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Chromosomes and the Sites of Five Albino Gene Loci in the *Rana nigromaculata* Group

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(With 12 Text-figures)

ABSTRACT

In order to detect the chromosomes bearing the loci for five groups of albino genes, the constitution of genotypes (homozygous or heterozygous) for albinism was compared with that of each of the 13 bivalents in the oocytes of female backcrosses produced from heterozygous female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with albinic males of one of these species. When the genotype for albinism agreed in constitution with a definite bivalent in all or most of the female backcrosses, this bivalent was considered to bear the gene for albinism. The results of comparison between the genotype for albinism and each bivalent permitted the following assumptions: albino genes *a*, *c* and *e* are located on chromosomes Nos. 2, 2 and 3, respectively. Although albino gene *b* seemed to be located on chromosome No. 1, the agreement in constitution between the genotype and bivalent No. I was of very slight significance. Albino gene *d* seemed to be located on the end portion of one of the 13 chromosomes, where crossing-over frequently occurs.

For the purpose of making genetic maps including albino genes, *a*, *b*, *c*, *d* and *e*, linkages among these albino genes and 23 genes controlling 16 blood proteins and enzymes were examined. Albino gene *a* was assumed to be located at the site of about 20.4% in recombination rate from the centromere on an arm of chromosome No. 2. The genes for ME-A and α-GDH were assumed to be linked with albino gene *a* on the same arm, while the gene for Pep-C was assumed to be located on the other arm. Albino gene *c* and the gene for SOD-B were assumed to be located on the same arm as the gene for Pep-C at the sites of about 15.7% and 24.3% in recombination rate from the centromere, respectively. Albino gene *b* was assumed to be located on chromosome No. 1, as it is linked with the genes for ADH-A and Ab, both of which are linked with each other on chromosome No. 1, although albino gene *b* agreed in constitution with bivalent No. I in only 61.5% of 104 female backcrosses. Albino gene *d* was assumed to be located on chromosome No. 9, as it is linked with the genes for ALD and Prot-C, both of which are located on this chromosome. By comparing the constitution of bivalent No. IX having a translocation with the genotypes for Prot-C and ALD in 18 female backcrosses, it was assumed that the genes for Prot-C and ALD together with albino gene *d* are located on the distal portion of the long arm of chromosome No. 9. Albino gene *e* was assumed to be located on the long arm of chromosome No. 3 at the site of about 1.8% in recombination rate from the centromere. The distances between albino gene *e* and the genes for MDH-B and ME-B were about 22.7% and 2.8% in recombination rate, respectively.

INTRODUCTION

NISHIOKA and UEDA (1977) have reported that ten stocks of albino treefrogs, *Hyla arborea japonica,* collected from an area of about 6000 square kilometers were divided into three groups. The albinos of each group are due to the presence of a single recessive gene, *f, s* or *t,* in the homozygous condition. It was evident that the locus for albinism in each of the three groups differs from those in the other groups, as the hybrids between the albinos of different groups become normal in coloration. In one stock of albinos belonging to the first group, a dominant black-eyed gene (*B*) was linked with the *f,* while a dominant colored gene (*C*) was linked with the *f* in another stock of the same group. The same authors (1985) have reported 13 albino stocks in two sibling species, *Rana nigromaculata* and *Rana brevipoda.* While three of them were induced by irradiating gametes of *Rana nigromaculata,* the other 10 were collected from the field. The 13 albino stocks were divided into five groups, whose loci differ from one another. The first group consists of eight albino stocks including the three stocks induced by irradiation of gametes and five stocks collected from the field. The eight albino stocks of the first group were genetically divided into four strains. The first strain includes the three stocks induced by irradiation of gametes. The second strain includes three of the five stocks collected from the field, while each of the third and fourth strains includes one of the remaining two stocks.

The determination of chromosomes bearing the genes for inherited characters in amphibians was made for the first time by NISHIOKA, OHTANI and SUMIDA (1980). They succeeded in detecting chromosomes which bear the genes for seven kinds of blood proteins and enzymes in two sibling species, *Rana nigromaculata* and *Rana brevipoda,* by comparing the genotypes for these chemical components with the constitutions of lampbrush chromosomes in mature female backcrosses produced from female hybrids between the two species by mating with males of the parental species. Recently, NISHIOKA and OHTANI (1986) have succeeded in detecting chromosomes bearing the loci for blue and olive mutations in *Rana nigromaculata* by comparing the genotypes at the loci for these mutations with the constitutions of lampbrush chromosomes in gynogenetic diploids or female backcrosses produced from female heterozygous hybrids between a green female *Rana brevipoda* and a male *Rana nigromaculata* homozygous for blue and olive genes by mating with this male *Rana nigromaculata.*

In the present study, the authors have detected the chromosomes bearing the five loci for albinism in *Rana nigromaculata* and *Rana brevipoda* by comparing the genotypes and the constitutions of lampbrush chromosomes in female backcrosses produced by a method similar to that reported by NISHIOKA and OHTANI (1986). In order to assume the sites of the loci for albinism on the respective chromosomes, the linkage groups of the loci for blood proteins and enzymes and the effect of translocations on the arrangement of loci were examined.

MATERIALS AND METHODS

A female (NN.W78♀, No. 1) of *Rana nigromaculata* HALLOWELL collected in the breeding season of 1978 from the suburbs of Hiroshima and two females (BB.W75♀, Nos. 1 and 2) of the F_3 offspring of *Rana brevipoda* ITO which were collected in 1970 from Konko, Okayama Prefecture, were used as wild-type frogs in the present study. A total of 13 albino stocks consisting of 12 stocks of *Rana nigromaculata* and a stock of *Rana brevipoda* were used. As described by NISHIOKA and UEDA (1985), these 13 albino stocks are divided into five groups whose loci differ from one another. Each of albinos is due to a single recessive gene. Eight of the 13 albino stocks, Ex, Sn I, Sn II, Ym II, Hr, Br, Tj and Fc, belong to the first group, while the Go, Ym I, and Km stocks belong to the second, third and fourth groups, respectively. The remaining Ty and Ns stocks belong to the fifth group. The first, second, third, fourth and fifth groups of albinos are due to five kinds of albino genes, *a, b, c, d* and *e,* located on different loci, respectively. The first albino group is again divided into four strains which differ from one another in allele and phenotype. The first strain of albinos consisting of the Ex, Sn I and Sn II stocks is due to one, a^r, of four allelomorphic genes and called the Ra-type in phenotype. The second strain consisting of the Ym II, Hr and Br stocks is due to another allelomorphic gene, a^h, and called the Hr-type in phenotype. The third and fourth strains consisting of the Tj and Fc stocks, respectively, are due to the remaining allelomorphic genes, a^t and a^f, and called the Tj- and Fc-type in phenotype, respectively. As these four allelomorphic genes are codominant, the six kinds of hybrids among the four strains are a^ra^h, a^ra^t, a^ra^f, a^ha^t, a^ha^f and a^ta^f in genotype and intermediate between the parental albinos in phenotype (Table 1).

A total of 13 male albinos belonging to the five groups were used as materials in the present study. In the first group (*aa*), four male albinos of *Rana brevipoda,* BB-Br.Alb.75♂, Nos. 1~4, were used. They were produced from a mating between a homozygous female (*aa*) and a homozygous male (*aa*) whose albino gene was discovered in a mature male collected in 1970 from Konko, Okayama Prefecture (cf. NISHIOKA and UEDA, 1985; Table 4, p. 10). In the second group (*bb*), three male albinos of *Rana nigromaculata,* NN-Go.Alb.76♂, Nos. 1~3, were used. They were produced from a mating between a heterozygous female (*Bb*) and a heterozygous male (*Bb*) collected in 1974 from Gion, Hiroshima Prefecture (cf. NISHIOKA and UEDA, 1985; Table 7, p. 13). In the third group (*cc*), two male albinos of *Rana nigromaculata,* NN-Ym I.Alb.77♂, Nos. 1 and 2, were used. They were produced from a mating between a heterozygous female (*Cc*) and a heterozygous male (*Cc*) collected in 1977 from Yabara, Yamaguchi Prefecture (cf. NISHIOKA and UEDA, 1985; Table 5, p. 11). In the fourth group (*dd*), two male albinos of *Rana nigromaculata,* NN-Km.Alb.77♂, Nos. 1 and 2, were used. They were produced from a mating between a heterozygous female (*Dd*) and a heterozygous male (*Dd*) collected in 1977 from Kamogata, Okayama Prefecture (cf. NISHIOKA and UEDA, 1985; Table 6, p. 12). In the fifth group (*ee*), two male albinos of *Rana nigromaculata,* NN-Ty.Alb.77♂, Nos. 1 and 2, were used. These

TABLE 1

Five groups of the 13 albino stocks and their phenotypes and genotypes together with the abbreviation of each stock in *Rana nigromaculata* and *Rana brevipoda*

Group	Stock	Origin	Phenotype	Genotype
1	Ex	Originated from an egg irradiated with 145 rads of X-rays	Ra	$a^r a^r$
	Sn I	Originated from a sperm irradiated with 50 rads of neutrons		
	Sn II	Originated from a sperm irradiated with 130 rads of neutrons		
	Ym II	Collected from field in Yamaguchi (II), Yamaguchi Prefecture	Hr	$a^h a^h$
	Hr	Collected from field in Hiro, Hiroshima Prefecture		
	Br	*Rana brevipoda* collected from field in Konko, Okayama Prefecture		
	Tj	Collected from field in Tojo, Hiroshima Prefecture	Tj	$a^t a^t$
	Fc	Collected from field in Fuchu, Hiroshima Prefecture	Fc	$a^f a^f$
2	Go	Collected from field in Gion, Hiroshima Prefecture	Go	*bb*
3	Ym I	Collected from field in Yamaguchi (I), Yamaguchi Prefecture	Ym I	*cc*
4	Km	Collected from field in Kamogata, Okayama Prefecture	Km	*dd*
5	Ty	Collected from field in Toyomatsu, Hiroshima Prefecture	Ty	*ee*
	Ns	Collected from field in Nishinomiya, Hyogo Prefecture		

albinos were collected in 1977 from Toyomatsu, Hiroshima Prefecture (cf. Nishioka and Ueda, 1985; Table 10, p. 18).

The interspecific hybrids between *Rana nigromaculata* and *Rana brevipoda* were produced by artificial fertilization. Eggs obtained from female *Rana nigromaculata* or *Rana brevipoda* by pituitary injection were fertilized with sperm of male albinos of these species. Tadpoles were fed on boiled spinach or chard. Frogs were fed on crickets from the froglet stage till sexual maturity.

