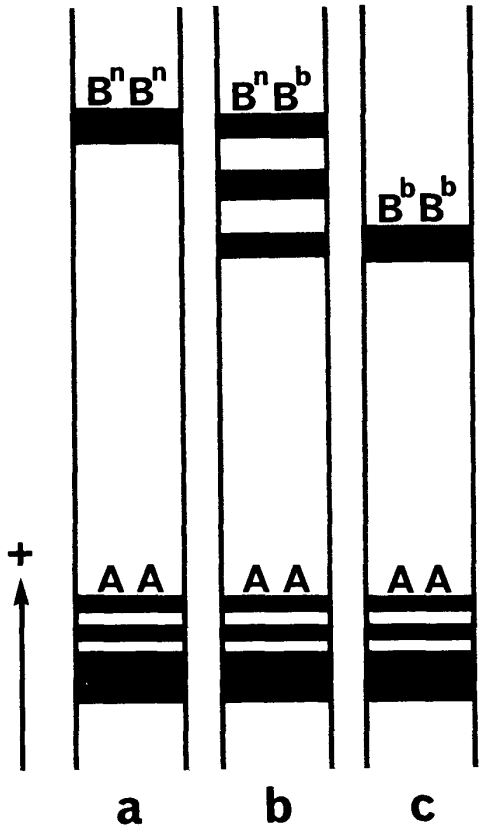

Fig. 14. Electrophoretic patterns of IDH.
a. *Rana nigromaculata* type b. Hybrid type c. *Rana brevipoda* type

Electrophoretic patterns of IDH were examined in 40 mature male and female reciprocal hybrids between the two species. It was found that all of them had a pattern characteristic of hybrids (Fig. 14b). Next, the IDH of 80 frogs produced from backcrosses between six female reciprocal hybrids and male *Rana nigromaculata* or *R. brevipoda* was electrophoretically examined. The result of analyses showed that 19, 43 and 18 of the 80 male and female frogs were of the *Rana nigromaculata,* the hybrid and the *R. brevipoda* type in IDH pattern, respectively. Thus, it was evident that the single faster band of IDH in *Rana nigromaculata* or *R. brevipoda* was expressed by a codominant allelic gene called B^n or B^b, in contrast with the group of slower bands which was expressed by a commom gene named *A*.

In order to identify the chromosome bearing gene B^n or B^b for IDH-B, electrophoretic patterns and lampbrush chromosomes were examined in 182 mature females produced from backcrosses between female reciprocal hybrids and males of the parental species (Table 7).

TABLE 7

Number of frogs whose genotypes of IDH-B agreed in constitution with each of the 13 lampbrush (bivalent) chromosomes in their oocytes

Parents		No. of	Bivalent chromosome no.												
Female	Male	frogs	1	2	3	4	5	6	7	8	9	10	11	12	13
N_1B_1, No. 1	N_1N_1, No. 1	6	4	3	4	4	3	6	1	3	2	3	4	4	3
N_1B_1, No. 2	N_1N_1, No. 2	31	14	15	18	17	16	31	16	15	16	13	18	11	15
N_2B_2, No. 3	N_2N_2, No. 3	5	2	1	2	1	4	5	4	2	1	4	3	2	4
NB	NN	42	20	19	24	22	23	42	21	20	19	20	25	17	22
N_1B_1, No. 1	B_1B_1, No. 1	10	6	3	3	4	2	10	5	5	5	5	7	5	4
N_1B_1, No. 2	B_1B_1, No. 2	31	18	11	16	23	14	30	22	21	13	19	15	15	12
N_2B_2, No. 3	B_2B_2, No. 3	4	2	4	1	3	3	4	2	4	1	2	3	3	2
NB	BB	45	26	18	20	30	19	44	29	30	19	26	25	23	18
B_1N_1, No. 1	N_1N_1, No. 1	6	3	3	1	3	3	6	3	3	4	5	3	5	3
B_1N_1, No. 2	N_1N_1, No. 2	33	14	16	12	15	15	32	16	12	18	19	15	15	13
B_2N_2, No. 3	N_2N_2, No. 3	8	4	4	6	4	5	8	6	2	6	5	4	6	3
BN	NN	47	21	23	19	22	23	46	25	17	28	29	22	26	19
B_1N_1, No. 1	B_1B_1, No. 1	12	7	6	7	5	8	12	7	7	10	6	5	5	7
B_1N_1, No. 2	B_1B_1, No. 2	36	21	25	19	17	15	36	18	15	16	16	13	19	20
BN	BB	48	28	31	26	22	23	48	25	22	26	22	18	24	27
Total		182	95	91	89	96	88	180	100	89	92	97	90	90	86
		(%)	(52.2)	(50.0)	(48.9)	(52.7)	(48.4)	(98.9)	(54.9)	(48.9)	(50.5)	(53.3)	(49.5)	(49.5)	(47.3)

1. Backcross offspring produced from NB♀ × NN♂

Of six mature females produced from a mating, N_1B_1, No. 1♀ × N_1N_1, No. 1♂, three were of the *Rana nigromaculata* type (B^nB^n) and three of the hybrid type (B^nB^b) in IDH pattern. Of 31 mature females produced from a mating, N_1B_1, No. 2♀ × N_1N_1, No. 2♂, 12 were of the *Rana nigromaculata* type (B^nB^n) and 19 of the hybrid type (B^nB^b). Three and two of five mature females produced from a mating, N_2B_2, No. 3♀ × N_2N_2, No. 3♂, were of the *Rana nigromaculata* type (B^nB^n) and the hybrid type (B^nB^b), respectively.

In summation, 18 and 24 of a total of 42 females were B^nB^n and B^nB^b in genotype for IDH pattern, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 6 in all the females. However, one of the three B^nB^b females produced from N_1B_1, No. 1♀ × N_1N_1, No. 1♂ had bivalent chromosome No. 6 whose constitution was N and N with a translocation from B (Figs. 15d and 16d). On the other hand, the genotype for the IDH pattern of each female agreed in constitution with each of the other 12 bivalent chromosomes in 17~25 females, that is, nearly half the number of females (Tables 8 and 15).

2. Backcross offspring produced from NB♀ × BB♂

Three and seven of 10 mature females produced from a mating, N_1B_1, No. 1♀ × B_1B_1, No. 1♂, were of the *Rana brevipoda* type (B^bB^b) and the hybrid type (B^nB^b) in IDH pattern, respectively. Of 31 mature females produced from a mating, N_1B_1, No. 2♀ × B_1B_1, No. 2♂, eight were of the *Rana brevipoda* type (B^bB^b) and 23 of the hybrid type (B^nB^b). Of four mature females produced from a mating, N_2B_2, No. 3♀ × B_2B_2, No. 3♂, three were of the *Rana brevipoda*

type (B^bB^b) and one was of the hybrid type (B^nB^b).

In summation, 14 and 31 of a total of 45 females were B^bB^b and B^nB^b in genotype for IDH pattern, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 6 in 13 and 31 females, respectively. In contrast, the genotype of each female agreed in constitution with each of the other 12 bivalent chromosomes (Nos. 1~5, 7~13) in 18~30 females (Tables 8 and 16).

TABLE 8
Relationship between the constitution of bivalent chromosome No. 6 and the genotypes of IDH-B

Kind	Bivalent chromosome		IDH-B			No. of frogs whose chromosomes were compared with the genotypes	
	Constitution	No. of frogs	Phenotype	Genotype	No. of frogs	Agreed	Disagreed
NB♀×NN♂	NN	18	*nigrom.*	B^nB^n	18	18	0
	NB	23	hybrid	B^nB^b	23	23	0
	$N\frac{B}{N}$	1	hybrid	B^nB^b	1	1	0
	Total	42			42	42	0
NB♀×BB♂	BB	13	*brevip.*	B^bB^b	14	13	0
	NB	32	hybrid	B^nB^b	31	31	1
	Total	45			45	44	1
BN♀×NN♂	NN	26	*nigrom.*	B^nB^n	27	26	0
	NB	21	hybrid	B^nB^b	20	20	1
	Total	47			47	46	1
BN♀×BB♂	BB	8	*brevip.*	B^bB^b	8	8	0
	NB	38	hybrid	B^nB^b	38	38	0
	$B\frac{N}{B}$	2	*brevip.*	B^bB^b	2	2	0
	Total	48			48	48	0
	NN	44	*nigrom.*	B^nB^n	45	44	0
	NB	114	hybrid	B^nB^b	112	112	2
	BB	21	*brevip.*	B^bB^b	22	21	0
	$N\frac{B}{N}$	1	hybrid	B^nB^b	1	1	0
	$B\frac{N}{B}$	2	*brevip.*	B^bB^b	2	2	0
	Total	182			182	180	2

3. Backcross offspring produced from BN♀×NN♂

Of six mature females produced from a mating, B_1N_1, No. 1♀×N_1N_1, No. 1♂, four were of the *Rana nigromaculata* type (B^nB^n) and two of the hybrid type (B^nB^b) in IDH pattern. Of 33 mature females produced from a mating, B_1N_1, No. 2♀×N_1N_1, No. 2♂, 18 were of the *Rana nigromaculata* type (B^nB^n) and 15 of the hybrid type (B^nB^b). Of eight females produced from a mating, B_2N_2, No. 3♀×N_2N_2, No. 3♂, five were of the *Rana nigromaculata* type (B^nB^n)

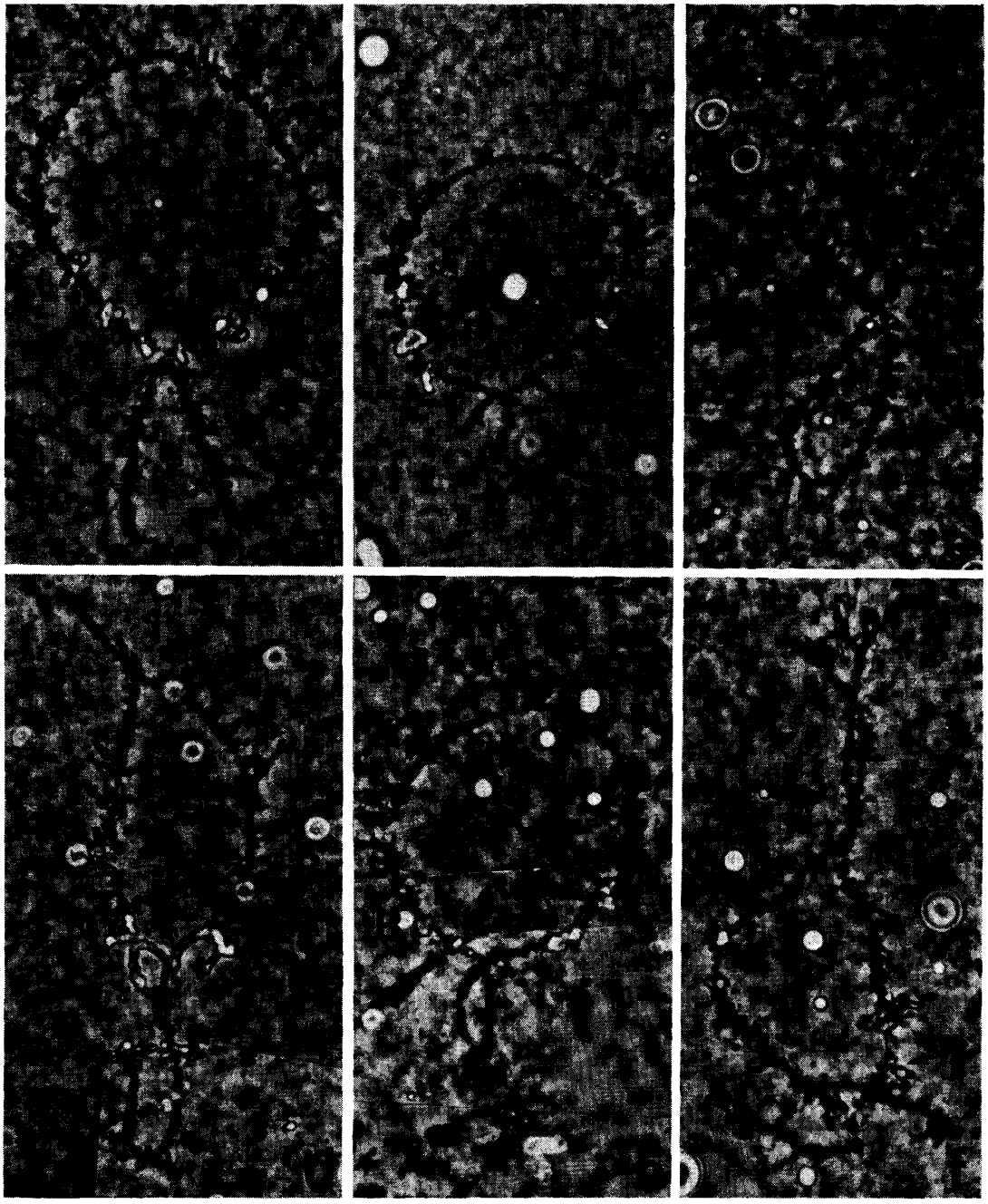

Fig. 15. Microphotographs of lampbrush (bivalent) chromosome No. 6 in oocytes of six female backcross offspring between *Rana nigromaculata* and *R. brevipoda*. ×500

a. A pair of *Rana nigromaculata* chromosomes in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 15
b. A pair of *Rana nigromaculata* and *R. brevipoda* chromosomes in a female, (N_1B_1, No. 1♀ × B_1B_1, No. 1♂) No. 1
c. A pair of *Rana brevipoda* chromosomes in a female, (B_1N_1, No. 2♀ × B_1B_1, No. 2♂) No. 28
d. A pair of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome with a translocation from a *R. brevipoda* chromosome in a female, (N_1B_1, No. 1♀ × N_1N_1, No. 1♂) No. 5
e. A pair of a *Rana brevipoda* chromosome and a *R. brevipoda* chromosome with an intercalated translocation from a *R. nigromaculata* chromosome in a female, (B_1N_1, No. 2♀ × B_1B_1, No. 2♂) No. 32
f. A pair of a *Rana brevipoda* chromosome and a *R. brevipoda* chromosome with an intercalated translocation from a *R. nigromaculata* chromosome in a female, (B_1N_1, No. 2♀ × B_1B_1, No. 2♂) No. 36

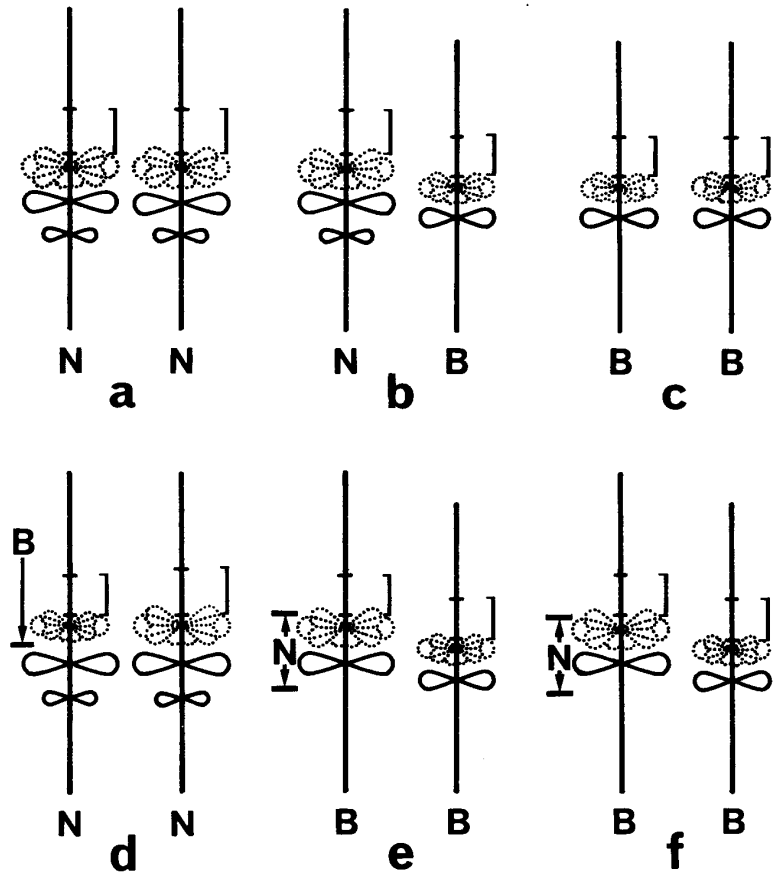

Fig. 16. Diagrams showing the constitution of bivalent chromosome No. 6 in the oocytes of six female backcross offspring between *Rana nigromaculata* and *R. brevipoda*.

a. A pair of *Rana nigromaculata* chromosomes in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 15
b. A pair of *Rana nigromaculata* and *R. brevipoda* chromosomes in a female, (N_1B_1, No. 1♀ × B_1B_1, No. 1♂) No. 1
c. A pair of *Rana brevipoda* chromosomes in a female, (B_1N_1, No. 2♀ × B_1B_1, No. 2♂) No. 28
d. A pair of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome with a translocation from a *R. brevipoda* chromosome in a female, (N_1B_1, No. 1♀ × N_1N_1, No. 1♂) No. 5
e. A pair of a *Rana brevipoda* chromosome and a *R. brevipoda* chromosome with an intercalated translocation from a *R. nigromaculata* chromosome in a female, (B_1N_1, No. 2♀ × B_1B_1, No. 2♂) No. 32
f. A pair of a *Rana brevipoda* chromosome and a *R. brevipoda* chromosome with an intercalated translocation from a *R. nigromaculata* chromosome in a female, (B_1N_1, No. 2♀ × B_1B_1, No. 2♂) No. 36

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

and three of the hybrid type (B^nB^b).

In summation, 27 and 20 of a total of 47 females were B^nB^n and B^nB^b in genotype for IDH–B, respectively. Except one female with B^nB^n, the genotype of each female agreed in constitution with bivalent chromosome No. 6 in all the 46 females (Table 8). In contrast, the genotype of each female agreed in

constitution with each of the other 12 bivalent chromosomes in 17~29 females (Tables 8 and 17).

4. Backcross offspring produced from BN♀×BB♂

Of 12 mature females produced from a mating, B_1N_1, No. 1♀×B_1B_1, No. 1♂, three were of the *Rana brevipoda* type (B^bB^b) and nine of the hybrid type (B^nB^b) in IDH pattern. Of 36 mature females produced from a mating, B_1N_1, No. 2♀×B_1B_1, No. 2♂, seven were of the *Rana brevipoda* type (B^bB^b) and 29 of the hybrid type (B^nB^b).

In summation, 10 and 38 of a total of 48 females were B^bB^b and B^nB^b in genotype for IDH. The genotype of each female agreed in constitution with bivalent chromosome No. 6 in all the females (Table 8). However, two of the seven B^bB^b females produced from B_1N_1, No. 2♀×B_1B_1, No. 2♂ had bivalent chromosome No. 6 whose constitution was B and B with an intercalated translocation from N (Figs. 15e, f and 16e, f). On the other hand, the genotype for IDH of each female agreed in constitution with each of the other 12 bivalent chromosomes in 18~31 females (Tables 8 and 18).

5. Summary of the experiments with IDH

Electrophoretic patterns of IDH and lampbrush chromosomes of oocytes were examined in 182 mature females produced from backcrosses between female hybrids and males of the parental species. Then, the constitution of the genotype for IDH of each female backcross offspring was collated with that of each of the 13 bivalent chromosomes in her oocytes. It was found that the genotype of each female agreed in constitution with bivalent chromosome No. 6 in 180 (98.9%) females. In contrast, the genotype for IDH of each female agreed in constitution with each of the other 12 bivalent chromosomes in 86 (47.3%)~100 (54.9%) females. Thus, the gene for IDH–B was assumed to be borne on chromosome No. 6 (Table 7).

The constitution of bivalent chromosome No. 6 was NN in 44, BB in 21, NB in 114, NN with a translocation from B in one and BB with an intercalated translocation from N in two of the total 182 females. These chromosome constitutions agreed with the genotypes for IDH in 44 (B^nB^n), 21 (B^bB^b), 112 (B^nB^b), one (B^nB^b) and two (B^bB^b) females, respectively, while they differed from the latter only in two females (Table 8).

VI. Hemoglobin (Hb)

Electrophoretic analyses of hemoglobin (Hb) were made in 30 males and females of *Rana nigromaculata* and *R. brevipoda* (Fig. 17a, c). These frogs included individuals collected from the field in 1973 and their offspring. It was found that the hemoglobin of each species had a characteristic pattern consisting of two bands that moved toward the anode. The hemoglobin of 40 male and female reciprocal hybrids between the two species showed an electrophoretic pattern corresponding to the sum of those of the two species (Fig. 17b). Then, the

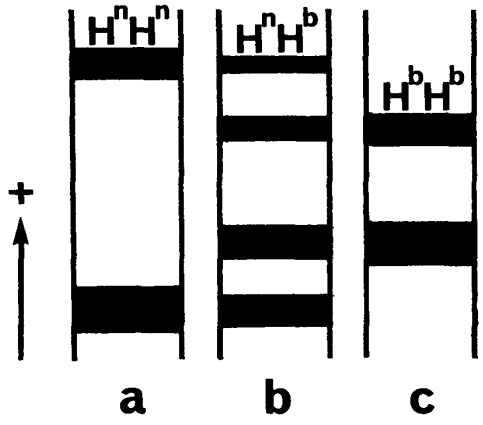

Fig. 17. Electrophoretic patterns of hemoglobin.
a. *Rana nigromaculata* type b. Hybrid type c. *Rana brevipoda* type

hemoglobin of 80 mature males and females produced from backcrosses between female reciprocal hybrids and male *Rana nigromaculata* or *R. brevipoda* was electrophoretically analyzed. It was found that 20, 40 and 20 of these frogs were of the *Rana nigromaculata*, the hybrid, and the *R. brevipoda* type in electrophoretic pattern, respectively. Thus, it was evident that the hemoglobin patterns of *Rana nigromaculata* and *R. brevipoda* were expressed by two codominant alleles H^n and H^b.

In order to identify the chromosome bearing the gene for hemoglobin, the electrophoretic pattern of hemoglobin and the lampbrush chromosomes in oocytes were examined in 182 mature female backcross offspring (Table 9).

TABLE 9
Number of frogs whose genotypes of hemoglobin agreed in constitution with each of the 13 lampbrush (bivalent) chromosomes in their oocytes

Parents		No. of	Bivalent chromosome no.												
Female	Male	frogs	1	2	3	4	5	6	7	8	9	10	11	12	13
N_1B_1, No. 1	N_1N_1, No. 1	6	4	3	4	4	3	6	1	3	2	3	4	4	3
N_1B_1, No. 2	N_1N_1, No. 2	31	14	15	18	17	16	31	16	15	16	13	18	11	15
N_2B_2, No. 3	N_2N_2, No. 3	5	2	1	2	1	4	5	4	2	1	4	3	2	4
NB	NN	42	20	19	24	22	23	42	21	20	19	20	25	17	22
N_1B_1, No. 1	B_1B_1, No. 1	10	6	3	3	4	2	10	5	5	5	5	7	5	4
N_1B_1, No. 2	B_1B_1, No. 2	31	18	11	16	23	14	30	22	21	13	19	15	15	12
N_2B_2, No. 3	B_2B_2, No. 3	4	2	4	1	3	3	4	2	4	1	2	3	3	2
NB	BB	45	26	18	20	30	19	44	29	30	19	26	25	23	18
B_1N_1, No. 1	N_1N_1, No. 1	6	3	3	1	3	3	6	3	3	4	5	3	5	3
B_1N_1, No. 2	N_1N_1, No. 2	33	15	17	13	18	18	31	15	13	18	18	18	14	16
B_2N_2, No. 3	N_2N_2, No. 3	8	4	4	6	4	5	8	6	2	6	5	4	6	3
BN	NN	47	22	24	20	25	26	45	24	18	28	28	25	25	22
B_1N_1, No. 1	B_1B_1, No. 1	12	7	6	7	5	8	12	7	7	10	6	5	5	7
B_1N_1, No. 2	B_1B_1, No. 2	36	20	24	20	16	14	35	17	14	17	17	14	18	19
BN	BB	48	27	30	27	21	22	47	24	21	27	23	19	23	26
Total		182	95	91	91	98	90	178	98	89	93	97	94	88	88
		(%)	(52.2)	(50.0)	(50.0)	(53.8)	(49.5)	(97.8)	(53.8)	(48.9)	(51.1)	(53.3)	(51.6)	(48.4)	(48.4)

1. Backcross offspring produced from NB♀×NN♂

Of six mature females produced from a mating, N_1B_1, No. 1♀×N_1N_1, No. 1♂, three were of the *Rana nigromaculata* type (H^nH^n) and three of the hybrid type (H^nH^b) in Hb pattern. Of 31 mature females produced from a mating, N_1B_1, No. 2♀×N_1N_1, No. 2♂, 12 were of the *Rana nigromaculata* type (H^nH^n) and 19 of the hybrid type (H^nH^b). Of five mature females produced from a mating, N_2B_2, No. 3♀×N_2N_2, No. 3♂, three were of the *Rana nigromaculata* type (H^nH^n) and two of the hybrid type (H^nH^b). The genotypes for these electrophoretic patterns agreed in constitution with bivalent chromosome No. 6 in all the 42 females. However, one of the three H^nH^b females produced from N_1B_1, No. 1♀×N_1N_1, No. 1♂ had bivalent chromosome No. 6 which was N and N with a translocation from B (Figs. 15d and 16d). In contrast, the genotype for hemoglobin of each female agreed in constitution with each of the other 12 bivalent chromosomes (Nos. 1～5 and 7～13) in 17～25 females, that is, about half the number of females (Tables 10 and 15).

2. Backcross offspring produced from NB♀×BB♂

Of ten mature females produced from a mating, N_1B_1, No. 1♀×B_1B_1, No. 1♂, three were of the *Rana brevipoda* type (H^bH^b) and seven of the hybrid type (H^nH^b) in Hb pattern. Of 31 mature females produced from a mating, N_1B_1, No. 2♀×B_1B_1, No. 2♂, eight were of the *Rana brevipoda* type (H^bH^b) and 23 of the hybrid type (H^nH^b). Of four mature females produced from a mating, N_2B_2, No. 3♀×B_2B_2, No. 3♂, three were of the *Rana brevipoda* type (H^bH^b) and one was of the hybrid type (H^nH^b).

In summation, 14 and 31 of a total of 45 female backcross offspring were H^bH^b and H^nH^b in genotype for hemoglobin, respectively. These genotypes agreed in constitution with bivalent chromosome No. 6 in 44 out of the 45 females (Tables 10 and 16). In contrast, the genotype of each female agreed in constitution with each of the other 12 bivalent chromosomes in 18～30 females, that is, about half the number of females.

3. Backcross offspring produced from BN♀×NN♂

Of six mature females produced from a mating B_1N_1, No. 1♀×N_1N_1, No. 1♂, four were of the *Rana nigromaculata* type (H^nH^n) and two of the hybrid type (H^nH^b) in Hb pattern. Of eight mature females produced from a mating, B_2N_2, No. 3♀×N_2N_2, No. 3♂, five were of the *Rana nigromaculata* type (H^nH^n) and three of the hybrid type (H^nH^b). In summation, nine and five of a total of 14 female backcross offspring were H^nH^n and H^nH^b in genotype for hemoglobin, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 6 in all the females.

Of 33 mature females produced from a mating, B_1N_1, No. 2♀×N_1N_1, No. 2♂, 15 were of the *Rana nigromaculata* type (H^nH^n) and 18 of the hybrid type (H^nH^b). Except two of the females with H^nH^b, the genotype of each female agreed in constitution with bivalent chromosome No. 6. The two exceptional

females had NN in place of NB (Tables 10 and 17). In contrast, the genotype for hemoglobin of each female agreed in constitution with each of the other 12 bivalent chromosomes in 18 to 28 of the 47 females in total.