Lampbrush chromosomes in the oocytes of mature females were observed by the methods of Gall (1966) and Nishioka, Ohtani and Sumida (1980). The analyses of serum proteins, hemoglobin and enzymes extracted from the skeletal muscles and livers were made by the method of starch-gel electrophoresis according to Brewer (1970), Harris and Hopkinson (1976) and Nishioka, Ohtani and Sumida (1980).

The detection of chromosomes bearing the loci of albino genes was made by examining the lampbrush chromosomes in oocytes. As the 13 bivalents of *Rana nigromaculata* can be distinguished from those of *Rana brevipoda* in chromosome length, centromere position and size, shape and number of landmarks, the chromosomes bearing the loci of albino genes were detected by the following way.

First of all, diploid hybrids heterozygous for an albino gene were produced by crossing albinic male *Rana nigromaculata* or *Rana brevipoda* with wild-type female *Rana brevipoda* or *Rana nigromaculata*. Then, backcrosses were produced by mating female hybrids with albinic male *Rana nigromaculata* or *Rana brevipoda*. These backcrosses were albinos or of the wild-type in phenotype, as they were homozygous (*aa, bb, cc, dd* or *ee*) or heterozygous (*Aa, Bb, Cc, Dd* or *Ee*) for albino

genes. Such genotypes of female backcrosses were compared with the constitutions of the 13 lampbrush chromosomes (bivalents) in their oocytes. When the homozygous or heterozygous state of an albino gene agrees with the constitution of a bivalent in all or most of the female backcrosses, this bivalent is considered to bear the locus of this albino gene.

Linkage groups of albino genes and various other genes controlling blood proteins and enzymes were established in order to assume the situation of the loci of the albino genes on the chromosomes by calculating the distances of the albino genes from the centromeres and the linked genes for enzymes or blood proteins.

The following abbreviations are used in the present paper.

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

The developmental stages described in this paper follow those of *Rana pipiens* established by SHUMWAY (1940) and TAYLOR and KOLLROS (1946) for convenience' sake.

OBSERVATION

I. Albinic Rana brevipoda (aa) of the first group

1. Backcrosses from female hybrids (*Aa*) between a wild-type female *Rana nigromaculata* (*AA*) and an albinic male *Rana brevipoda* (*aa*), by mating with three albinic male *Rana brevipoda* (*aa*)

In 1978, a wild-type female, NN.W78♀, No. 1 (*AA*), of *Rana nigromaculata* collected from the suburbs of Hiroshima was crossed with an albinic male *Rana brevipoda,* BB-BR.Alb.75♂, No. 1 (*aa*), collected from Konko, Okayama Prefecture. From this crossing, 29 wild-type hybrids (*Aa*) including females and males were produced. As most of the females of these hybrids sexually matured in November of 1978, lampbrush chromosomes were observed in the oocytes of their ovaries. There were always 13 bivalents, each of which consisted of N derived from *Rana nigromaculata* and B derived from *Rana brevipoda* (Figs. 1, 2).

In three years, 1979~1981, four female hybrids, NB.W78♀, Nos. 1~4 (*Aa*), were backcrossed with three albinic male *Rana brevipoda,* BB-BR.Alb.75♂, Nos. 2~4 (*aa*), and produced 683 tadpoles at stage 25. Of these tadpoles, 338 (49.5%) were of the wild-type (*Aa*) and 345 (50.5%) were albinos (*aa*). A part of the backcrosses was reared until their sexual maturity. The constitution of lamp-

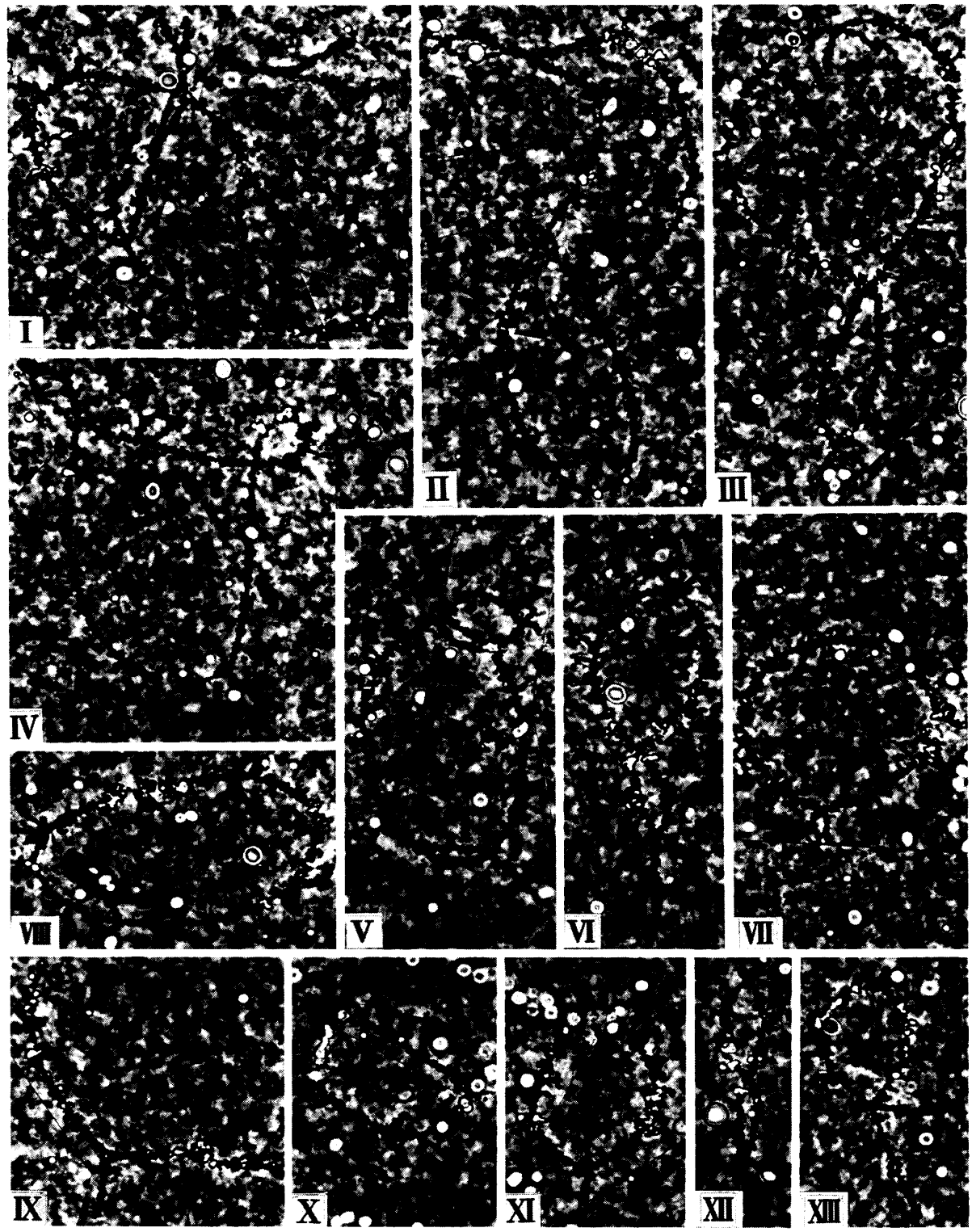


Fig. 1. Microphotographs of bivalents Nos. I~XIII in an oocyte of a female hybrid, *Rana nigromaculata* ♀×*Rana brevipoda* ♂. ×450

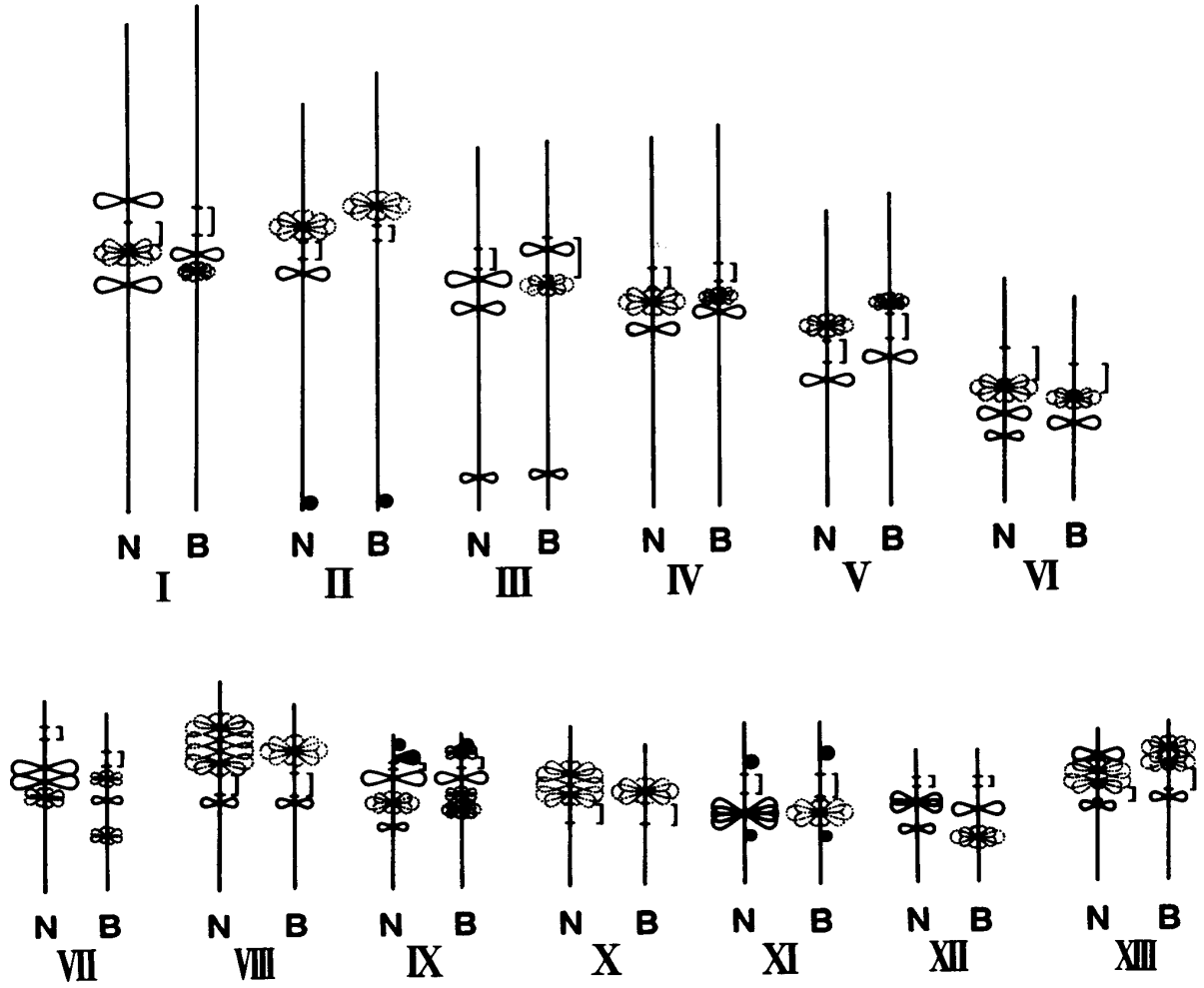


Fig. 2. Diagrams showing the constitutions of bivalents Nos. I～XIII in oocytes of a female hybrid, *Rana nigromaculata* ♀×*Rana brevipoda* ♂.