TABLE 10
Relationship between the constitution of bivalent chromosome No. 6 and the genotypes of hemoglobin

Kind	Bivalent chromosome		Hemoglobin			No. of frogs whose chromosomes were compared with the genotypes	
	Constitution	No. of frogs	Phenotype	Genotype	No. of frogs	Agreed	Disagreed
NB♀ × NN♂	NN	18	*nigrom.*	H^nH^n	18	18	0
	NB	23	hybrid	H^nH^b	23	23	0
	$N\frac{B}{N}$	1	hybrid	H^nH^b	1	1	0
	Total	42			42	42	0
NB♀ × BB♂	BB	13	*brevip.*	H^bH^b	14	13	0
	NB	32	hybrid	H^nH^b	31	31	1
	Total	45			45	44	1
BN♀ × NN♂	NN	26	*nigrom.*	H^nH^n	24	24	2
	NB	21	hybrid	H^nH^b	23	21	0
	Total	47			47	45	2
BN♀ × BB♂	BB	8	*brevip.*	H^bH^b	9	8	0
	NB	38	hybrid	H^nH^b	37	37	1
	$B\frac{N}{B}$	2	*brevip.*	H^bH^b	2	2	0
	Total	48			48	47	1
	NN	44	*nigrom.*	H^nH^n	42	42	2
	NB	114	hybrid	H^nH^b	114	112	2
	BB	21	*brevip.*	H^bH^b	23	21	0
	$N\frac{B}{N}$	1	hybrid	H^nH^b	1	1	0
	$B\frac{N}{B}$	2	*brevip.*	H^bH^b	2	2	0
	Total	182			182	178	4

4. Backcross offspring produced from BN♀ × BB♂

Of 12 mature females produced from a mating, B_1N_1, No. 1♀ × B_1B_1, No. 1♂, three were of the *Rana brevipoda* type (H^bH^b) and nine of the hybrid type (H^nH^b) in Hb pattern. The genotype for hemoglobin of each female agreed in constitution with bivalent chromosome No. 6 in her oocytes in all the females. Of 36 mature females produced from a mating, B_1N_1, No. 2♀ × B_1B_1, No. 2♂, eight were of the *Rana brevipoda* type (H^bH^b) and 28 of the hybrid type (H^nH^b). While the genotype of each female with H^bH^b agreed in constitution with bivalent chromosome No. 6 in five of the eight females, two other females had bivalent chromosome No. 6 which was BB with an intercalated translocation from N

(Figs. 15e, f and 16e, f). The remaining female was NB in the constitution of bivalent chromosome No. 6. The genotype of each female with H^nH^b completely agreed in constitution with bivalent chromosome No. 6 in all the 28 females (Tables 10 and 18).

In summation, 11 and 37 of a total of 48 female backcross offspring were H^bH^b and H^nH^b in genotype for hemoglobin, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 6 in 47 of the females. The remaining female was NB in the constitution of bivalent chromosome No. 6 in spite of H^bH^b. On the other hand, the genotype for hemoglobin of each female agreed in constitution with each of the other 12 bivalent chromosomes in 19 to 30 out of the 48 females.

5. Summary of the experiments with hemoglobin

Electrophoretic patterns of hemoglobin and lampbrush chromosomes were examined in 182 mature female backcross offspring. The genotype for hemoglobin of each female was collated with the constitution of each of the 13 bivalent chromosomes in her oocytes. It was observed that the genotype agreed in constitution with bivalent chromosome No. 6 in 178 (97.8%) out of the 182 females. In contrast, it agreed with each of the other 12 bivalent chromosomes in 88 (48.4%) to 98 (53.8%) females, that is, about half the number of females. Thus, it was assumed that the gene for hemoglobin was borne on chromosome No. 6 (Table 9).

VII. Albumin (Ab)

Electrophoretic patterns of serum albumin were examined in 23 male and female *Rana nigromaculata* and 23 male and female *Rana brevipoda*. These frogs of each species were produced in 1975 from matings of two pairs of frogs which were in turn obtained in 1973 from two pairs collected from the field. Of the 23 *Rana nigromaculata*, 12 and 11 were produced from matings, N_1N_1, No. 1♀ × N_1N_1, No. 1♂ and N_2N_2, No. 2♀ × N_2N_2, No. 3♂, respectively, while of the 23 *R. brevipoda*, 12 and 11 were produced from matings, B_1B_1, No. 1♀ × B_1B_1, No. 1♂ and B_2B_2, No. 2♀ × B_2B_2, No. 3♂, respectively. The albumin patterns of the 23 *Rana nigromaculata* consisted of a single band, while those of the 23 *R. brevipoda* consisted of one or two bands. While the 11 *Rana brevipoda* produced from B_2B_2, No. 2♀ × B_2B_2, No. 3♂ and seven of the 12 *R. brevipoda* produced from B_1B_1, No. 1♀ × B_1B_1, No. 1♂ showed a single band which was remarkably slower in mobility than that of *R. nigromaculata*, the remaining five *R. brevipoda* produced from the latter mating showed two bands. While one of these two bands was the same in mobility as the single band shown by *Rana brevipoda* described above, the other band was slightly slower than this band (Fig. 18a, d, e).

The electrophoretic patterns of reciprocal hybrids produced in 1973 from crosses of the two species showed two bands. While one of these bands was the same in mobility as the single band of *Rana nigromaculata*, the other was the same as the faster or slower band of *R. brevipoda*. When these two bands of *Rana brevipoda* were described separately, their frequency was as follows. Of seven

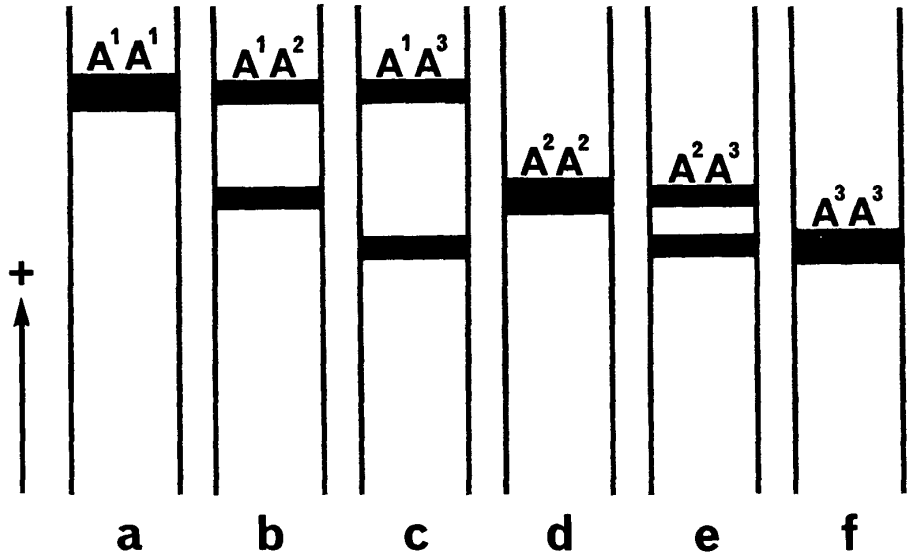

Fig. 18. Electrophoretic patterns of albumin.
a. *Rana nigromaculata* type b and c. Hybrid type
d, e and f. *Rana brevipoda* type

hybrids produced from 73NN, No. 1♀ × 73BB, No. 1♂, two showed the faster band and five showed the slower band (Fig. 18b, c). All 15 hybrids produced from 73NN, No. 2♀ × 73BB, No. 2♂ showed the faster band. Of ten hybrids produced from 73BB, No. 1♀ × 73NN, No. 1♂, seven showed the faster band and three showed the slower band. All 15 hybrids produced from 73BB, No. 2♀ × 73NN, No. 2♂ showed the faster band. Thus, it was evident that the electrophoretic patterns of serum albumin in *Rana nigromaculata* and *R. brevipoda* were determined by three alleles, A^1, A^2 and A^3, each of which was codominant. While the two males and two females of *Rana nigromaculata* were A^1A^1 in genotype, a male and a female *R. brevipoda*, 73BB, No. 2♂ and 73BB, No. 2♀, were A^2A^2 and the other male and female *R. brevipoda*, 73BB, No. 1♂ and 73BB, No. 1♀, were A^2A^3.

In 1975, a female hybrid, N_2B_2, No. 3, produced from a mating, 73NN, No. 2♀ × 73BB, No. 2♂, was mated with a male *Rana nigromaculata*, N_2N_2, No. 3♂. Of 15 backcross offspring produced from this mating, eight were of the *R. nigromaculata* type (A^1A^1) and seven of the hybrid type (A^1A^2) in electrophoretic pattern of albumin. The same female hybrid was mated with a male *Rana brevipoda*, B_2B_2, No. 3♂. Of 14 backcross offspring produced from this mating, seven were of the *Rana brevipoda* type (A^2A^2) and seven of the hybrid type (A^1A^2). On the other hand, a female reciprocal hybrid, B_2N_2, No. 3 obtained from a mating, 73BB, No. 2 ♀ × 73NN, No. 2♂, was mated with a male *Rana nigromaculata*, N_2N_2, No. 3♂. Of 18 backcross offspring produced from this mating, eleven were of the *Rana nigromaculata* type (A^1A^1) and seven of the hybrid type (A^1A^2). When the same female hybrid was mated with a male *Rana brevipoda*, B_2B_2, No. 3♂, six of ten backcross offspring were of the *R. brevipoda* type (A^2A^2) and the other four were of the hybrid type (A^1A^2). From these experiments, it was evident that the

hybrid, N_2B_2, No. 3 was A^1A^2 in genotype, while the male *Rana nigromaculata* and *R. brevipoda* were A^1A^1 and A^2A^2, respectively.

In order to identify the chromosome bearing the gene for albumin, the electrophoretic patterns of albumin were examined in 180 mature female backcross offspring and collated with the lampbrush chromosomes in the oocytes of these females (Table 11).

TABLE 11

Number of frogs whose genotypes of albumin agreed in constitution with each of the 13 lampbrush (bivalent) chromosomes in their oocytes

Parents		No. of	Bivalent chromosome no.												
Female	Male	frogs	1	2	3	4	5	6	7	8	9	10	11	12	13
N_1B_1, No. 1	N_1N_1, No. 1	6	6	1	4	4	3	4	3	1	0	1	2	4	3
N_1B_1, No. 2	N_1N_1, No. 2	31	29	19	19	13	10	13	12	11	16	15	16	17	17
N_2B_2, No. 3	N_2N_2, No. 3	5	5	2	1	4	1	2	3	5	2	1	4	1	3
NB	NN	42	40	22	24	21	14	19	18	17	18	17	22	22	23
N_1B_1, No. 1	B_1B_1, No. 1	10	10	7	5	8	6	6	5	5	5	7	5	5	4
N_1B_1, No. 2	B_1B_1, No. 2	30	30	14	17	19	15	16	14	15	12	15	17	15	19
N_2B_2, No. 3	B_2B_2, No. 3	4	3	3	2	2	2	3	3	3	0	1	4	3	3
NB	BB	44	43	24	24	29	23	25	22	23	17	23	26	23	26
B_1N_1, No. 1	N_1N_1, No. 1	6	6	4	4	2	4	3	2	4	3	4	2	4	4
B_1N_1, No. 2	N_1N_1, No. 2	33	33	17	17	18	18	14	12	17	20	17	18	15	20
B_2N_2, No. 3	N_2N_2, No. 3	8	7	7	7	5	4	6	5	3	5	4	3	5	4
BN	NN	47	46	28	28	25	26	23	19	24	28	25	23	24	28
B_1N_1, No. 1	B_1B_1, No. 1	12	12	9	4	4	7	7	10	6	5	9	2	8	8
B_1N_1, No. 2	B_1B_1, No. 2	35	33	15	17	19	15	23	16	16	16	19	13	23	16
BN	BB	47	45	24	21	23	22	30	26	22	21	28	15	31	24
Total		180	174	98	97	98	85	97	85	86	84	93	86	100	101
		(%)	(96.7)	(54.4)	(53.9)	(54.4)	(47.2)	(53.9)	(47.2)	(47.8)	(46.7)	(51.7)	(47.8)	(55.6)	(56.1)

1. Backcross offspring produced from NB♀ × NN♂

Of six mature female backcross offspring produced from a mating, N_1B_1, No. 1♀ × N_1N_1, No. 1♂, three were of the *Rana nigromaculata* type (A^1A^1) and three of the hybrid type (A^1A^3) in electrophoretic pattern of albumin. The genotypes for these albumin patterns agreed in constitution with bivalent chromosome No. 1 in all the females. Of 31 mature females produced from a mating, N_1B_1, No. 2♀ × N_1N_1, No. 2♂, 16 were of the *Rana nigromaculata* type (A^1A^1) and 15 of the hybrid type (A^1A^3). The genotypes agreed in constitution with bivalent chromosome No. 1 in 16 and 13 females, respectively, while they differed from the latter in the remaining two females with A^1A^3. Besides, one of the 16 females with A^1A^1 had bivalent chromosome NB, No. 1 with a translocation from N (Figs. 19d and 20d). Of five mature females produced from a mating, N_2B_2, No. 3♀ × N_2N_2, No. 3♂, four were of the *Rana nigromaculata* type (A^1A^1) and one was of the hybrid type (A^1A^2). The genotypes agreed in constitution with bivalent chromosome No. 1 in all the females (Tables 12 and 15).

In summation, 23 and 19 of a total of 42 mature female backcross offspring were A^1A^1 and A^1A^2 or A^1A^3 in genotype for albumin, respectively. The genotype of each female agreed in constitution with bivalent chromosome No. 1 in her

oocytes in all the 23 females and 17 of the 19 females, respectively. In contrast, the genotype for albumin of each female agreed in constitution of each of the other 12 bivalent chromosomes (Nos. 2～13) in 14～24 of the 42 females in total.