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.

brush chromosomes (bivalents) in oocytes was examined in 103 mature female backcrosses consisting of 60 wild-type frogs and 43 albinos (Table 2).

2. Identification of the chromosome bearing albino gene *a* in the Hr-type *Rana brevipoda*

While albino gene *a* is located on B, wild-type gene *A* is located on N. Thus, in the backcrosses produced from NB(*Aa*) ♀×BB(*aa*) ♂, the bivalent bearing a pair of albino genes (*aa*) in an albino should be BB in constitution, while the corresponding bivalent bearing an albino gene (*a*) in a wild-type frog (*Aa*) should be NB in constitution. The genotype, *Aa* or *aa*, of the 103 female backcrosses consisting of 60 wild-type frogs and 43 albinos was compared with the constitution, NB or BB, of each of the 13 bivalents in the oocytes of these female backcrosses (Table 3).

The results showed that bivalent No. II consisted of NB in 49 and of BB in 11 of

TABLE 2

Phenotypes of mature female backcrosses produced from heterozygous female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with albinic males of the parental species

Group	Year	Parents		No. of mature females		
		Female	Male	Total	Wild	Albino
1	1979	NB.W78, No. 1 (*Aa*)	BB-Br.Alb.75, No. 2 (*aa*)	8	5	3
	1980	NB.W78, No. 2 (*Aa*)	BB-Br.Alb.75, No. 3 (*aa*)	50	29	21
	〃	NB.W78, No. 3 (*Aa*)	〃	28	14	14
	1981	NB.W78, No. 4 (*Aa*)	BB-Br.Alb.75, No. 4 (*aa*)	17	12	5
	Total			103	60	43
2	1980	BN.W79, Nos. 1~5 (*Bb*)	NN-Go.Alb.76, No. 2 (*bb*)	65	26	39
	1981	BN.W79, No. 6 (*Bb*)	NN-Go.Alb.76, No. 3 (*bb*)	39	32	7
	Total			104	58	46
3	1980	BN.W79, No. 1 (*Cc*)	NN-YmI.Alb.77, No. 2 (*cc*)	102	49	53
4	1981	BN.W80, No. 1 (*Dd*)	NN-Km.Alb.77, No. 2 (*dd*)	43	24	19
	〃	BN.W80, No. 2 (*Dd*)	〃	31	16	15
	〃	BN.W80, No. 3 (*Dd*)	〃	27	16	11
	Total			101	56	45
5	1981	BN.W79, Nos. 1~3 (*Ee*)	NN-Ty.Alb.79, No. 2 (*ee*)	112	65	47
	〃	〃	NN-Ty.Het.79, No. 1 (*Ee*)	35	35	—
	Total			147	100	47

the 60 wild-type (*Aa*) females. The other 12 bivalents, Nos. I and III~XIII, consisted of NB in 24~46 of the 60 wild-type females. On the other hand, bivalent No. II consisted of BB in 33 and of NB in 10 of the 43 albinos (*aa*). The other 12 bivalents, Nos. I and III~XIII, consisted of BB in 16~26 of the 43 albinos. In total, the constitutions of bivalent No. II agreed with the genotypes in 82 (79.6%) of the 103 female backcrosses and disagreed in the remaining 21 (20.4%) female backcrosses ($\chi^2=36.1$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I and III~XIII, agreed with the genotypes in 43 (41.7%)~62 (60.2%), and disagreed with the genotypes in 60~41 of the female backcrosses ($P>0.05$). Thus, it is assumed that albino gene *a* is located on chromosome No. 2 with a recombination rate of about 20.4% (Tables 3, 4).

II. Albinic Rana nigromaculata (bb) of the second group

1. Backcrosses from female hybrids (*Bb*) between a wild-type female *Rana brevipoda* (*BB*) and an albinic male *Rana nigromaculata* (*bb*), by mating with two albinic male *Rana nigromaculata* (*bb*)

In 1979, a wild-type female, BB.W75♀, No. 1 (*BB*), of the third-generation offspring of *Rana brevipoda* collected from Konko, Okayama Prefecture, was mated

TABLE 3

Number of female backcrosses whose genotypes for albinism (*Aa* or *aa*) agreed in constitution with each of the 13 bivalents in their oocytes

Group	Parents		Phenotype and genotype	No. of frogs	Bivalent number												
	Female	Male			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
1	NB (*Aa*), Nos. 1~4	BB (*aa*), Nos. 2~4	Wild (*Aa*)	60	39	**49**	46	27	32	35	32	31	31	29	34	30	24
			Albino (*aa*)	43	17	**33**	16	20	25	26	21	23	20	26	21	23	19
			Total	103	56	**82**	62	47	57	61	53	54	51	55	55	53	43
			(%)		(54.4)	(**79.6**)	(60.2)	(45.6)	(55.3)	(59.2)	(51.5)	(52.4)	(49.5)	(53.4)	(53.4)	(51.5)	(41.7)
2	BN (*Bb*), Nos. 1~6	NN (*bb*), Nos. 2, 3	Wild (*Bb*)	58	**37**	32	17	18	28	25	33	25	39	27	38	30	33
			Albino (*bb*)	46	**27**	14	29	19	20	22	19	17	23	22	14	19	19
			Total	104	**64**	46	46	37	48	47	52	42	62	49	52	49	52
			(%)		(**61.5**)	(44.2)	(44.2)	(35.6)	(46.2)	(45.2)	(50.0)	(40.4)	(59.6)	(47.1)	(50.0)	(47.1)	(50.0)
3	BN (*Cc*), No. 1	NN (*cc*), No. 2	Wild (*Cc*)	49	25	**41**	22	28	27	20	22	26	18	19	28	25	32
			Albino (*cc*)	53	25	**45**	34	32	27	29	21	25	32	25	22	20	10
			Total	102	50	**86**	56	60	54	49	43	51	50	44	50	45	42
			(%)		(49.0)	(**84.3**)	(54.9)	(58.8)	(52.9)	(48.0)	(42.2)	(50.0)	(49.0)	(43.1)	(49.0)	(44.1)	(41.2)
4	BN (*Dd*), Nos. 1~3	NN (*dd*), No. 2	Wild (*Dd*)	56	23	27	24	35	29	22	36	22	30	30	31	31	22
			Albino (*dd*)	45	18	16	33	19	24	18	22	23	22	26	17	23	26
			Total	101	41	43	57	54	53	40	58	45	52	56	48	54	48
			(%)		(40.6)	(42.6)	(56.4)	(53.5)	(52.5)	(39.6)	(57.4)	(44.6)	(51.5)	(55.4)	(47.5)	(53.5)	(47.5)
5	BN (*Ee*), Nos. 1~3	NN (*ee*), No. 2	Wild (*Ee*)	65	36	33	**65**	35	33	30	32	26	39	28	29	29	39
			Albino (*ee*)	47	24	21	**45**	16	22	16	22	24	26	24	21	26	18
			Total	112	60	54	**110**	51	55	46	54	50	65	52	50	55	57
			(%)		(53.6)	(48.2)	(**98.2**)	(45.5)	(49.1)	(41.1)	(48.2)	(44.6)	(58.0)	(46.4)	(44.6)	(49.1)	(50.9)

with an albinic male *Rana nigromaculata* of the Go-type, NN-Go.Alb.76♂, No. 1 (*bb*), collected from Gion, Hiroshima Prefecture. From this crossing, 43 wild-type hybrids (*Bb*), including females and males, were produced. As the females of these hybrids mostly attained sexual maturity in November of this year, the lampbrush chromosomes of their oocytes were observed. There were always 13 bivalents, each of which consisted of BN.

In 1980 and 1981, 892 feeding tadpoles were produced from backcrossing of six female hybrids, BN.W79♀, Nos. 1~6 (*Bb*), with two albinic male *Rana nigromaculata*, NN-Go.Alb.76♂, Nos. 2 and 3 (*bb*). Of these tadpoles, 451 (50.6%) were of the wild-type (*Bb*) and 441 (49.4%) were albinos (*bb*). Thereafter, 650 tadpoles attained completion of metamorphosis. Of these froglets, 333 (51.2%) were of the wild-type and 317 (48.8%) were albinos. About half of the froglets were reared until their sexual maturity. Lampbrush chromosomes were observed in a total of 104 mature female backcrosses including 58 wild-type frogs and 46 albinos (Table 2).

2. Identification of the chromosome bearing albino gene *b* in the Go-type *Rana nigromaculata*

While albino gene *b* is located on N, wild-type gene *B* is located on B. Thus, in the backcrosses produced from BN(*Bb*)♀×NN(*bb*)♂, the bivalent bearing a pair of albino genes (*bb*) in an albino should be NN in constitution, while the corresponding bivalent bearing an albino gene (*b*) in a wild-type frog (*Bb*) should be BN. The genotype, *Bb* or *bb*, of the 104 female backcrosses consisting of 58 wild-type frogs and 46 albinos was compared with the constitution, BN or NN, of each of the 13 bivalents in the oocytes of these female backcrosses (Table 3).

It was found that bivalents Nos. IX, XI and I consisted of BN in 39, 38 and 37 of the 58 wild-type frogs, respectively, while the other 10 bivalents, Nos. II~VIII, X, XII and XIII, consisted of BN in 17~33 of the wild-type frogs. On the other hand, bivalents Nos. III and I consisted of NN in 29 and 27 of the 46 albinos, respectively, while the other 11 bivalents, Nos. II and IV~XIII, consisted of NN in 14~23 of the albinos. In total, the constitution of bivalent No. I agreed with the genotype in 64 (61.5%) and disagreed in 40 (38.5%) of the 104 female backcrosses ($\chi^2=5.54$, $0.05>P>0.01$). The constitutions of the other 12 bivalents, Nos. II~XIII, agreed with the genotype in 62 (59.6%)~37 (35.6%) and disagreed in 42~67 of the female backcrosses ($P>0.05$) (Tables 3, 4). These findings indicate that albino gene *b* is probably located on chromosome No. 1, although the agreement between the constitution of bivalent No. I and the genotype of the female backcrosses is of very slight significance. When albino gene *b* is located on chromosome No. 1 in reality, it is assumed that this gene is located at the end portion of the chromosome where crossing-over frequently occurs. The results of comparison between the constitution of each bivalent and the genotype for albinism in female backcrosses also show the possibility that albino gene *b* is located near the end portion of any other chromosome.

TABLE 4

Relationship between the constitution of the applicable bivalent and the genotype for albinism in female backcrosses

Group	Bivalent no. applicable	Kind of backcrossing	Bivalent		Albinism			No. of frogs whose bivalents were compared with the genotype		χ^2	P	Recombination rate (%)
			Constitution	No. of frogs	Phenotype	Genotype	No. of frogs	Agree (%)	Disagree			
1	II	NB (*Aa*) ♀ × BB (*aa*) ♂ Hr-type	NB	59	Wild	*Aa*	60	49	10			
			BB	44	Albino	*aa*	43	33	11			
			Total	103			103	82 (79.6)	21	36.1	<0.00001	20.4
2	I	BN (*Bb*) ♀ × NN (*bb*) ♂ Go-type	BN	56	Wild	*Bb*	58	37	19			
			NN	48	Albino	*bb*	46	27	21			
			Total	104			104	64 (61.5)	40	5.54	0.02	38.5
3	II	BN (*Cc*) ♀ × NN (*cc*) ♂ YmI-type	BN	49	Wild	*Cc*	49	41	8			
			NN	53	Albino	*cc*	53	45	8			
			Total	102			102	86 (84.3)	16	48.0	<0.00001	15.7
4	VII	BN (*Dd*) ♀ × NN (*dd*) ♂ Km-type	BN	59	Wild	*Dd*	56	36	23			
			NN	42	Albino	*dd*	45	22	20			
			Total	101			101	58 (57.4)	43	2.23	0.14	42.6
	IX	BN (*Dd*) ♀ × NN (*dd*) ♂ Km-type	BN	53	Wild	*Dd*	56	30	23			
			NN	47	Albino	*dd*	44	21	26			
			$\frac{B}{N}$N	1	Albino	*dd*	1	1	0			
			Total	101			101	52 (51.5)	49	0.09	0.77	48.5
5	III	BN (*Ee*) ♀ × NN (*ee*) ♂ Ty-type	BN	65	Wild	*Ee*	63	63	2			
			NN	43	Albino	*ee*	45	43	0			
			$\frac{B}{N}$N	2	Albino	*ee*	2	2	0			
			$\frac{N}{B}$N	2	Wild	*Ee*	2	2	0			
			Total	112			112	110 (98.2)	2	104.1	<0.00001	1.8

III. Albinic Rana nigromaculata (cc) of the third group

1. Backcrosses from female hybrids (*Cc*) between a wild-type female *Rana brevipoda* (*CC*) and an albinic male *Rana nigromaculata* (*cc*), by mating with an albinic male *Rana nigromaculata* (*cc*)

In 1979, the same wild-type female *Rana brevipoda,* BB.W75♀, No. 1 (*CC*), as used in the foregoing experiment (*II,* 1) was mated with an albinic male *Rana nigromaculata* of the YM I-type, NN-YM I.Alb.77♂, No. 1 (*cc*), collected from Yamaguchi, Yamaguchi Prefecture. From this crossing, 116 wild-type hybrids (*Cc*) including females and males were obtained. As the females of these hybrids mostly attained sexual maturity in November of this year, the lampbrush chromosomes of their oocytes were observed. The results showed that there were always 13 bivalents, each of which consisted of BN.

In 1980, a female hybrid, BN.W79♀, No. 1 (*Cc*), was backcrossed with an albinic male *Rana nigromaculata,* NN-YM I.Alb.77♂, No. 2 (*cc*), and produced 544 tadpoles at stage 21. Of these backcrosses, 270 (49.6%) were of the wild-type (*Cc*) and 274 (50.4%) were albinos (*cc*). Thereafter, 483 tadpoles began to eat and 429 completed metamorphosis. While 218 (45.1%) and 265 (54.9%) of the feeding tadpoles were of wild-type and albinic ones, respectively, 188 (43.8%) and 241 (56.2%) of the metamorphosed frogs were of wild-type and albinic ones, respectively. These froglets were reared up to the stage of sexual maturity. Lampbrush chromosomes were observed in the oocytes of a total of 102 mature females consisting of 49 wild-type and 53 albinic ones (Table 2).

2. Identification of the chromosome bearing albino gene *c* in the YM I-type *Rana nigromaculata*

While albino gene *c* is located on N, wild-type gene *C* is located on B. Thus, in the backcrosses produced from BN(*Cc*)♀×NN(*cc*)♂, the bivalent bearing a pair of albino genes (*cc*) in an albino should be NN in constitution, while the corresponding bivalent bearing an albino gene (*c*) in a wild-type frog (*Cc*) should be BN in constitution. The genotype, *Cc* or *cc,* of the 102 female backcrosses consisting of 49 wild-type frogs and 53 albinos was compared with the constitution, BN or NN, of each of the 13 bivalents in the oocytes of these backcrosses (Table 3).

It was found that bivalent No. II consisted of BN in 41 and of NN in the other eight of the 49 wild-type backcrosses. On the other hand, the other 12 bivalents, Nos. I and III~XIII, consisted of BN in 18~32 and of NN in 31~17 of the 49 wild-type backcrosses. Bivalent No. II consisted of NN in 45 and of BN in eight of the 53 albinic backcrosses. The other 12 bivalents, Nos. I and III~XIII, consisted of NN in 10~34 and of BN in 43~19 of the 53 albinic backcrosses. In total, the constitution of bivalent No. II agreed with the genotype in 86 (84.3%) and disagreed in 16 (15.7%) of the 102 female backcrosses ($\chi^2=48.0$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I and III~XIII, agreed with the genotype in 42 (41.2%)~60 (58.8%) and disagreed in 60~42 of

the female backcrosses. These findings seem to show that albino gene *c* is located on chromosome No. 2 with a recombination rate of about 15.7% (Tables 3, 4).

IV. Albinic Rana nigromaculata (dd) of the fourth group

1. Backcrosses from female hybrids (*Dd*) between a wild-type female *Rana brevipoda* (*DD*) and an albinic male *Rana nigromaculata* (*dd*), by mating with an albinic male *Rana nigromaculata* (*dd*)

In 1980, a wild-type female, BB.W75♀, No. 2 (*DD*), of the third-generation offspring of *Rana brevipoda* collected from Konko, Okayama Prefecture, was mated with an albinic male *Rana nigromaculata* of the KM-type, NN-KM.Alb.77♂, No. 1 (*dd*), collected from Kamogata, Okayama Prefecture. From this crossing, 104 female and male wild-type hybrids (*Dd*) were produced. As the female hybrids mostly attained sexual maturity, the lampbrush chromosomes of their oocytes were observed. It was found that the oocytes contained 13 bivalents, each of which consisted of BN.

In 1981, three female hybrids, BN.W80♀, Nos. 1~3 (*Dd*), were backcrossed with an albinic male *Rana nigromaculata,* NN-KM.Alb.77♂, No. 2 (*dd*), and produced 789 feeding tadpoles, including 391 (49.6%) wild-type (*Dd*) and 398 (50.4%) albinic (*dd*) ones. Of these tadpoles, 225 wild-type and 225 albinic ones were continuously reared. The results showed that 200 (52.8%) wild-type and 179 (47.2%) albinic tadpoles completed metamorphosis. As these froglets attained sexual maturity, lampbrush chromosomes of oocytes were observed in a total of 101 mature females including 56 wild-type and 45 albinic ones (Table 2).

2. Identification of the chromosome bearing albino gene *d* in the KM-type *Rana nigromaculata*

While albino gene *d* is located on N, wild-type gene *D* is located on B. Thus, in the backcrosses produced from BN(*Dd*)♀×NN(*dd*)♂, the bivalent bearing a pair of albino genes (*dd*) in an albino should be NN in constitution, while the corresponding bivalent bearing an albino gene (*d*) in a wild-type frog (*Dd*) should be BN in constitution. The genotype, *Dd* or *dd,* of the 101 female backcrosses consisting of 56 wild-type frogs and 45 albinos was compared with the constitution, BN or NN, of each of the 13 bivalents in the oocytes of these backcrosses (Table 3).

The results showed that bivalent No. VII consisted of BN in 36 of the 56 wild-type backcrosses, while the other 12 bivalents consisted of BN in 22~35 of these backcrosses. On the other hand, bivalent No. III consisted of NN in 33 of the 45 albinic backcrosses, while the other 12 bivalents consisted of NN in 16~26 of these backcrosses. In total, the constitution of bivalent No. VII agreed with the genotype in 58 (57.4%) and disagreed in 43 (42.6%) of the 101 female backcrosses ($\chi^2=2.23$, $P>0.1$). The constitutions of the other 12 bivalents, Nos. I~VI and VIII~XIII, agreed with the genotype in 40 (39.6%)~57 (56.4%) and disagreed in 61~44 of the female backcrosses. These findings seem to show that

the location of albino gene *d* on chromosome No. 7 is not always reliable and that albino gene *d* is located on the end portion of any of the 13 chromosomes (Nos. 1~13) where crossing-over frequently occurs (Tables 3, 4).

V. Albinic Rana nigromaculata (ee) of the fifth group

1. Backcrosses from female hybrids (*Ee*) between a wild-type female *Rana brevipoda* (*EE*) and an albinic male *Rana nigromaculata* (*ee*), by mating with an albinic male *Rana nigromaculata* (*ee*)

In 1979, a wild-type female, BB.W75♀, No. 1 (*EE*), of the F_3 offspring of wild-type *Rana brevipoda* collected from Konko, Okayama Prefecture, was mated with an albinic male *Rana nigromaculata* of the Ty-type, NN-Ty.Alb.77♂, No. 1 (*ee*), collected from Toyomatsu, Hiroshima Prefecture. From this crossing, 70 female and male wild-type hybrids (*Ee*) were produced. As the females of these hybrids mostly attained sexual maturity in November of this year, the lampbrush chromosomes of their oocytes were observed. It was found that there were always 13 bivalents, each of which consisted of BN.