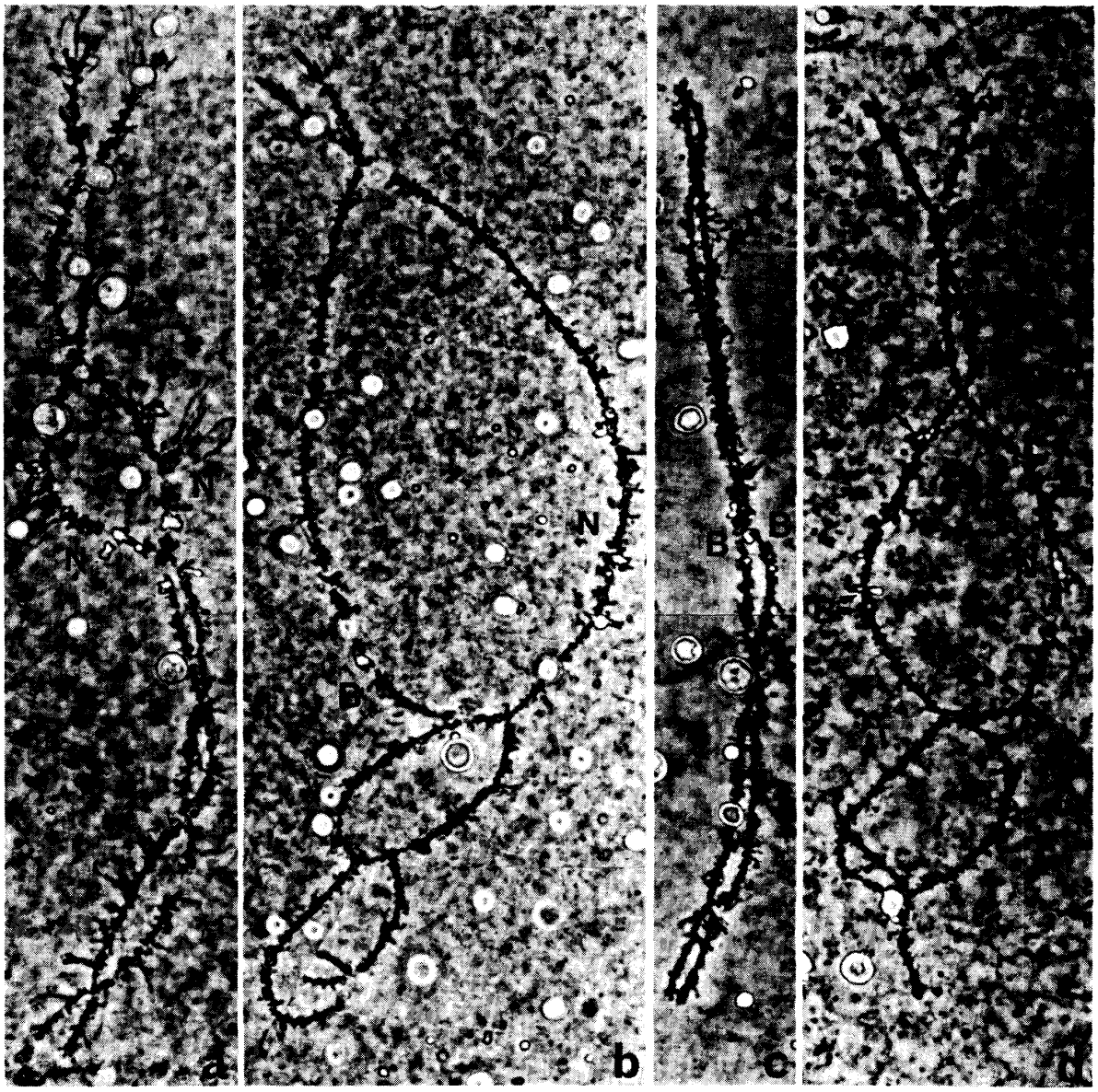

Fig. 19. Microphotographs of lampbrush (bivalent) chromosome No. 1 in oocytes of four female backcross offspring between *Rana nigromaculata* and *R. brevipoda*. ×430

a. A pair of *Rana nigromaculata* chromosomes in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 18
b. A pair of *Rana nigromaculata* and *R. brevipoda* chromosomes in a female, (N_2B_2, No. 3♀ × B_2B_2, No. 3♂) No. 2
c. A pair of *Rana brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ × B_1B_1, No. 2♂) No. 3
d. A pair of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome with a translocation from a *R. nigromaculata* chromosome in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 31

2. Backcross offspring produced from NB♀ × BB♂

Of ten mature females produced from a mating, N_1B_1, No. 1♀ × B_1B_1,

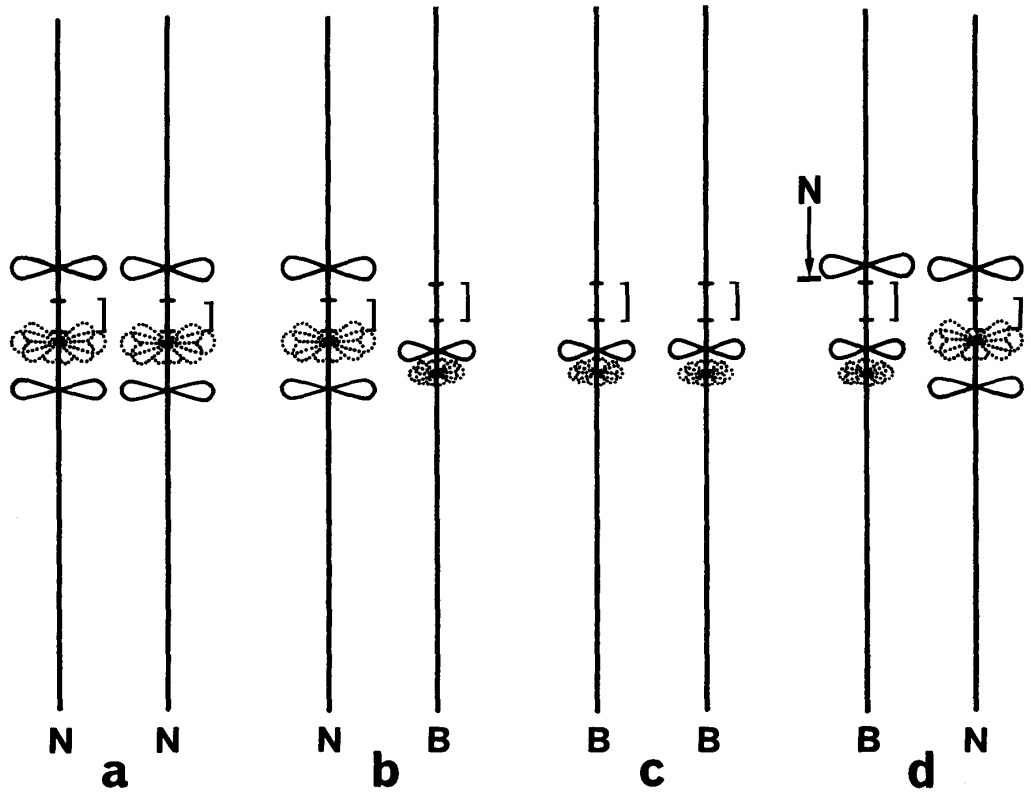

Fig. 20. Diagrams showing the constitution of bivalent chromosome No. 1 in the oocytes of four female backcross offspring between *Rana nigromaculata* and *R. brevipoda*.

a. A pair of *Rana nigromaculata* chromosomes in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 18
b. A pair of *Rana nigromaculata* and *R. brevipoda* chromosomes in a female, (N_2B_2, No. 3♀ × B_2B_2, No. 3♂) No. 2
c. A pair of *Rana brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ × B_1B_1, No. 2♂) No. 3
d. A pair of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome with a translocation from a *R. nigromaculata* chromosome in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 31

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

No. 1♂, three were of the *Rana brevipoda* type (A^2A^3) and seven of the hybrid type (A^1A^2) in albumin pattern. The genotypes for these albumin patterns agreed in constitution with bivalent chromosome No. 1 in all the females. Of 30 mature females produced from a mating, N_1B_1, No. 2♀ × B_1B_1, No. 2♂, nine were of the *Rana brevipoda* type (A^2A^3 in 5 and A^3A^3 in 4 females) and 21 of the hybrid type (A^1A^2 in 13 and A^1A^3 in 8 females). The genotypes for these albumin patterns completely agreed in constitution with bivalent chromosome No. 1 in all the females. Of four females produced from a mating, N_2B_2, No. 3♀ × B_2B_2, No. 3♂, two were of the *Rana brevipoda* type (A^2A^2) and two of the hybrid type (A^1A^2). These genotypes agreed in constitution with bivalent chromosome No. 1 in one and two females, respectively (Tables 12 and 16).

In summation, 14 and 30 of a total of 44 females produced from NB♀ × BB♂ were of the *Rana brevipoda* type (A^2A^2, A^2A^3 or A^3A^3) and the hybrid type (A^1A^2 or

A^1A^3) in albumin pattern, respectively. The genotype for the albumin pattern of each female agreed in constitution with bivalent chromosome No. 1 in 13 of the 14 females and all the 30 females, respectively. In contrast, the genotype of each female agreed with each of the other 12 bivalent chromosomes in 17～29 of the 44 females in total.

3. Backcross offspring produced from BN♀ × NN♂

Of six mature females produced from a mating, B_1N_1, No. 1♀ × N_1N_1, No. 1♂, three were of the *Rana nigromaculata* type (A^1A^1) and three of the hybrid type (A^1A^3) in albumin pattern. The genotypes for these albumin patterns agreed in constitution with bivalent chromosome No. 1 in all the females. Of 33 females produced from a mating, B_1N_1, No. 2♀ × N_1N_1, No. 2♂, 21 were of the *Rana nigromaculata* type (A^1A^1) and 12 of the hybrid type (A^1A^2). The genotypes agreed in constitution with bivalent chromosome No. 1 in all the females. Of eight mature females produced from a mating, B_2N_2, No. 3♀ × N_2N_2, No. 3♂, six were of the *Rana nigromaculata* type (A^1A^1) and two of the

TABLE 12
Relationship between the constitution of bivalent chromosome No. 1 and the genotypes of albumin

Kind	Bivalent chromosome		Albumin			No. of frogs whose chromosomes were compared with the genotypes	
	Constitution	No. of frogs	Phenotype	Genotype	No. of frogs	Agreed	Disagreed
NB♀ × NN♂	NN	24	*nigrom.*	A^1A^1	22	22	2
	NB	17	hybrid	A^1A^2, A^1A^3	19	17	0
	$N\frac{N}{B}$	1	*nigrom.*	A^1A^1	1	1	0
	Total	42			42	40	2
NB♀ × BB♂	BB	13	*brevip.*	A^2A^2, A^2A^3 A^3A^3	14	13	0
	NB	31	hybrid	A^1A^2, A^1A^3	30	30	1
	Total	44			44	43	1
BN♀ × NN♂	NN	29	*nigrom.*	A^1A^1	30	29	0
	NB	18	hybrid	A^1A^2, A^1A^3	17	17	1
	Total	47			47	46	1
BN♀ × BB♂	BB	14	*brevip.*	A^2A^2, A^2A^3	12	12	2
	NB	33	hybrid	A^1A^2, A^1A^3	35	33	0
	Total	47			47	45	2
	NN	53	*nigrom.*	A^1A^1	52	51	2
	NB	99	hybrid	A^1A^2, A^1A^3	101	97	2
	BB	27	*brevip.*	A^2A^2, A^2A^3 A^3A^3	26	25	2
	$N\frac{N}{B}$	1	*nigrom.*	A^1A^1	1	1	0
	Total	180			180	174	6

hybrid type (A^1A^2). These genotypes agreed in constitution with bivalent chromosome No. 1 in five and two females, respectively (Tables 12 and 17).

In summation, 30 and 17 of a total of 47 females produced from BN♀ × NN♂ were of the *Rana nigromaculata* type (A^1A^1) and the hybrid type (A^1A^2 or A^1A^3), respectively. The genotype for albumin of each female agreed in constitution with bivalent chromosome No. 1 in 29 of the 30 females and all the 17 females, respectively. In contrast, the genotype of each female agreed with each of the 12 bivalent chromosomes in 19~28 of the 47 females.

4. Backcross offspring produced from BN♀ × BB♂

Of 12 mature females produced from a mating, B_1N_1, No. 1♀ × B_1B_1, No. 1♂, two were of the *Rana brevipoda* type (A^2A^3) and ten of the hybrid type (A^1A^2) in albumin pattern. The genotypes for these albumin patterns agreed in constitution with bivalent chromosome No. 1 in all the females. Of 35 mature females produced from a mating, B_1N_1, No. 2♀ × B_1B_1, No. 2♂, ten were of the *Rana brevipoda* type (A^2A^2 in 3 and A^2A^3 in 7 females) and 25 of the hybrid type (A^1A^2 in 18 and A^1A^3 in 7 females). Except for two females with A^1A^2, the genotypes for these albumin patterns agreed in constitution with bivalent chromosome No. 1 (Tables 12 and 18).

In summation, 12 and 35 of a total of 47 females produced from BN♀ × BB♂, were of the *Rana brevipoda* type (A^2A^2 or A^2A^3) and the hybrid type (A^1A^2 or A^1A^3) in albumin pattern, respectively. The genotypes agreed in constitution with bivalent chromosome No. 1 in all the 12 females and 33 of the 35 females. In contrast, they agreed with each of the other 12 bivalent chromosomes in 15~31 of the 47 females in total.

5. Summary of the experiments with serum albumin

Electrophoretic patterns of serum albumin and lampbrush chromosomes in oocytes were examined in 180 mature females produced from backcrosses between female hybrids and males of the parental species. Then, the genotype for albumin of each female backcross offspring was collated with the constitution of each of the 13 bivalent chromosomes in her oocytes. It was observed that the genotype of each female agreed in constitution with bivalent chromosome No. 1 in 174 (96.7%) out of 180 females. In contrast, the genotype of each female agreed with each of the other 12 bivalent chromosomes in 84 (46.7%)~101 (56.1%) females, that is, nearly half the number of females. Thus, the gene for albumin was assumed to be borne on chromosome No. 1 (Table 11).

The constitution of bivalent chromosome No. 1 was NN in 53, BB in 27, NB in 99, and NN with a translocation from B in one of a total of 180 females (Figs. 19 and 20). These constitutions agreed with those of the genotypes for serum albumin in 51 (A^1A^1), 25 (A^2A^2, A^3A^3 or A^2A^3), 97 (A^1A^2 or A^1A^3) and one (A^1A^1) females, respectively, while they differed from the latter only in six females (Table 12).

VIII. Serum protein C

Electrophoretic analyses of crude blood serum were made in 40 mature frogs in 1975, including 10 frogs from N_1N_1, No. 1♀ × N_1N_1, No. 1♂, 10 frogs from N_2N_2, No. 2♀ × N_2N_2, No. 3♂, 10 frogs from B_1B_1, No. 1♀ × B_1B_1, No. 1♂ and 10 frogs from B_2B_2, No. 2♀ × B_2B_2, No. 3♂. It was found that each electrophoretic pattern consisted of five kinds of bands that moved toward the anode (Fig. 21). These bands were called A, B, C, D and E in the order of mobility, according to TUNNER (1970). Band A was the fastest and darkest among these bands; this fraction was albumin. Band C was the next in darkness. Band C of *Rana brevipoda* was distinctly faster in mobility than that of *R. nigromaculata*. As the substance indicated by band C was not identified, it was tentatively named serum protein C. At the same time, the gene for band C of *Rana nigromaculata* and *R. brevipoda* was called C^n and C^b, respectively (Fig. 22a, c). The fact that the slowest band named E indicated the position of transferrin was confirmed by the method of acrylamide-gel or starch-gel electrophoresis with acrynol pretreatment (MORIWAKI, SADAIE and HIRASAWA, 1974). Crude blood serum of 45 reciprocal hybrids, including seven N_1B_1 from 73NN, No. 1♀ × 73BB, No. 1♂, 13 N_2B_2 from 73NN, No. 2♀ × 73BB, No. 2♂, 10 B_1N_1 from 73BB, No. 1♀ × 73NN, No. 1♂ and 15 B_2N_2 from 73BB, No. 2♀ × 73NN, No. 2♂, was then electro-

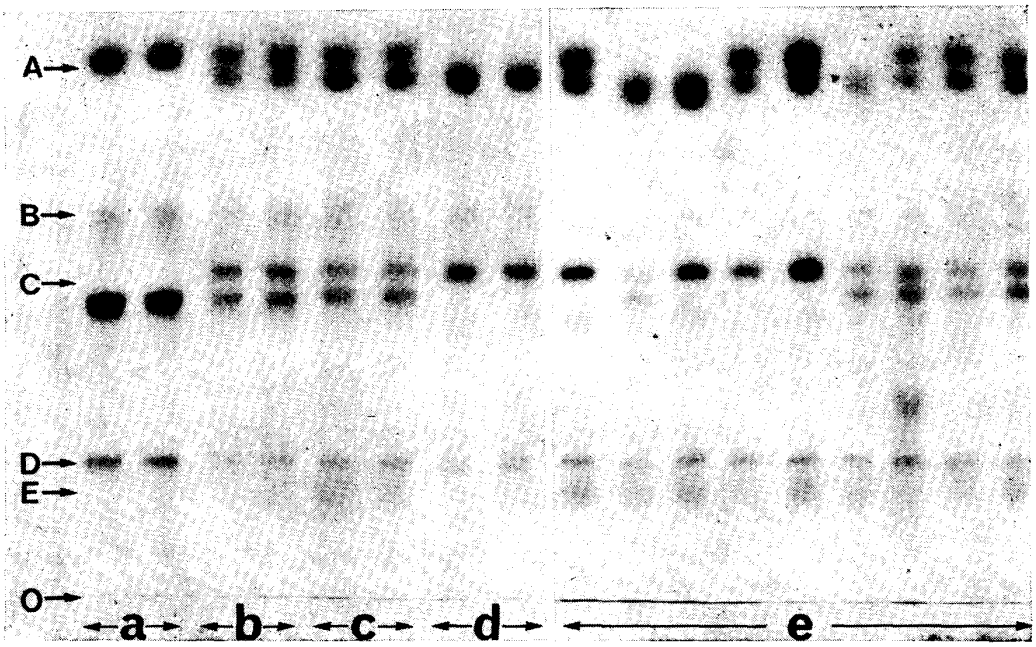

Fig. 21. Electrophoretic patterns of crude blood serum.

a. *Rana nigromaculata* (NN)
b. Hybrids between female *Rana nigromaculata* and male *R. brevipoda* (NB)
c. Hybrids between female *Rana brevipoda* and male *R. nigromaculata* (BN)
d. *Rana brevipoda* (BB)
e. Backcross offspring produced from NB♀ × BB♂

A, Albumin B, Protein B C, Protein C D, Protein D E, Transferrin O, Origin

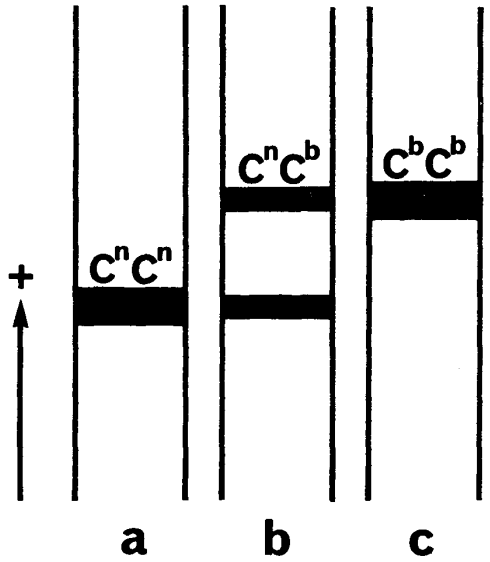

Fig. 22. Electrophoretic patterns of serum protein C.
a. *Rana nigromaculata* type b. Hybrid type c. *Rana brevipoda* type

phoretically analyzed. The results showed that the electrophoretic patterns of serum protein C always consisted of two bands (Fig. 22b).

Electrophoretic patterns of serum protein C were examined in 40 mature males and females produced from backcrosses between two female reciprocal hybrids (N_2B_2, No. 3♀ and B_2N_2, No. 3♀) and a male of *Rana nigromaculata* (N_2N_2, No. 3♂) or *R. brevipoda* (B_2B_2, No. 3♂). It was found that 12 frogs were of the *Rana nigromaculata* type (C^nC^n), 21 of the hybrid type (C^nC^b) and seven of the *R. brevipoda* type (C^bC^b). Thus, it was evident that the electrophoretic patterns of serum protein C were expressed by codominant alleles C^n and C^b.

In order to identify the chromosome bearing the gene for serum protein C, the electrophoretic patterns of this substance were examined in 176 mature female backcross offspring and collated with the lampbrush chromosomes in the oocytes of these females (Table 13).

1. Backcross offspring produced from NB♀ × NN♂

Of 42 mature female backcross offspring, including six from N_1B_1, No. 1♀ × N_1N_1, No. 1♂, 31 from N_1B_1, No. 2♀ × N_1N_1, No. 2♂ and five from N_2B_2, No. 3♀ × N_2N_2, No. 3♂, 16 were of the *Rana nigromaculata* type (C^nC^n) and 26 of the hybrid type (C^nC^b) in electrophoretic pattern of serum protein C. The genotypes for these patterns agreed in constitution with bivalent chromosome No. 9 in 13 and 23 females, respectively, while they differed from the latter in three and three females, respectively. In one female with C^nC^b, bivalent chromosome No. 9 was NB with a translocation from N (Table 14, Fig. 24d). In contrast, the genotypes for these electrophoretic patterns agreed in constitution with each of the other 12 bivalent chromosomes (Nos. 1~8, 10~13) in 16~29 out of the 42 females in total (Tables 14 and 15).

TABLE 13

Number of frogs whose genotypes of serum protein C agreed in constitution with each of the 13 lampbrush (bivalent) chromosomes in their oocytes

Parents		No. of	Bivalent chromosome no.												
Female	Male	frogs	1	2	3	4	5	6	7	8	9	10	11	12	13
N_1B_1, No. 1	N_1N_1, No. 1	6	2	3	2	4	5	3	3	5	4	3	4	4	1
N_1B_1, No. 2	N_1N_1, No. 2	31	14	22	14	14	19	14	15	16	28	16	14	16	14
N_2B_2, No. 3	N_2N_2, No. 3	5	3	4	3	4	1	0	1	3	4	1	2	3	1
NB	NN	42	19	29	19	22	25	17	19	24	36	20	20	23	16
N_1B_1, No. 1	B_1B_1, No. 1	10	6	5	5	6	6	6	5	5	9	5	5	5	6
N_1B_1, No. 2	B_1B_1, No. 2	29	11	10	18	15	18	13	17	13	27	11	17	17	15
N_2B_2, No. 3	B_2B_2, No. 3	4	1	1	2	2	2	1	1	1	4	3	0	2	1
NB	BB	43	18	16	25	23	26	20	23	19	40	19	22	24	22
B_1N_1, No. 1	N_1N_1, No. 1	6	3	3	3	3	5	4	3	3	6	3	3	3	5
B_1N_1, No. 2	N_1N_1, No. 2	31	20	15	18	21	18	14	13	18	25	19	22	15	18
B_2N_2, No. 3	N_2N_2, No. 3	8	6	6	6	4	5	5	6	4	6	5	4	4	5
BN	NN	45	29	24	27	28	28	23	22	25	37	27	29	22	28
B_1N_1, No. 1	B_1B_1, No. 1	12	5	4	5	5	6	10	7	7	12	8	5	3	7
B_1N_1, No. 2	B_1B_1, No. 2	34	13	23	17	19	19	18	17	17	27	19	15	19	20
BN	BB	46	18	27	22	24	25	28	24	24	39	27	20	22	27
Total		176	84	96	93	97	104	88	88	92	152	93	91	91	93
		(%)	(47.7)	(54.5)	(52.8)	(55.1)	(59.1)	(50.0)	(50.0)	(52.3)	(86.4)	(52.8)	(51.7)	(51.7)	(52.8)

2. Backcross offspring produced from NB♀ × BB♂

Of 43 mature female backcross offspring, including ten from N_1B_1, No. 1♀ × B_1B_1, No. 1♂, 29 from N_1B_1, No. 2♀ × B_1B_1, No. 2♂ and four from N_2B_2, No. 3♀ × B_2B_2, No. 3♂, 18 were of the *Rana brevipoda* type (C^bC^b) and 25 of the hybrid type (C^nC^b) in serum protein C pattern. The genotypes for these electrophoretic patterns agreed in constitution with bivalent chromosome No. 9 in 17 and 23 females, respectively. In one female with C^bC^b, bivalent chromosome No. 9 was BB with a translocation from N (Tables 14 and 16, Figs. 23d and 24e). The genotypes for electrophoretic patterns of serum protein C agreed in constitution with each of the other 12 bivalent chromosomes in 16~26 of the 43 females.

3. Backcross offspring produced from BN♀ × NN♂

Of 45 mature female backcross offspring, including six from B_1N_1, No. 1♀ × N_1N_1, No. 1♂, 31 from B_1N_1, No. 2♀ × N_1N_1, No. 2♂ and eight from B_2N_2, No. 3♀ × N_2N_2, No. 3♂, 21 were of the *Rana nigromaculata* type (C^nC^n) and 24 of the hybrid type (C^nC^b) in serum protein C pattern. The genotypes for these electrophoretic patterns agreed in constitution with bivalent chromosome No. 9 in 17 and 20 females, respectively. However, one of the 17 C^nC^n type females was NN with an intercalated translocation from B (Figs. 23f and 24h), while two of the 20 C^nC^b type females were NB with a translocation from N (Figs. 23e and 24f, g). The genotype for the electrophoretic pattern of each female agreed in constitution with each of the other 12 bivalent chromosomes in 22~29 of the total females (Tables 14 and 17).

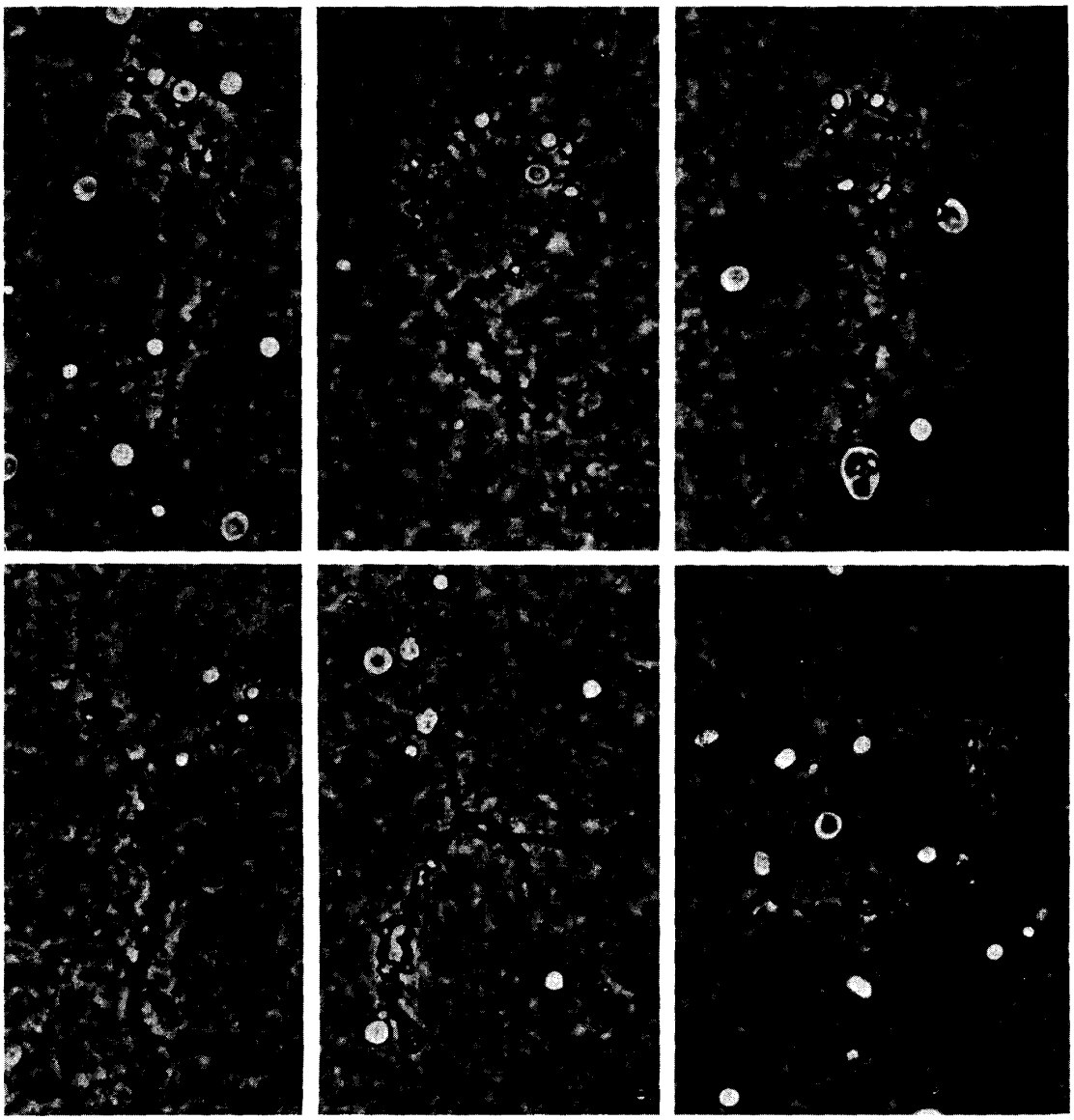

Fig. 23. Microphotographs of lampbrush (bivalent) chromosome No. 9 in oocytes of six female backcross offspring between *Rana nigromaculata* and *R. brevipoda*. ×500