In 1981, three female hybrids, BN.W79♀, Nos. 1~3 (*Ee*), were backcrossed with a Ty-type albinic male, NN-Ty.Alb.79♂, No. 2 (*ee*), and produced 290 feeding tadpoles at stage III. Of these backcrosses, 143 (49.3%) were of the wild-type (*Ee*) and 147 (50.7%) were albinos (*ee*). Of 184 normally metamorphosed backcrosses, 101 (54.9%) were of the wild-type and 83 (45.1%) were albinos. These frogs were reared until sexual maturity. The lampbrush chromosomes of oocytes were observed in a total of 112 mature females consisting of 65 wild-type and 47 albinic backcrosses. Besides, lampbrush chromosomes were also observed in 35 mature wild-type females (*Ee, EE*) obtained from the foregoing three female hybrids by mating with a male *Rana nigromaculata,* NN-Ty.Het.79♂, No. 1, which was heterozygous for the Ty-type albino gene (Table 2).

2. Identification of the chromosome bearing albino gene *e* in the Ty-type *Rana nigromaculata*

Albino gene *e* and wild-type gene *E* are located on N and B, respectively. Thus, in the backcrosses produced from BN(*Ee*)♀×NN(*ee*)♂, the bivalent bearing a pair of albino genes (*ee*) in an albino should be NN in constitution, while the corresponding bivalent bearing an albino gene (*e*) in a wild-type frog (*Ee*) should be BN in constitution. The genotype, *Ee* or *ee*, of the 112 female backcrosses consisting of 65 wild-type frogs (*Ee*) and 47 albinos (*ee*) was compared with the constitution, BN or NN, of each of the 13 bivalents in the oocytes of these female backcrosses (Table 3).

It was found that bivalent No. III consisted of BN in constitution in all the 65 wild-type (*Ee*) backcrosses, including two in which bivalent No. III had a translocation. In these two backcrosses, bivalent No. III was $\frac{N}{B}N$ in constitu-

tion, that is, a part of N was translocated to the B of the original BN. The other 12 bivalents, Nos. I, II and IV~XIII, were BN in constitution in 26~39 and NN in 39~26 of the 65 wild-type backcrosses. On the other hand, bivalent No. III was NN in 45 of the 47 albinos, although in two of the 45, bivalent No. III was $\frac{B}{N}$N in constitution, that is, a part of B was translocated to one chromosome of the original NN. The other bivalents, Nos. I, II and IV~XIII, consisted of NN in 16~26 and of BN in 31~21 of the 47 albinos. In total, the constitution of bivalent No. III agreed with the genotype in 110 (98.2%) of the 112 female backcrosses and disagreed in two (1.8%) ($\chi^2=104.1$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I, II and IV~XIII, agreed with the genotype in 46 (41.1%)~65 (58.0%) and disagreed in 66~47 ($P>0.05$). These findings seem to show that albino gene *e* is located on chromosome No. 3 with a recombination rate of about 1.8% (Tables 3, 4).

VI. Chromosomes bearing the 23 loci for 16 blood proteins and enzymes

From the foregoing experiments, it was assumed that albino genes *a* and *c* are located on chromosome No. 2 and albino gene *e* is on chromosome No. 3. In contrast, the chromosomes bearing albino genes *b* and *d* were not identified from comparison between the genotype of female backcrosses and the constitution of each of the 13 bivalents in their oocytes.

Nishioka, Ohtani and Sumida (1980) and Nishioka and Ohtani (1986) have assumed that the loci for serum albumin (Ab) and α-GDH are located on chromosomes Nos. 1 and 2, respectively. The loci for MDH-B, ME-B and olive mutant gene *i* are assumed to be located on chromosome No. 3. It has also been assumed that the loci for LDH-B, IDH-B and Hb, and Protein-C are located on chromosomes Nos. 4, 6 and 9, respectively.

In order to elucidate the linkage groups of albino genes *a, b, c, d* and *e,* the genotypes for blood proteins and enzymes extracted from the muscles and livers were compared with the constitution of each of the 13 bivalents of each oocyte in a total of 557 mature female backcrosses, including 103 obtained from NB(*Aa*)♀×BB(*aa*)♂, 104 obtained from BN(*Bb*)♀×NN(*bb*)♂, 102 obtained from BN(*Cc*)♀×NN(*cc*)♂, 101 obtained from BN(*Dd*)♀×NN(*dd*)♂, 112 obtained from BN(*Ee*)♀×NN(*ee*)♂ and 35 obtained from BN(*Ee*)♀×NN(*Ee*)♂. The loci analyzed were those for ME-A, SOD-B, ALD, ADH-A, HK, MPI, ADA, Pep-A, -B, -C and -D, and Est-1, -2, -4 and -5, in addition to the eight loci for Ab, Hb, Prot-C, α-GDH, MDH-B, ME-B, LDH-B and IDH-B which have been reported previously.

1. Chromosome No. 1

a. Albumin (Ab)

The genotype for Ab was compared with the constitution of each of the 13 bivalents of each oocyte in 549 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two

TABLE 5

Number of female backcrosses whose genotype for enzyme or blood protein agreed in constitution with each of the 13 bivalents in their oocytes. I

Component	Kind of backcrossing	No. of analyzed frogs	Bivalent number												
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Ab	NB (*Aa*) ♀×BB (*aa*) ♂	98	**94**	45	56	52	42	54	57	41	51	37	55	51	53
	BN (*Bb*) ♀×NN (*bb*) ♂	104	**98**	52	52	49	54	59	50	50	60	49	50	57	52
	BN (*Cc*) ♀×NN (*cc*) ♂	102	**96**	48	44	54	50	56	43	49	56	58	51	53	54
	BN (*Dd*) ♀×NN (*dd*) ♂	100	**96**	51	54	47	45	52	47	53	49	52	50	53	55
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	145	**131**	60	80	81	67	80	76	67	77	68	80	77	69
	Total	549	**515**	256	286	283	258	301	273	260	293	264	286	291	283
		(%)	(**93.8**)	(46.6)	(52.1)	(51.5)	(47.0)	(54.8)	(49.7)	(47.4)	(53.4)	(48.1)	(52.1)	(53.0)	(51.5)
ADH–A	NB (*Aa*) ♀×BB (*aa*) ♂	99	**91**	49	59	52	45	58	58	44	49	41	56	52	48
	BN (*Bb*) ♀×NN (*bb*) ♂	104	**93**	52	51	46	53	58	53	49	61	47	50	56	48
	BN (*Cc*) ♀×NN (*cc*) ♂	99	**84**	42	40	48	51	52	41	44	53	55	54	50	53
	BN (*Dd*) ♀×NN (*dd*) ♂	101	**95**	50	54	49	48	53	47	54	49	53	48	51	57
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	145	**115**	66	74	81	71	79	76	64	84	72	75	86	72
	Total	548	**478**	259	278	276	268	300	275	255	296	268	283	295	278
		(%)	(**87.2**)	(47.3)	(50.7)	(50.4)	(48.9)	(54.7)	(50.2)	(46.5)	(54.0)	(48.9)	(51.6)	(53.8)	(50.7)
α–GDH	NB (*Aa*) ♀×BB (*aa*) ♂	99	53	**75**	59	44	57	58	51	54	46	49	52	52	42
	BN (*Bb*) ♀×NN (*bb*) ♂	104	54	**75**	52	57	44	61	56	46	48	46	55	51	59
	BN (*Cc*) ♀×NN (*cc*) ♂	102	56	**61**	51	53	43	47	52	52	52	41	50	52	51
	BN (*Dd*) ♀×NN (*dd*) ♂	101	57	**67**	59	56	49	50	56	47	48	54	54	52	52
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	145	61	**99**	72	68	66	76	64	71	65	79	77	72	71
	Total	551	281	**377**	293	278	259	292	279	270	259	269	288	279	275
		(%)	(51.0)	(**68.4**)	(53.2)	(50.5)	(47.0)	(53.0)	(50.6)	(49.0)	(47.0)	(48.8)	(52.3)	(50.6)	(49.9)
Pep–C	NB (*Aa*) ♀×BB (*aa*) ♂	99	49	**93**	57	48	57	60	50	52	51	55	48	47	48
	BN (*Bb*) ♀×NN (*bb*) ♂	104	50	**99**	46	49	50	67	48	44	48	50	49	57	55
	BN (*Cc*) ♀×NN (*cc*) ♂	102	46	**98**	58	56	56	53	45	53	50	48	50	53	48
	BN (*Dd*) ♀×NN (*dd*) ♂	101	45	**93**	51	50	51	52	52	57	54	48	56	45	52
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	118	51	**97**	60	63	51	54	60	60	54	66	60	56	60