a. A pair of *Rana nigromaculata* chromosomes in a female, (B_2N_2, No. 3♀ × N_2N_2, No. 3♂) No. 8

b. A pair of *Rana nigromaculata* and *Rana brevipoda* chromosomes in a female, (N_1B_1, No. 1♀ × N_1N_1, No. 1♂) No. 2

c. A pair of *Rana brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ × B_1B_1, No. 2♂) No. 7

d. A pair of a *Rana brevipoda* chromosome and a *R. brevipoda* chromosome with a translocation from a *R. nigromaculata* chromosome in a female, (N_1B_1, No. 2♀ × B_1B_1, No. 2♂) No. 24

e. A pair of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome with a translocation from a *R. nigromaculata* chromosome in a female, (B_1N_1, No. 2♀ × N_1N_1, No. 2♂) No. 15

f. A pair of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome with an intercalated translocation from a *R. brevipoda* chromosome in a female, (B_2N_2, No. 3♀ × N_2N_2, No. 3♂) No. 5

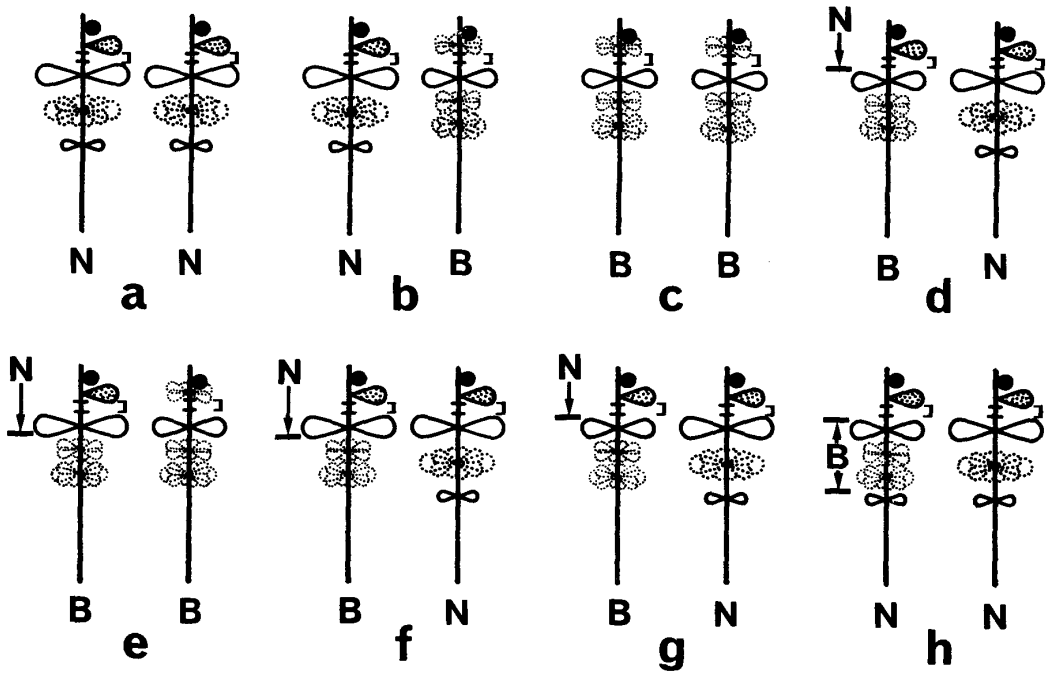

Fig. 24. Diagrams showing the constitution of bivalent chromosome No. 9 in the oocytes of eight female backcross offspring between *Rana nigromaculata* and *R. brevipoda*.

a. A pair of *Rana nigromaculata* chromosomes in a female, (B_2N_2, No. 3♀ × N_2N_2, No. 3♂) No. 8
b. A pair of *Rana nigromaculata* and *R. brevipoda* chromosomes in a female, (N_1B_1, No. 1♀ × N_1N_1, No. 1♂) No. 2
c. A pair of *Rana brevipoda* chromosomes in a female, (N_1B_1, No. 2♀ × B_1B_1, No. 2♂) No. 7
d. A pair of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome with a translocation from a *R. nigromaculata* chromosome in a female, (N_1B_1, No. 2♀ × N_1N_1, No. 2♂) No. 4
e. A pair of a *Rana brevipoda* chromosome and a *R. brevipoda* chromosome with a translocation from a *R. nigromaculata* chromosome in a female, (N_1B_1, No. 2♀ × B_1B_1, No. 2♂) No. 24
f. A pair of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome with a translocation from a *R. nigromaculata* chromosome in a female, (B_1N_1, No. 2♀ × N_1N_1, No. 2♂) No. 11
g. A pair of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome with a translocation from a *R. nigromaculata* chromosome in a female, (B_1N_1, No. 2♀ × N_1N_1, No. 2♂) No. 15
h. A pair of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome with an intercalated translocation from a *R. brevipoda* chromosome in a female, (B_2N_2, No. 3♀ × N_2N_2, No. 3♂) No. 5

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

4. Backcross offspring produced from BN♀ × BB♂

Of 46 mature female backcross offspring, including 12 from B_1N_1, No. 1♀ × B_1B_1, No. 1♂ and 34 from B_1N_1, No. 2♀ × B_1B_1, No. 2♂, 23 were of the *Rana brevipoda* type (C^bC^b) and 23 of the hybrid type (C^nC^b) in serum protein C pattern. The genotypes for these electrophoretic patterns agreed with the constitution of bivalent chromosome No. 9 in 20 and 19 females, respectively. In contrast, these genotypes agreed with the constitution of each of the other 12 bivalent chromosomes in 18~28 of the total females (Tables 14 and 18).

TABLE 14

Relationship between the constitution of bivalent chromosome No. 9 and the genotypes of serum protein C

Kind	Bivalent chromosome		Protein C			No. of frogs whose chromosomes were compared with the genotypes	
	Constitution	No. of frogs	Phenotype	Genotype	No. of frogs	Agreed	Disagreed
NB♀ × NN♂	NN	16	*nigrom.*	C^nC^n	16	13	3
	NB	25	hybrid	C^nC^b	25	22	3
	$N\frac{N}{B}$	1	hybrid	C^nC^b	1	1	0
	Total	42			42	36	6
NB♀ × BB♂	BB	18	*brevip.*	C^bC^b	17	16	2
	NB	24	hybrid	C^nC^b	25	23	1
	$B\frac{N}{B}$	1	*brevip.*	C^bC^b	1	1	0
	Total	43			43	40	3
BN♀ × NN♂	NN	20	*nigrom.*	C^nC^n	20	16	4
	NB	22	hybrid	C^nC^b	22	18	4
	$N\frac{B}{N}$	1	*nigrom.*	C^nC^n	1	1	0
	$N\frac{N}{B}$	2	hybrid	C^nC^b	2	2	0
	Total	45			45	37	8
BN♀ × BB♂	BB	24	*brevip.*	C^bC^b	23	20	4
	NB	22	hybrid	C^nC^b	23	19	3
	Total	46			46	39	7
	NN	36	*nigrom.*	C^nC^n	36	29	7
	NB	93	hybrid	C^nC^b	95	82	11
	BB	42	*brevip.*	C^bC^b	40	36	6
	$N\frac{B}{N}$	1	*nigrom.*	C^nC^n	1	1	0
	$N\frac{N}{B}$	3	hybrid	C^nC^b	3	3	0
	$B\frac{N}{B}$	1	*brevip.*	C^bC^b	1	1	0
	Total	176			176	152	24

5. Summary of the experiments with serum protein C

Electrophoretic patterns of serum protein C and lampbrush chromosomes in oocytes were examined in 176 mature female backcross offspring between female reciprocal hybrids and males of the two parental species. It was found that 37 females were of the *Rana nigromaculata* type (C^nC^n), 98 of the hybrid type (C^nC^b) and 41 of the *Rana brevipoda* type (C^bC^b) in electrophoretic patterns of serum protein C. The genotype for serum protein C of each female agreed in constitution with bivalent chromosome No. 9 in her oocytes in 152 (86.4%) out of 176 females. In contrast, the genotype of each female agreed in constitution with each of the other 12 bivalent chromosomes in 84 (47.7%) ~ 104 (59.1%) females. Thus, the gene for serum protein C was assumed to be carried on bivalent

chromosome No. 9.

The constitution of bivalent chromosome No. 9 was NN in 36, NB in 93, BB in 42, NB with a translocation from N in three, NN with an intercalated translocation from B in one and BB with a translocation from N in one of the total 176 females.

TABLE 15

Relationship between the genotype for each kind of protein and the constitution of the bivalent chromosome (B. ch.)* bearing the locus in each of the analyzed frogs produced from matings, NB♀ × NN♂

Parents		Individual no.	Albumin		α-GDH		MDH-B		LDH-B		IDH-B and Hb			Protein C	
Female	Male		Genotype	B. ch. No. 1	Genotype	B. ch. No. 2	Genotype	B. ch. No. 3	Genotype	B. ch. No. 4	Genotype	B. ch. No. 6	Genotype	Genotype	B. ch. No. 9
N_1B_1, No. 1	N_1N_1, No. 1	1	A^1A^1	NN	G^nG^b	BN	B^nB^b	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		2	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		3	A^1A^1	NN	G^nG^b	BN	B^nB^n	NN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		4	A^1A^3	BN	G^nG^n	NN	B^nB^n	NN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^b	NN
		5	A^1A^3	BN	G^nG^n	NN	B^nB^b	BN	B^nB^b	BN	B^nB^b	$\frac{B}{N}N$	H^nH^b	C^nC^n	NN
		6	A^1A^3	BN	G^nG^n	NN	B^nB^n	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	NN
N_1B_1, No. 2	N_1N_1, No. 2	1	A^1A^1	NN	G^nG^b	BN	B^nB^b	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		2	A^1A^3	BN	G^nG^n	BN	B^nB^n	NN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		3	A^1A^1	NN	G^nG^b	BN	B^nB^n	NN	B^nB^n	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		4	A^1A^1	NN	G^nG^n	BN	B^nB^n	NN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	$\frac{N}{B}N$
		5	A^1A^3	BN	G^nG^b	NN	B^nB^b	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		6	A^1A^1	NN	G^nG^n	NN	B^nB^b	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		7	A^1A^1	NN	G^nG^n	BN	B^nB^b	BN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		8	A^1A^3	BN	G^nG^n	NN	B^nB^n	BN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		9	A^1A^3	BN	G^nG^n	NN	B^nB^b	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		10	A^1A^3	BN	G^nG^n	NN	B^nB^b	BN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		11	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		12	A^1A^3	BN	G^nG^n	BN	B^nB^n	NN	B^nB^b	NN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		13	A^1A^3	BN	G^nG^b	BN	B^nB^b	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		14	A^1A^3	BN	G^nG^n	NN	B^nB^b	BN	B^nB^b	NN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		15	A^1A^3	BN	G^nG^b	BN	B^nB^n	NN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^n	BN
		16	A^1A^1	NN	G^nG^n	BN	B^nB^b	NN	B^nB^n	BN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		17	A^1A^1	NN	G^nG^n	NN	B^nB^b	NN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		18	A^1A^1	NN	G^nG^n	NN	B^nB^b	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		19	A^1A^1	NN	G^nG^n	NN	B^nB^b	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		20	A^1A^3	BN	G^nG^b	BN	B^nB^b	BN	B^nB^n	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		21	A^1A^3	BN	G^nG^n	BN	B^nB^b	BN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		22	A^1A^3	NN	G^nG^n	NN	B^nB^b	BN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^b	NN
		23	A^1A^1	NN	G^nG^n	NN	B^nB^b	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		24	A^1A^1	NN	G^nG^b	BN	B^nB^b	NN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		25	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		26	A^1A^1	NN	G^nG^n	NN	B^nB^b	BN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		27	A^1A^1	NN	G^nG^n	NN	B^nB^b	BN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^n	BN
		28	A^1A^3	NN	G^nG^n	BN	B^nB^b	BN	B^nB^n	BN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		29	A^1A^3	BN	G^nG^n	BN	B^nB^b	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		30	A^1A^3	BN	G^nG^n	BN	B^nB^n	NN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		31	A^1A^1	$\frac{B}{N}N$	G^nG^n	NN	B^nB^b	$\frac{B}{N}N$	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^n	NN
N_2B_2, No. 3	N_2N_2, No. 3	1	A^1A^1	NN	G^nG^b	BN	B^nB^b	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		2	A^1A^1	NN	G^nG^b	BN	B^nB^b	BN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		3	A^1A^2	BN	G^nG^b	BN	B^nB^n	NN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		4	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^n	BN
		5	A^1A^1	NN	G^nG^n	BN	B^nB^b	BN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^b	BN

* The right letter of each symbol indicates the chromosome from the male parent.

These constitutions agreed with the genotypes for serum protein C in 29 (C^nC^n), 82 (C^nC^b), 36 (C^bC^b), 3 (C^nC^b), 1 (C^nC^n) and 1 (C^bC^b), respectively, while they differed from the latter only in 24 females.

TABLE 16

Relationship between the genotype for each kind of protein and the constitution of the bivalent chromosome (B. ch.)* bearing the locus in each of the analyzed frogs produced from matings, NB♀ × BB♂

Parents		Individual no.	Albumin		α-GDH		MDH-B		LDH-B		IDH-B and Hb			Protein C	
Female	Male		Genotype	B. ch. No. 1	Genotype	B. ch. No. 2	Genotype	B. ch. No. 3	Genotype	B. ch. No. 4	Genotype	B. ch. No. 6	Genotype	Genotype	B. ch. No. 9
N_1B_1, No. 1	B_1B_1, No. 1	1	A^1A^2	NB	G^bG^b	BB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		2	A^1A^2	NB	G^nG^b	NB	B^bB^b	BB	B^nB^b	NB	B^bB^b	BB	H^bH^b	C^nC^b	NB
		3	A^1A^2	NB	G^bG^b	BB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		4	A^1A^2	NB	G^nG^b	NB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	BB
		5	A^2A^3	BB	G^bG^b	BB	B^nB^b	NB	B^nB^b	NB	B^bB^b	BB	H^bH^b	C^nC^b	NB
		6	A^1A^2	NB	G^nG^b	NB	B^nB^b	NB	B^bB^b	NB	B^bB^b	BB	H^bH^b	C^nC^b	NB
		7	A^2A^3	BB	G^bG^b	BB	B^bB^b	BB	B^nB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		8	A^1A^2	NB	G^bG^b	BB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		9	A^1A^2	NB	G^nG^b	NB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		10	A^2A^3	BB	G^bG^b	BB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
N_1B_1, No. 2	B_1B_1, No. 2	1	A^3A^3	BB	G^bG^b	BB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		2	A^2A^3	BB	G^bG^b	BB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		3	A^2A^3	BB	G^nG^b	NB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		4	A^1A^2	NB	G^bG^b	NB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		5	A^3A^3	BB	G^bG^b	BB	B^bB^b	BB	B^bB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		6	A^1A^3	NB	G^nG^b	BB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		7	A^1A^2	NB	G^bG^b	BB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		8	A^3A^3	BB	G^nG^b	BB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		9	A^2A^3	BB	G^bG^b	NB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		10	A^1A^2	NB	G^bG^b	BB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		11	A^1A^3	NB	G^nG^b	BB	B^nB^b	NB	B^bB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	BB
		12	A^1A^3	NB	G^nG^b	NB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		13	A^1A^2	NB	G^nG^b	NB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	NB
		14	A^1A^3	NB	G^nG^b	NB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		15	A^1A^3	NB	G^bG^b	BB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		16	A^1A^2	NB	G^nG^b	NB	B^bB^b	BB	B^nB^b	NB	B^bB^b	BB	H^bH^b	C^nC^b	NB
		17	A^1A^2	NB	G^bG^b	BB	B^bB^b	BB	B^bB^b	BB	B^bB^b	BB	H^bH^b	C^nC^b	NB
		18	A^1A^3	NB	G^bG^b	BB	B^bB^b	BB	B^bB^b	BB	B^bB^b	BB	H^bH^b	C^bC^b	BB
		19	A^1A^2	NB	G^bG^b	BB	B^nB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		20	A^1A^2	NB	G^nG^b	BB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		21	A^1A^2	NB	G^nG^b	NB	B^bB^b	BB	B^bB^b	BB	B^bB^b	BB	H^bH^b	C^bC^b	BB
		22	A^2A^3	BB	G^nG^b	NB	B^nB^b	NB	B^bB^b	BB	B^bB^b	BB	H^bH^b	C^nC^b	NB
		23	A^2A^3	BB	G^nG^b	NB	B^nB^b	NB	B^bB^b	BB	B^bB^b	NB	H^bH^b	C^nC^b	NB
		24	A^1A^2	NB	G^nG^b	NB	B^nB^b	NB	B^nB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	$\frac{N}{B}B$
		25	A^1A^2	NB	G^bG^b	BB	B^bB^b	BB	B^bB^b	BB	B^bB^b	BB	H^bH^b	C^bC^b	BB
		26	A^3A^3	BB	G^nG^b	BB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		27		NB	G^bG^b	BB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b		BB
		28	A^1A^2	NB	G^nG^b	NB	B^bB^b	NB	B^nB^b	NB	B^bB^b	BB	H^bH^b	C^nC^b	NB
		29	A^1A^3	NB	G^nG^b	NB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		30	A^1A^3	NB	G^nG^b	BB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		31	A^1A^2	NB	G^nG^b	BB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b		BB
N_2B_2, No. 3	B_2B_2, No. 3	1	A^2A^2	NB	G^bG^b	BB	B^bB^b	BB	B^nB^b	NB	B^bB^b	BB	H^bH^b	C^nC^b	NB
		2	A^1A^2	NB	G^bG^b	BB	B^bB^b	NB	B^bB^b	BB	B^bB^b	BB	H^bH^b	C^bC^b	BB
		3	A^2A^2	BB	G^bG^b	BB	B^nB^b	NB	B^bB^b	BB	B^bB^b	BB	H^bH^b	C^nC^b	NB
		4	A^1A^2	NB	G^nG^b	NB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB

* The right letter of each symbol indicates the chromosome from the male parent.

When the bivalent chromosome No. 9 with a translocation in each of the five

TABLE 17

Relationship between the genotype for each kind of protein and the constitution of the bivalent chromosome (B. ch.)* bearing the locus in each of the analyzed frogs produced from matings, BN♀ × NN♂

Parents		Individual no.	Albumin		α-GDH		MDH-B		LDH-B		IDH-B and Hb			Protein C	
Female	Male		Genotype	B. ch. No. 1	Genotype	B. ch. No. 2	Genotype	B. ch. No. 3	Genotype	B. ch. No. 4	Genotype	B. ch. No. 6	Genotype	Genotype	B. ch. No. 9
B_1N_1, No. 1	N_1N_1, No. 1	1	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		2	A^1A^3	BN	G^nG^b	BN	B^nB^b	BN	B^nB^b	NN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		3	A^1A^1	NN	G^nG^b	NN	B^nB^b	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		4	A^1A^3	BN	G^nG^b	NN	B^nB^b	BN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		5	A^1A^1	NN	G^nG^b	BN	B^nB^b	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		6	A^1A^3	BN	G^nG^b	BN	B^nB^b	BN	B^nB^n	BN	B^nB^b	BN	H^nH^b	C^nC^b	BN
B_1N_1, No. 2	N_1N_1, No. 2	1	A^1A^1	NN	G^nG^b	BN	B^nB^n	NN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		2	A^1A^1	NN	G^bG^n	BN	B^nB^b	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		3	A^1A^1	NN	G^nG^b	BN	B^nB^b	BN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		4	A^1A^1	NN	G^nG^b	BN	B^nB^n	NN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		5	A^1A^2	BN	G^nG^n	NN	B^nB^n	NN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		6	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		7	A^1A^2	BN	G^nG^b	NN	B^nB^b	BN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		8	A^1A^2	BN	G^nG^b	BN	B^nB^b	BN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^b	NN
		9	A^1A^1	NN	G^nG^b	NN	B^nB^b	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		10	A^1A^2	BN	G^nG^b	NN	B^nB^n	NN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		11	A^1A^2	BN	G^nG^n	NN	B^nB^b	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	$\frac{N}{B}N$
		12	A^1A^1	NN	G^nG^b	BN	B^nB^b	BN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		13	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^n	BN
		14	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		15	A^1A^1	NN	G^nG^n	NN	B^nB^b	BN	B^nB^b	BN	B^nB^n	NN	H^nH^b	C^nC^b	$\frac{N}{B}N$
		16	A^1A^1	NN	G^nG^n	NN	B^nB^b	BN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		17	A^1A^1	NN	G^nG^n	BN	B^nB^n	NN	B^nB^n	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		18	A^1A^1	NN	G^nG^b	NN	B^nB^b	NN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^b	NN
		19	A^1A^1	NN	G^nG^b	NN	B^nB^b	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^b	NN
		20	A^1A^2	BN	G^nG^b	BN	B^nB^b	BN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		21	A^1A^1	NN	G^nG^b	NN	B^nB^b	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b		BN
		22	A^1A^1	NN	G^nG^b	NN	B^nB^n	NN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^n	NN
		23	A^1A^1	NN	G^nG^b	BN	B^nB^n	BN	B^nB^n	BN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		24	A^1A^2	BN	G^nG^b	BN	B^nB^n	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		25	A^1A^2	BN	G^nG^n	BN	B^nB^b	NN	B^nB^b	BN	B^nB^n	NN	H^nH^b	C^nC^b	NN
		26	A^1A^2	BN	G^nG^b	BN	B^nB^b	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		27	A^1A^1	NN	G^nG^b	BN	B^nB^n	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n		NN
		28	A^1A^1	NN	G^nG^b	BN	B^nB^n	NN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^n	BN
		29	A^1A^2	BN	G^nG^b	BN	B^nB^n	NN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		30	A^1A^1	NN	G^nG^b	BN	B^nB^b	BN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		31	A^1A^2	BN	G^nG^n	NN	B^nB^n	NN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		32	A^1A^1	NN	G^nG^n	BN	B^nB^n	NN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		33	A^1A^2	BN	G^nG^n	BN	B^nB^b	BN	B^nB^b	BN	B^nB^n	BN	H^nH^b	C^nC^b	BN
B_2N_2, No. 3	N_2N_2, No. 3	1	A^1A^2	BN	G^nG^b	BN	B^nB^b	BN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		2	A^1A^1	BN	G^nG^b	NN	B^nB^n	NN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^b	BN
		3	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^n	NN
		4	A^1A^2	BN	G^nG^b	NN	B^nB^n	BN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^n	BN
		5	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^b	BN	B^nB^n	NN	H^nH^n	C^nC^n	$\frac{B}{N}N$
		6	A^1A^1	NN	G^nG^b	NN	B^nB^b	BN	B^nB^n	NN	B^nB^b	BN	H^nH^b	C^nC^b	BN
		7	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^b	BN	B^nB^b	BN	H^nH^b	C^nC^n	BN
		8	A^1A^1	NN	G^nG^n	NN	B^nB^n	NN	B^nB^n	NN	B^nB^n	NN	H^nH^n	C^nC^n	NN

* The right letter of each symbol indicates the chromosome from the male parent.

females was observed in detail, it was found that the break occurred at the same

TABLE 18

Relationship between the genotype for each kind of protein and the constitution of the bivalent chromosome (B. ch.)* bearing the locus in each of the analyzed frogs produced from matings, BN♀ × BB♂

Parents		Individual no.	Albumin		α-GDH		MDH-B		LDH-B		IDH-B and Hb			Protein C	
Female	Male		Genotype	B. ch. No. 1	Genotype	B. ch. No. 2	Genotype	B. ch. No. 3	Genotype	B. ch. No. 4	Genotype	B. ch. No. 6	Genotype	Genotype	B. ch. No. 9
B_1N_1, No. 1	B_1B_1, No. 1	1	A^1A^2	NB	G^nG^b	NB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		2	A^1A^2	NB	G^nG^b	NB	B^nB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		3	A^2A^3	BB	G^bG^b	BB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		4	A^1A^2	NB	G^nG^b	NB	B^bB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		5	A^1A^2	NB	G^nG^b	NB	B^bB^b	BB	B^bB^b	BB	B^bB^b	BB	H^bH^b	C^bC^b	BB
		6	A^1A^2	NB	G^bG^b	NB	B^nB^b	NB	B^nB^b	BB	B^bB^b	BB	H^bH^b	C^bC^b	BB
		7	A^1A^2	NB	G^nG^b	NB	B^bB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		8	A^1A^2	NB	G^bG^b	NB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		9	A^2A^3	BB	G^bG^b	NB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		10	A^1A^2	NB	G^nG^b	BB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		11	A^1A^2	NB	G^nG^b	NB	B^bB^b	BB	B^bB^b	BB	B^bB^b	BB	H^bH^b	C^bC^b	BB
		12	A^1A^2	NB	G^bG^b	BB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
B_1N_1, No. 2	B_1B_1, No. 2	1	A^1A^3	NB	G^nG^b	NB	B^bB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		2	A^1A^3	NB	G^nG^b	NB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		3	A^1A^2	NB	G^nG^b	NB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		4	A^1A^2	NB	G^nG^b	NB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	BB
		5	A^1A^2	NB	G^bG^b	BB	B^nB^b	NB	B^bB^b	BB	B^bB^b	BB	H^bH^b	C^nC^b	NB
		6	A^2A^2	BB	G^bG^b	BB	B^nB^b	NB	B^nB^b	NB	B^bB^b	BB	H^bH^b	C^bC^b	BB
		7	A^1A^2	NB	G^bG^b	BB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		8	A^2A^3	BB	G^bG^b	NB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		9	A^1A^2	NB	G^bG^b	NB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b		BB
		10	A^2A^3	BB	G^nG^b	NB	B^bB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		11	A^1A^2	NB	G^bG^b	NB	B^nB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		12	A^1A^3	NB	G^nG^b	NB	B^bB^b	BB	B^nB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		13	A^1A^2	BB	G^nG^b	BB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		14	A^2A^2	BB	G^nG^b	NB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		15	A^1A^2	NB	G^bG^b	BB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	NB
		16	A^1A^3	NB	G^nG^b	NB	B^nB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		17	A^1A^3	NB	G^bG^b	BB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		18	A^2A^3	BB	G^bG^b	NB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	BB
		19	A^1A^2	NB	G^nG^b	NB	B^nB^b	NB	B^bB^b	BB	B^bB^b	BB	H^bH^b	C^bC^b	BB
		20	A^1A^3	NB	G^bG^b	BB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		21	A^1A^2	NB	G^bG^b	NB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	BB
		22	A^2A^3	BB	G^bG^b	NB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	BB
		23	A^1A^2	NB	G^nG^b	BB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	NB
		24	A^1A^2	NB	G^bG^b	NB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		25	A^1A^3	NB	G^nG^b	NB	B^bB^b	BB	B^nB^b	BB	B^bB^b	BB	H^bH^b	C^nC^b	NB
		26	A^1A^2	NB	G^bG^b	BB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		27	A^2A^3	BB	G^nG^b	NB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		28	A^1A^2	NB	G^nG^b	BB	B^nB^b	NB	B^nB^b	NB	B^bB^b	BB	H^bH^b	C^bC^b	BB
		29		NB	G^nG^b	NB	B^bB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b		NB
		30	A^1A^2	NB	G^bG^b	BB	B^nB^b	NB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	NB
		31	A^2A^2	BB	G^nG^b	NB	B^bB^b	BB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		32	A^1A^2	NB	G^bG^b	BB	B^bB^b	BB	B^nB^b	NB	B^bB^b	$\frac{N}{B}$B	H^bH^b	C^nC^b	NB
		33	A^1A^2	BB	G^bG^b	BB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^nH^b	C^bC^b	BB
		34	A^1A^2	NB	G^nG^b	NB	B^bB^b	BB	B^nB^b	NB	B^nB^b	NB	H^bH^b	C^bC^b	BB
		35	A^2A^3	BB	G^bG^b	NB	B^nB^b	NB	B^bB^b	BB	B^nB^b	NB	H^nH^b	C^nC^b	NB
		36	A^2A^3	BB	G^nG^b	BB	B^bB^b	BB	B^nB^b	NB	B^bB^b	$\frac{N}{B}$B	H^bH^b	C^nC^b	NB

* The right letter of each symbol indicates the chromosome from the male parent.

definite point of a homologue in three females (Figs. 23e, f and 24d, g, h). In the other two females, the break occurred also at a definite point differing from that of the above three females (Figs. 23d and 24e, f).