TABLE 5 Continued

Component	Kind of backcrossing	No. of analyzed frogs	Bivalent number											
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Pep-C	Total	524	241	**480**	272	266	265	286	255	266	257	267	263	258	263
		(%)	(46.0)	(**91.6**)	(51.9)	(50.8)	(50.6)	(54.6)	(48.7)	(50.8)	(49.0)	(51.0)	(50.2)	(49.2)	(50.2)
SOD-B	NB (*Aa*) ♀×BB (*aa*) ♂	53	28	**41**	30	26	33	31	31	30	23	25	28	33	25
	BN (*Dd*) ♀×NN (*dd*) ♂	58	21	**43**	33	26	24	34	38	28	28	20	31	34	36
	Total	111	49	**84**	63	52	57	65	69	58	51	45	59	67	61
		(%)	(44.1)	(**75.7**)	(56.8)	(46.8)	(51.4)	(58.6)	(62.2)	(52.3)	(45.9)	(40.5)	(53.2)	(60.4)	(55.0)
ME-A	NB (*Aa*) ♀×BB (*aa*) ♂	99	55	**79**	61	44	55	58	51	52	50	51	52	50	42
	BN (*Bb*) ♀×NN (*bb*) ♂	102	51	**71**	47	58	46	56	55	48	47	45	55	58	49
	BN (*Cc*) ♀×NN (*cc*) ♂	101	57	**61**	47	54	46	43	50	50	51	44	53	57	53
	BN (*Ee*) ♀×NN (*Ee*) ♂	22	11	**16**	10	11	13	13	9	14	10	11	11	13	9
	Total	324	174	**227**	165	167	160	170	165	164	158	151	171	178	153
		(%)	(53.7)	(**70.1**)	(50.9)	(51.5)	(49.4)	(52.5)	(50.9)	(50.6)	(48.8)	(46.6)	(52.8)	(54.9)	(47.2)
ME-B	NB (*Aa*) ♀×BB (*aa*) ♂	99	56	54	**90**	53	52	55	55	53	55	52	53	52	39
	BN (*Cc*) ♀×NN (*cc*) ♂	102	42	54	**90**	52	50	47	63	61	48	48	40	50	52
	BN (*Dd*) ♀×NN (*dd*) ♂	101	48	48	**90**	49	44	46	41	46	55	51	44	46	55
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	143	79	65	**137**	69	68	60	69	71	80	73	62	68	73
	Total	445	225	221	**407**	223	214	208	228	231	238	224	199	216	219
		(%)	(50.6)	(49.7)	(**91.5**)	(50.1)	(48.1)	(46.7)	(51.2)	(51.9)	(53.5)	(50.3)	(44.7)	(48.5)	(49.2)
MDH-B	NB (*Aa*) ♀×BB (*aa*) ♂	99	47	57	**88**	46	55	50	49	52	53	49	50	57	40
	BN (*Bb*) ♀×NN (*bb*) ♂	104	52	47	**90**	55	54	49	52	58	44	62	49	49	59
	BN (*Cc*) ♀×NN (*cc*) ♂	102	48	51	**81**	49	47	48	54	54	43	47	42	51	51
	BN (*Dd*) ♀×NN (*dd*) ♂	101	47	55	**87**	44	53	53	48	47	50	52	51	43	54
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	145	81	70	**123**	73	67	69	76	70	81	76	71	64	80
	Total	551	275	280	**469**	267	276	269	279	281	271	286	263	264	284
		(%)	(49.9)	(50.8)	(**85.1**)	(48.5)	(50.1)	(48.8)	(50.6)	(51.0)	(49.2)	(51.9)	(47.7)	(47.9)	(51.5)

parental species. The constitution of bivalents was BB or NB in the backcrosses produced from NB♀×BB♂, and NN or BN in the backcrosses produced from BN♀×NN♂. The genotype for Ab was A^bA^b or A^nA^b in the backcrosses produced from NB♀×BB♂, and A^nA^n or A^bA^n in the backcrosses produced from BN♀×NN♂, as gene A^n is located on N, and gene A^b is on B.

It was found that the genotype for Ab agreed with the constitution of bivalent No. I in 515 (93.8%) of the female backcrosses, while disagreed in 34 (6.2%) of the latter ($\chi^2=421.4$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. II~XIII, agreed with the genotype for Ab in 256 (46.6%)~301 (54.8%). Thus, the gene controlling Ab in the 549 female backcrosses is assumed to be located on chromosome No. 1, as reported previously by NISHIOKA, OHTANI and SUMIDA (1980), and to be about 6.2% in recombination rate (Tables 5, 9).

b. Alcohol dehydrogenase-A (ADH-A)

The constitution of each of the 13 bivalents of each oocyte was compared with the genotype for ADH-A in 548 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. The constitution of each bivalent was BB or NB in the backcrosses produced from NB♀×BB♂, and NN or BN in the backcrosses produced from BN♀×NN♂. The genotype for ADH-A is A^bA^b or A^nA^b in the backcrosses produced from NB♀×BB♂ and A^nA^n or A^bA^n in the backcrosses produced from BN♀×NN♂, as gene A^n of ADH-A is situated on N and gene A^b is on B.

The results showed that the genotype for ADH-A agreed with the consitiution of bivalent No. I in 478 (87.2%) and disagreed in 70 (12.8%) of the female backcrosses ($\chi^2=303.8$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. II~XIII, agreed with the genotype for ADH-A in 255 (46.5%)~300 (54.7%). Thus, it is assumed that the gene controlling ADH-A is situated on chromosome No. 1 with a recombination rate of about 12.8% (Tables 5, 9).

2. Chromosome No. 2

a. α-Glycerophosphate dehydrogenase (α-GDH)

The genotype for α-GDH which is G^bG^b or G^nG^b, or G^nG^n or G^bG^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 551 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. It was found that the genotype for α-GDH agreed with the constitution of bivalent No. II in 377 (68.4%) and disagreed in 174 (31.6%) of the backcrosses ($\chi^2=74.8$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I and III~XIII, agreed with the genotype for α-GDH in 259 (47.0%)~293 (53.2%) of the female backcrosses. Thus, it is assumed from the results of examining 551 female backcrosses that the gene controlling α-GDH is located on chromosome No. 2, as described previously by NISHIOKA, OHTANI and SUMIDA (1980). While the gene was about 28.6% in recombination rate in 182 female

backcrosses examined by these authors (1980), it was assumed to be 31.6% in the present 551 female backcrosses (Tables 5, 8).

b. Peptidase-C (Pep-C)

The genotype for Pep-C which is C^bC^b or C^nC^b, or C^nC^n or C^bC^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 524 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. The results showed that the constitution of bivalent No. II agreed with the genotype for Pep-C in 480 (91.6%) and disagreed in 44 (8.4%) of the female backcrosses ($\chi^2=362.8$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I and III~XIII, agreed with the genotype for Pep-C in 241 (46.0%)~286 (54.6%) of the backcrosses. Thus, the gene controlling Pep-C is assumed to be located on chromosome No. 2 with a recombination rate of about 8.4% (Tables 5, 8).

c. Superoxide dismutase-B (SOD-B)

The genotype for SOD-B which is B^bB^b or B^nB^b, or B^nB^n or B^bB^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 111 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. The results showed that the constitution of bivalent No. II agreed with the genotype for SOD-B in 84 (75.7%) and disagreed in 27 (24.3%) of the backcrosses ($\chi^2=29.3$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I and III~XIII, agreed with the genotype for SOD-B in 45 (40.5%)~69 (62.2%) of the female backcrosses. Thus, it is assumed that the gene controlling SOD-B is located on chromosome No. 2 with a recombination rate of about 24.3% (Tables 5, 8).

d. Malic enzyme-A (ME-A)

The genotype for ME-A which is A^bA^b or A^nA^b, or A^nA^n or A^bA^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 324 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. The results showed that the constitution of bivalent No. II agreed with the genotype for ME-A in 227 (70.1%) and disagreed in 97 (29.9%) of the backcrosses ($\chi^2=52.2$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I and III~XIII, agreed with the genotype for ME-A in 151 (46.6%)~178 (54.9%) of the backcrosses ($P>0.05$). Thus, it is assumed that the gene controlling ME-A is located on chromosome No. 2 with a recombination rate of about 29.9% (Tables 5, 8).

3. Chromosome No. 3

a. Malic enzyme-B (ME-B)

The genotype for ME-B which is B^bB^b or B^nB^b, or B^nB^n or B^bB^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 445 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. The results showed that the constitution of bivalent No. III agreed with the genotype for ME-B in 407 (91.5%) and disagreed in 38 (8.5%) of the backcrosses ($\chi^2=306.0$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I, II and IV~XIII, agreed with the genotype for ME-B in 199 (44.7%)~238 (53.5%) of the backcrosses ($P>0.05$). Thus, it is assumed that the gene controlling ME-B is located on chromosome No. 3 with a recombination rate of about 8.5%, as described by NISHIOKA and OHTANI (1986) (Tables 5, 12).

b. Malate dehydrogenase-B (MDH-B)

The genotype for MDH-B which is B^bB^b or B^nB^b, or B^nB^n or B^bB^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 551 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. It was found that the constitution of bivalent No. III agreed with the genotype for MDH-B in 469 (85.1%) and disagreed in 82 (14.9%) of the backcrosses ($\chi^2=271.8$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I, II and IV~XIII, agreed with the genotype for MDH-B in 263 (47.7%)~286 (51.9%) of the backcrosses. Thus, it is assumed from the present examination of 551 female backcrosses that the gene controlling MDH-B is located on chromosome No. 3, as described previously by NISHIOKA and OHTANI (1986) and the gene locus is about 14.9% in recombination rate (Tables 5, 12).

4. Chromosome No. 4

a. Lactate dehydrogenase-B (LDH-B)

The genotype for LDH-B which is B^bB^b or B^nB^b, or B^nB^n or B^bB^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 551 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. It was found that the constitution of bivalent No. IV agreed with the genotype for LDH-B in 486 (88.2%) and disagreed in 65 (11.8%) of the backcrosses ($\chi^2=321.7$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~III and V~XIII, agreed with the genotype for LDH-B in 259 (47.0%)~310 (56.3%) of the female backcrosses. Thus, it is assumed from the present examination of 551 female backcrosses that the gene controlling LDH-B is located on chromosome No. 4, as reported previously by NISHIOKA, OHTANI and SUMIDA (1980), and is about 11.8% in recombination rate (Table 6).

b. Hexokinase (HK)

The genotype for HK which is H^nH^n or H^bH^n was compared with the constitution, NN or BN, of each of the 13 bivalents of each oocyte in 168 female backcrosses produced from female hybrids between female *Rana brevipoda* and male *Rana nigromaculata* by mating with male *Rana nigromaculata*. It was found that the constitution of bivalent No. IV agreed with the genotype for HK in 133 (79.2%) and disagreed in 35 (20.8%) of the backcrosses ($\chi^2=57.2$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~III and V~XIII, agreed with the genotype for HK in 79 (47.0%)~101 (60.1%) of the backcrosses. Thus, it is assumed that the gene controlling HK is located on chromosome No. 4 with a recombination rate of about 20.8% (Table 6).

c. Mannose phosphate isomerase (MPI)

The genotype for MPI which is M^bM^b or M^nM^b, or M^nM^n or M^bM^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 142 backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. It was found that the constitution of bivalent No. IV agreed with the genotype for MPI in 95 (66.9%) and disagreed in 47 (33.1%) of the backcrosses ($\chi^2=16.2$, $P<0.0001$). The constitutions of the other 12 bivalents, Nos. I~III and V~XIII, agreed with the genotype for MPI in 59 (41.5%)~78 (54.9%) of the backcrosses. Thus, it is assumed that the gene controlling MPI is located on chromosome No. 4 with a recombination rate of about 33.1% (Table 6).

d. Peptidase-B (Pep-B)

The genotype for Pep-B which is B^bB^b or B^nB^b, or B^nB^n or B^bB^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 447 backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. It was found that the constitution of bivalent No. IV agreed with the genotype for Pep-B in 361 (80.8%) and disagreed in 86 (19.2%) of the female backcrosses ($\chi^2=169.2$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~III and V~XIII, agreed with the genotype for Pep-B in 208 (46.5%)~245 (54.8%) of the female backcrosses. Thus, it is assumed that the gene controlling Pep-B is located on chromosome No. 4 with a recombination rate of about 19.2% (Table 6).