DISCUSSION

Since the studies by MOORE (1941, 1942, 1943), the inheritance of spotting patterns in *Rana pipiens* have been reported by many investigators (BAKER, 1950, 1951; VOLPE, 1954, 1955, 1956, 1960, 1961a, b; VOLPE and DASGUPTA, 1962a, b; ANDERSON and VOLPE, 1958; DAVIDSON, 1961, 1963, 1964; MERRELL, 1965; BROWDER, 1968, 1972; UNDERHILL, 1968). Although it is evident that the two variants, burnsi and kandiyohi, are expressed by two dominant non-allelic genes, VOLPE (1961b) and VOLPE and DASGUPTA (1962a, b) have found that the burnsi, nonspotted phenotype, is a manifestation of genic interaction between a major pigmentary locus and a complex of modifiers (minor-spotting genes). BROWDER (1972) obtained a line of *Rana pipiens* in which a subvital gene was linked to the burnsi locus. Except for these investigations on the spotting patterns, there have been only a few reports on the inheritance of albinism (BROWDER, 1972; SMITH-GILL, RICHARDS and NACE, 1972) and melanoid mutation (RICHARDS, TARTOF and NACE, 1969) in *Rana pipiens.*

In frogs belonging to genus *Rana*, the inheritances of albinism in *R. temporaria* (SMALLCOMBE, 1949; SLÁDEČEK, 1964), a black marbly pattern of the venter in *R. nigromaculata* (MORIYA, 1952), the mid-dorsal stripe in *R. limnocharis* (MORIWAKI, 1953) and *R. sylvatica* (BROWDER, UNDERHILL and MERRELL, 1966) and digital abnormalities in *R. temporaria* (DUBOIS, 1977) have been described by several authors. Recently, NISHIOKA (1977) has reported on the inheritance of nine color variants, albino, gray-eyed, black-eyed, blue, grayish-brown, greenish-olive, bluish-olive, yellowish-olive and brownish-olive, induced by irradiation of sperm or oviducal eggs with X-rays or neutrons in *Rana nigromaculata.* The electron-microscopic structures of the dorsal skins from six of the above nine color variants have been described in detail by NISHIOKA and UEDA (1977a) together with those from two normal types of frogs.

As a matter of fact, comparatively numerous mutations have been discovered in *Xenopus laevis* and *Ambystoma mexicanum*, as these two species have been bred for many generations in laboratories as experimental animals. While some interesting mutations like periodic albinism (HOPERSKAYA, 1975) and mutation reducing nucleolar number (ELSDALE, FISCHBERG and SMITH, 1958) in *Xenopus laevis* or color variant (HUMPHREY and BAGNARA, 1967) and albinism (HUMPHREY, 1967) in *Ambystoma mexicanum* have been reported, most of the mutations described in these species were lethal or semilethal (UEHLINGER and REYNAUD, 1965; HUMPHREY, 1948, 1959, 1962, 1964, 1967, 1973; etc.). A few lethal or semilethal mutations have also been found in *Pleurodeles waltl* by GALLIEN and GOLLENOT (1964), BEETSCHEN and JAYLET (1965), SIGNORET, COLLENOT and GALLIEN (1966), etc.

There are a few reports on the inheritance of mid-dorsal stripe and albinism in

frogs belonging to Hylidae. PYBURN (1961) has shown that the presence of the green stripe is determined by a single dominant gene and that the recessive homozygote is gray-striped. DAITO (1968) has reported on albinism in *Hyla arborea japonica*. Albinism in the same species has been extensively studied by NISHIOKA and UEDA (1977b). Ten stocks of albinos collected from the field were sorted into three groups which differed from one another in number, size, shape or minute structure of premelanosomes contained in dermal melanophores and pigment cells of the eye. These three groups of albinos differed from one another in the loci of their genes, although each of them was expressed by the presence of recessive genes in the homozygous condition. These authors, moreover, discovered two dominant genes linked to one of the three kinds of albino genes.

Since about thirteen years ago, various enzymes extracted from frogs have been electrophoretically analyzed, as the genotype for each enzyme can be easily elucidated from the electrophoretic pattern. Such analyses have been made for lactate dehydrogenase (LDH) of two subspecies of *Rana pipiens*, *R. palustris* and *R. sylvatica* by WRIGHT and MOYER (1966, 1968), and afterwards for malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6-PGD) and some other enzymes of the *Rana pipiens* group by WRIGHT and his colleagues (WRIGHT and SUBTELNY, 1971, 1973; SUBTELNY, 1974; WRIGHT, 1975, 1976, 1978; WRIGHT, HUANG and CHUOKE, 1976). Similar analyses have been made during these ten years in the *Rana esculenta* group to elucidate mainly the interrelations of the members belonging to this group. Serum proteins of these frogs have been electrophoretically analyzed by TUNNER (1970, 1972, 1973, 1974), ENGELMANN (1972, 1973), HEMMER (1973) and VOGEL (1973), while albumin, LDH and some other enzymes have been analyzed by TUNNER and UZZELL (1974), UZZELL and BERGER (1975), TUNNER and DOBROWSKY (1976), VOGEL and CHEN (1976a, 1976b, 1977) and VOGEL (1977). Besides the genus *Rana*, electrophoretic analyses of proteins have been made in the *Bufo americanus* species group as well as some other *Bufo* species by GUTTMAN (1967, 1969, 1975) and ROGERS (1973), in *Acris crepitans* and *A. gryllus* by DESSAUER and NEVO (1969), in *Pleurodeles waltl* by AIMAR and CHALUMEAU-LE FOULGOC (1969) and in some other European species belonging to *Triturus, Bombina, Alytes* and *Bufo* by CHEN (1967, 1968).

In spite of a large accumulation of the genes for morphological or biochemical phenotypes as stated above, none of these genes has been related to a definite chromosome. In the present study the chromosomes carrying seven kinds of genes were elucidated for the first time. These genes are those for LDH, MDH, α-glycerophosphate dehydrogenase (α-GDH), IDH, hemoglobin (Hb), albumin (Ab) and serum protein C. The electrophoretic patterns of these proteins were examined in two sibling species, *Rana nigromaculata* and *R. brevipoda*, reciprocal hybrids between these two species and female backcross offspring between female reciprocal hybrids and males of the parental species. The genotypes for the electrophoretic patterns obtained from each female backcross offspring were collated with each of the 13 pairs of lampbrush chromosomes in her oocytes in

order to identify the chromosome bearing the locus for each protein. It was found in the present study that the genotypes for each protein agreed in constitution with one of the bivalent chromosomes in the following percentage of a total number of analyzed females: LDH and chromosome No. 4 in 90.7%, MDH and chromosome No. 3 in 87.9%, α–GDH and chromosome No. 2 in 71.4%, IDH and chromosome No. 6 in 98.9%, hemoglobin and chromosome No. 6 in 97.8%, albumin and chromosome No. 1 in 96.7%, and serum protein C and chromosome No. 9 in 86.4%. Thus, each of the genes for the seven kinds of proteins is assumed to be borne on the respective chromosome. The fact that the genotypes for each protein did not completely agree with the respective bivalent chromosome seems to be attributable to crossing-over between a *Rana nigromaculata* and a *R. brevipoda* chromosome during meiosis in the female hybrid used as the mother of backcrossing. It is very probable that a larger percentage of agreement found in the above seven cases shows a smaller frequency of crossing-over. In other words, a larger percentage of agreement seems to indicate that the locus for this protein occupies a closer position to the centromere on the chromosome. The loci for IDH, hemoglobin and albumin seem to be situated near the centromere, as the agreement was found in 96% or more of analyzed females. In contrast, the locus for α–GDH seems to be located distal from the centromere, as the agreement was found in 71%. From the present authors' unpublished data, the locus for transferrin is considered to be situated near the end of a chromosome, as the agreement of the genotype in constitution with each of the 13 bivalent chromosomes was always found in about 50% of the total number of analyzed females. In this case, the locus could not be related with a definite chromosome.

While the two loci for IDH and hemoglobin are assumed to be located near the centromere on chromosome No. 6, the locus for IDH seems to be a little closer to the centromere than that for hemoglobin. Moreover, the two loci for IDH and hemoglobin seem to be situated on the landmark-free part of the short arm from the following findings on chromosomes with translocations shown in Figs. 15 and 16. (1) Both the IDH and hemoglobin patterns were of the hybrid type in a female whose chromosome was NN with a translocation from B (Figs. 15d and 16d). (2) They were of the *Rana brevipoda* type in two females whose chromosome was BB with an intercalated translocation from N (Figs. 15e, f and 16e, f). (3) They were of the *Rana brevipoda* type in a female whose chromosome was NB. (4) The IDH and hemoglobin patterns were of the *Rana nigromaculata* and the hybrid type, respectively, in two females whose chromosome was NN.

The locus for serum protein C also seems to be situated on the landmark-free part of the long arm of chromosome No. 9 from the following findings. (1) Three out of five females whose chromosome was NB with a translocation from N were of the hybrid type in electrophoretic pattern (Figs. 23e and 24d, f, g). (2) Another female whose chromosome was a pair of B and B with a translocation from N was of the *Rana brevipoda* type (Figs. 23d and 24e). (3) The remaining female whose chromosome was a pair of N and N with an intercalated translocation from B was of the *Rana nigromaculata* type (Figs. 23f and 24h). (4) The genotypes

for serum protein C patterns did not agree in constitution with bivalent chromosome No. 9 in 24 out of 176 females.

The first step in making a chromosome map in amphibians has been taken by the present study. Hereafter, the number of genes which are related to definite chromosomes will rapidly increase by the method described in the present study. At the same time the positions of the loci for various mutations that have already been reported will be elucidated by studies of linkage groups.

SUMMARY

1. Electrophoretic patterns of seven chemical extracts, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), α-glycerophosphate dehydrogenase (α-GDH), isocitrate dehydrogenase (IDH), hemoglobin (Hb), albumin (Ab) and serum protein C on the one hand and lampbrush chromosomes in oocytes on the other hand were examined in mature females produced from backcrosses between female reciprocal hybrids of *Rana nigromaculata* and *R. brevipoda* and males of these parental species, in order to determine the relation between the genes for these substances and the chromosomes.

2. The genotype for LDH-B of each female agreed in constitution with bivalent chromosome No. 4 in her oocytes in 165 (90.7%) out of 182 females. Thus, chromosome No. 4 was assumed to bear the gene for LDH-B.

3. The genotype for MDH-B of each female agreed in constitution with bivalent chromosome No. 3 in her oocytes in 160 (87.9%) out of 182 females. Thus, chromosome No. 3 was assumed to bear the gene for MDH-B.

4. The genotype for α-GDH of each female agreed in constitution with bivalent chromosome No. 2 in her oocytes in 130 (71.4%) out of 182 females. Thus, chromosome No. 2 was assumed to bear the gene for α-GDH.

5. The genotype for IDH-B of each female agreed in constitution with bivalent chromosome No. 6 in her oocytes in 180 (98.9%) out of 182 females. Thus, chromosome No. 6 was assumed to bear the gene for IDH-B.

6. The genotype for Hb of each female agreed in constitution with bivalent chromosome No. 6 in her oocytes in 178 (97.8%) out of 182 females. Thus, chromosome No. 6 was assumed to bear the gene for Hb.

7. The genotype for Ab of each female agreed in constitution with bivalent chromosome No. 1 in her oocytes in 174 (96.7%) out of 180 females. Thus, chromosome No. 1 was assumed to bear the gene for Ab.

8. The genotype for serum protein C of each female agreed in constitution with bivalent chromosome No. 9 in 152 (86.4%) out of 176 females. Thus, chromosome No. 9 was assumed to bear the gene for serum protein C.

9. On the basis of the percentages of females whose genotype agreed in constitution with a definite bivalent chromosome, the loci for IDH, hemoglobin and albumin appeared to be situated near the centromeres, while the locus for α-GDH appeared to be distal from the centromere. Moreover, each of the loci for IDH and hemoglobin seemed to be located on the landmark-free part of the short arm

of chromosome No. 6, while the locus for serum protein C seemed to be located on the landmark-free part of the long arm of chromosome No. 9.

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