5. Chromosome No. 5

a. Peptidase-A (Pep-A)

The genotype for Pep-A which is A^bA^b or A^nA^b, or A^nA^n or A^bA^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 551 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. It was found that the constitution of bivalent No. V agreed with the genotype for

TABLE 6

Number of female backcrosses whose genotype for enzyme or blood protein agreed in constitution with each of the 13 bivalents in their oocytes. II

Component	Kind of backcrossing	No. of analyzed frogs	Bivalent number												
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
LDH-B	NB (*Aa*) ♀×BB (*aa*) ♂	99	57	41	47	**88**	47	54	57	52	54	47	58	51	44
	BN (*Bb*) ♀×NN (*bb*) ♂	104	54	49	58	**87**	62	49	48	58	65	54	51	51	57
	BN (*Cc*) ♀ ×NN (*cc*) ♂	102	59	53	47	**89**	59	57	48	50	61	51	60	50	53
	BN (*Dd*) ♀×NN (*dd*) ♂	101	51	48	48	**89**	46	57	61	46	53	55	55	43	47
	BN (*Ee*) ♀ ×NN (*ee, Ee*) ♂	145	83	72	75	**133**	77	68	84	78	77	78	66	64	86
	Total	551	304	263	275	**486**	291	285	298	284	310	285	290	259	287
		(%)	(55.2)	(47.7)	(49.9)	(**88.2**)	(52.8)	(51.7)	(54.1)	(51.5)	(56.3)	(51.7)	(52.6)	(47.0)	(52.1)
HK	BN (*Bb*) ♀×NN (*bb*) ♂	69	35	36	39	**48**	41	35	36	38	42	31	32	30	36
	BN (*Cc*) ♀ ×NN (*cc*) ♂	99	48	54	43	**85**	58	55	46	45	59	54	52	49	47
	Total	168	83	90	82	**133**	99	90	82	83	101	85	84	79	83
		(%)	(49.4)	(53.6)	(48.8)	(**79.2**)	(58.9)	(53.6)	(48.8)	(49.4)	(60.1)	(50.6)	(50.0)	(47.0)	(49.4)
MPI	NB (*Aa*) ♀×BB (*aa*) ♂	99	45	47	46	**66**	47	52	55	54	62	53	48	50	42
	BN (*Dd*) ♀×NN (*dd*) ♂	43	25	25	21	**29**	20	19	23	22	16	21	23	26	17
	Total	142	70	72	67	**95**	67	71	78	76	78	74	71	76	59
		(%)	(49.3)	(50.7)	(47.2)	(**66.9**)	(47.2)	(50.0)	(54.9)	(53.5)	(54.9)	(52.1)	(50.0)	(53.5)	(41.5)
Pep-B	NB (*Aa*) ♀×BB (*aa*) ♂	99	54	52	51	**81**	46	55	53	49	57	54	57	47	43
	BN (*Cc*) ♀ ×NN (*cc*) ♂	102	46	58	48	**86**	62	54	45	49	57	54	57	49	52
	BN (*Dd*) ♀×NN (*dd*) ♂	101	57	46	38	**79**	54	53	57	52	49	51	51	51	43
	BN (*Ee*) ♀ ×NN (*ee, Ee*) ♂	145	88	78	71	**115**	71	64	76	76	70	78	68	66	82
	Total	447	245	234	208	**361**	233	226	231	226	233	237	233	213	220
		(%)	(54.8)	(52.3)	(46.5)	(**80.8**)	(52.1)	(50.6)	(51.7)	(50.6)	(52.1)	(53.0)	(52.1)	(47.7)	(49.2)

TABLE 6 Continued

Component	Kind of backcrossing	No. of analyzed frogs	Bivalent number											
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Pep-A	NB (*Aa*) ♀×BB (*aa*) ♂	99	45	53	57	48	**91**	52	51	50	51	53	44	51	50
	BN (*Bb*) ♀×NN (*bb*) ♂	104	51	52	57	56	**91**	60	59	53	57	55	48	50	54
	BN (*Cc*) ♀×NN (*cc*) ♂	102	47	52	44	56	**88**	46	49	51	59	50	61	52	52
	BN (*Dd*) ♀×NN (*dd*) ♂	101	51	49	47	46	**91**	57	40	57	51	52	46	46	50
	BN (*Ee*) ♀×NN (*ee*, *Ee*) ♂	145	76	60	71	75	**127**	72	80	84	71	68	78	79	82
	Total	551	270	266	276	281	**488**	287	279	295	289	278	277	278	288
		(%)	(49.0)	(48.3)	(50.1)	(51.0)	(**88.6**)	(52.1)	(50.6)	(53.5)	(52.5)	(50.5)	(50.3)	(50.5)	(52.3)
Hb	NB (*Aa*) ♀×BB (*aa*) ♂	101	58	61	55	52	49	**88**	57	52	54	43	51	55	52
	BN (*Bb*) ♀×NN (*bb*) ♂	104	57	67	51	50	57	**98**	53	57	54	52	47	52	57
	BN (*Cc*) ♀×NN (*cc*) ♂	102	59	49	47	53	51	**100**	48	48	52	51	48	48	47
	BN (*Dd*) ♀×NN (*dd*) ♂	101	50	49	39	52	53	**95**	48	61	47	54	55	45	46
	BN (*Ee*) ♀×NN (*ee*, *Ee*) ♂	145	76	67	68	66	68	**138**	80	74	70	69	68	81	62
	Total	553	300	293	260	273	278	**519**	286	292	277	269	269	281	264
		(%)	(54.2)	(53.0)	(47.0)	(49.4)	(50.3)	(**93.9**)	(51.7)	(52.8)	(50.1)	(48.6)	(48.6)	(50.8)	(47.7)
IDH-B	NB (*Aa*) ♀×BB (*aa*) ♂	99	59	59	51	54	45	**90**	58	48	53	45	50	52	50
	BN (*Bb*) ♀×NN (*bb*) ♂	104	58	65	48	53	58	**99**	50	52	57	56	53	51	55
	BN (*Cc*) ♀×NN (*cc*) ♂	102	66	52	46	54	50	**96**	47	47	52	48	47	51	48
	BN (*Dd*) ♀×NN (*dd*) ♂	101	51	50	42	55	54	**98**	51	62	49	51	50	42	49
	BN (*Ee*) ♀×NN (*ee*, *Ee*) ♂	145	77	68	61	67	63	**136**	75	70	70	66	78	74	62
	Total	551	311	294	248	283	270	**519**	281	279	281	266	278	270	264
		(%)	(56.4)	(53.4)	(45.0)	(51.4)	(49.0)	(**94.2**)	(51.0)	(50.6)	(51.0)	(48.3)	(50.5)	(49.0)	(47.9)

Pep-A in 488 (88.6%) and disagreed in 63 (11.4%) of the female backcrosses ($\chi^2=327.8$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~IV and VI~XIII, agreed with the genotype for Pep-A in 266 (48.3%)~295 (53.5%) of the female backcrosses. Thus, it is assumed that the gene controlling Pep-A is located on chromosome No. 5 with a recombination rate of about 11.4% (Table 6).

6. Chromosome No. 6

a. Hemoglobin (Hb)

The genotype for Hb which is H^bH^b or H^nH^b, or H^nH^n or H^bH^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 553 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. The results showed that the constitution of bivalent No. VI agreed with the genotype for Hb in 519 (93.9%) and disagreed in 34 (6.1%) of the female backcrosses ($\chi^2=425.4$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~V and VII~XIII, agreed with the genotype for Hb in 260 (47.0%)~300 (54.2%) of the backcrosses. Thus, it is assumed from the present examination of the 553 female backcrosses that the gene controlling Hb is located on chromosome No. 6, as NISHIOKA, OHTANI and SUMIDA (1980) have reported, and is about 6.1% in recombination rate (Table 6).

b. Isocitrate dehydrogenase-B (IDH-B)

The genotype for IDH-B which is B^bB^b or B^nB^b, or B^nB^n or B^bB^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 551 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. The results showed that the constitution of bivalent No. VI agreed with the genotype for IDH-B in 519 (94.2%) and disagreed in 32 (5.8%) of the female backcrosses ($\chi^2=430.4$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~V and VII~XIII, agreed with the genotype for IDH-B in 248 (45.0%)~311 (56.4%) of the female backcrosses. Thus, it is assumed from the present examination of 551 female backcrosses that the gene controlling IDH-B is located on chromosome No. 6, as NISHIOKA, OHTANI and SUMIDA (1980) have reported previously, and is about 5.8% in recombination rate (Table 6).

7. Chromosome No. 9

a. Protein-C (Prot-C)

The genotype for Prot-C which is C^bC^b or C^nC^b, or C^nC^n or C^bC^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 547 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. The results showed that the constitution of bivalent No. IX agreed with the genotype for Prot-C in 414 (75.7%) and disagreed in 133 (24.3%) of the female backcrosses ($\chi^2=144.4$, $P<0.00001$). The constitutions of the

other 12 bivalents, Nos. I~VIII and X~XIII, agreed with the genotype for Prot-C in 251 (45.9%)~291 (53.2%) of the female backcrosses ($P>0.05$). Thus, it is assumed from the present examination of 547 female backcrosses that the gene controlling Prot-C is located on chromosome No. 9, as NISHIOKA, OHTANI and SUMIDA (1980) have reported previously, and is about 24.3% in recombination rate (Tables 7, 10).

b. Aldolase (ALD)

The genotype for ALD which is A^bA^b or A^nA^b, or A^nA^n or A^bA^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 547 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. The results showed that the constitution of bivalent No. IX agreed with the genotype for ALD in 302 (55.2%) and disagreed in 245 (44.8%) of the female backcrosses ($\chi^2=5.94$, $0.05>P>0.01$). The constitutions of the other 12 bivalents, Nos. I~VIII and X~XIII, agreed with the genotype for ALD in 255 (46.6%)~288 (52.7%) of the female backcrosses (Table 7). Although it is questionable from the agreement value of only 55.2% that the gene controlling ALD is located on chromosome No. 9, it is evident that the locus for ALD is linked with the locus for Prot-C. The linkage between these two loci is found in 357 (66.5%) of 537 female backcrosses ($\chi^2=58.3$, $P<0.00001$), as described later (Table 10).

8. Chromosome No. 10

a. Esterase-1 (Est-1)

The genotype for Est-1 which is 1^n1^n or 1^b1^n was compared with the constitution, NN or BN, of each of the 13 bivalents of each oocyte in 446 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with male *Rana nigromaculata*. The results showed that the constitution of bivalent No. X agreed with the genotype for Est-1 in 327 (73.3%) and disagreed in 119 (26.7%) of the female backcrosses ($\chi^2=97.0$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~IX and XI~XIII, agreed with the genotype for Est-1 in 212 (47.5%)~231 (51.8%) of the female backcrosses. Thus, it is assumed that the gene controlling Est-1 is located on chromosome No. 10 with a recombination rate of about 26.7% (Table 7).

b. Esterase-2 (Est-2)

The genotype for Est-2 which is 2^n2^n or 2^b2^n was compared with the constitution, NN or BN, of each of the 13 bivalents of each oocyte in 269 female backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with male *Rana nigromaculata*. It was found that the constitution of bivalent No. X agreed with the genotype for Est-2 in 203 (75.5%) and disagreed in 66 (24.5%) of the female backcrosses ($\chi^2=69.8$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~IX and XI~XIII, agreed with

TABLE 7

Number of female backcrosses whose genotype for enzyme or blood protein agreed in constitution with each of the 13 bivalents in their oocytes. III

Component	Kind of backcrossing	No. of analyzed frogs	Bivalent number											
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Prot-C	NB (*Aa*) ♀×BB (*aa*) ♂	97	53	46	47	59	47	47	55	38	**82**	44	61	48	45
	BN (*Bb*) ♀×NN (*bb*) ♂	104	54	50	46	57	56	59	56	46	**77**	49	54	53	46
	BN (*Cc*) ♀×NN (*cc*) ♂	102	51	49	53	49	49	57	54	56	**75**	55	52	53	43
	BN (*Dd*) ♀×NN (*dd*) ♂	100	45	43	50	57	57	48	49	51	**73**	56	49	42	49
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	144	78	71	74	68	67	80	74	83	**107**	64	70	64	68
	Total	547	281	259	270	290	276	291	288	274	**414**	268	286	260	251
		(%)	(51.4)	(47.3)	(49.4)	(53.0)	(50.5)	(53.2)	(52.7)	(50.1)	(**75.7**)	(49.0)	(52.3)	(47.5)	(45.9)
ALD	NB (*Aa*) ♀×BB (*aa*) ♂	99	54	48	51	55	46	55	47	41	**64**	48	55	50	47
	BN (*Bb*) ♀×NN (*bb*) ♂	104	59	52	51	52	49	52	55	51	**53**	51	58	52	54
	BN (*Cc*) ♀×NN (*cc*) ♂	101	45	59	57	56	52	54	53	53	**55**	46	55	53	56
	BN (*Dd*) ♀×NN (*dd*) ♂	98	38	40	58	52	49	40	55	41	**52**	48	45	53	52
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	145	85	75	71	70	74	70	67	69	**78**	65	71	65	71
	Total	547	281	274	288	285	270	271	277	255	**302**	258	284	273	280
		(%)	(51.4)	(50.1)	(52.7)	(52.1)	(49.4)	(49.5)	(50.6)	(46.6)	(**55.2**)	(47.2)	(51.9)	(49.9)	(51.2)
Est-1	BN (*Bb*) ♀×NN (*bb*) ♂	98	43	47	56	50	49	43	51	55	46	**72**	46	49	47
	BN (*Cc*) ♀×NN (*cc*) ♂	102	52	47	51	47	43	53	44	50	56	**79**	54	57	55
	BN (*Dd*) ♀×NN (*dd*) ♂	101	50	49	55	46	49	51	48	53	50	**66**	50	44	50
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	145	73	74	68	69	73	74	74	72	75	**110**	75	81	72
	Total	446	218	217	230	212	214	221	217	230	227	**327**	225	231	224
		(%)	(48.9)	(48.7)	(51.6)	(47.5)	(48.0)	(49.6)	(48.7)	(51.6)	(50.9)	(**73.3**)	(50.4)	(51.8)	(50.2)
Est-2	BN (*Bb*) ♀×NN (*bb*) ♂	24	11	9	12	13	10	9	13	15	13	**16**	12	10	11
	BN (*Cc*) ♀×NN (*cc*) ♂	101	51	47	51	46	42	53	44	50	56	**78**	53	56	54
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	144	73	73	68	69	72	74	73	72	74	**109**	75	80	72

TABLE 7 Continued

Component	Kind of backcrossing	No. of analyzed frogs	Bivalent number												
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Est-2	Total	269	135	129	131	128	124	136	130	137	143	**203**	140	146	137
		(%)	(50.2)	(48.0)	(48.7)	(47.6)	(46.1)	(50.6)	(48.3)	(50.9)	(53.2)	(**75.5**)	(52.0)	(54.3)	(50.9)
Est-4	NB (*Aa*) ♀×BB (*aa*) ♂	99	50	44	47	55	54	53	49	53	53	**78**	55	46	45
		(%)	(50.5)	(44.4)	(47.5)	(55.6)	(54.5)	(53.5)	(49.5)	(53.5)	(53.5)	(**78.8**)	(55.6)	(46.5)	(45.5)
Est-5	BN (*Bb*) ♀×NN (*bb*) ♂	104	50	53	54	47	52	45	56	54	52	**70**	55	55	47
	BN (*Cc*) ♀×NN (*cc*) ♂	102	54	47	49	49	45	55	46	50	56	**79**	52	55	55
	BN (*Dd*) ♀×NN (*dd*) ♂	93	47	46	51	41	47	46	43	50	46	**60**	46	40	49
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	145	68	77	65	70	76	69	77	71	72	**107**	78	82	75
	Total	444	219	223	219	207	220	215	222	225	226	**316**	231	232	226
		(%)	(49.3)	(50.2)	(49.3)	(46.6)	(49.5)	(48.4)	(50.0)	(50.7)	(50.9)	(**71.2**)	(52.0)	(52.3)	(50.9)
Pep-D	NB (*Aa*) ♀×BB (*aa*) ♂	99	51	45	46	60	55	52	48	50	51	**75**	52	47	46
	BN (*Bb*) ♀×NN (*bb*) ♂	104	46	47	58	53	56	45	56	54	48	**70**	49	57	51
	BN (*Cc*) ♀×NN (*cc*) ♂	102	52	43	53	47	43	53	48	54	56	**77**	52	55	57
	BN (*Dd*) ♀×NN (*dd*) ♂	101	51	52	54	45	46	52	51	52	51	**61**	47	44	49
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	145	75	70	63	71	71	78	72	68	79	**102**	73	77	80
	Total	551	275	257	274	276	271	280	275	278	285	**385**	273	280	283
		(%)	(49.9)	(46.6)	(49.7)	(50.1)	(49.2)	(50.8)	(49.9)	(50.5)	(51.7)	(**69.9**)	(49.5)	(50.8)	(51.4)
ADA	NB (*Aa*) ♀×BB (*aa*) ♂	99	53	55	52	50	41	44	49	48	50	47	**76**	50	50
	BN (*Bb*) ♀×NN (*bb*) ♂	104	52	53	50	49	42	51	54	52	52	50	**77**	61	55
	BN (*Cc*) ♀×NN (*cc*) ♂	102	47	57	41	43	53	51	48	60	52	53	**67**	59	43
	BN (*Dd*) ♀×NN (*dd*) ♂	101	39	58	46	51	52	56	51	58	49	49	**73**	56	47
	BN (*Ee*) ♀×NN (*ee, Ee*) ♂	145	68	77	65	70	78	70	65	73	77	73	**114**	70	73
	Total	551	259	300	254	263	266	272	267	291	280	272	**407**	296	268
		(%)	(47.0)	(54.4)	(46.1)	(47.7)	(48.3)	(49.4)	(48.5)	(52.8)	(50.8)	(49.4)	(**73.9**)	(53.7)	(48.6)

the genotype for Est-2 in 124 (46.1%)~146 (54.3%) of the female backcrosses. Thus, it is assumed that the gene controlling Est-2 is located on chromosome No. 10 with a recombination rate of about 24.5% (Table 7).

c. Esterase-4 (Est-4)

The genotype for Est-4 which is 4^b4^b or 4^n4^b was compared with the constitution, BB or NB, of each of the 13 bivalents of each oocyte in 99 backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with male *Rana brevipoda* . The results showed that the constitution of bivalent No. X agreed with the genotype for Est-4 in 78 (78.8%) and disagreed in 21 (21.2%) of the female backcrosses ($\chi^2=32.8$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~IX and XI~XIII, agreed with the genotype for Est-4 in 44 (44.4%)~55 (55.6%) of the female backcrosses. Thus, it is assumed that the gene controlling Est-4 is located on chromosome No. 10 with a recombination rate of about 21.2% (Table 7).

d. Esterase-5 (Est-5)

The genotype for Est-5 which is 5^n5^n or 5^b5^n was compared with the constitution, NN or BN, of each of the 13 bivalents of each oocyte in 444 backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with male *Rana nigromaculata*. The results showed that the constitution of bivalent No. X agreed with the genotype for Est-5 in 316 (71.2%) and disagreed in 128 (28.8%) of the female backcrosses ($\chi^2=79.6$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~IX and XI~XIII, agreed with the genotype for Est-5 in 207 (46.6%)~232 (52.3%) of the female backcrosses. Thus, it is assumed that the gene controlling Est-5 is located on chromosome No. 10 with a recombination rate of about 28.8% (Table 7).

e. Peptidase-D (Pep-D)

The genotype for Pep-D which is D^bD^b or D^nD^b, or D^nD^n or D^bD^n was compared with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 551 backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. The results showed that the constitution of bivalent No. X agreed with the genotype for Pep-D in 385 (69.9%) and disagreed in 166 (30.1%) of the female backcrosses ($\chi^2=87.0$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~IX and XI~XIII, agreed with the genotype for Pep-D in 257 (46.6%)~285 (51.7%) of the female backcrosses. Thus, it is assumed that the gene controlling Pep-D is located on chromosome No. 10 with a recombination rate of about 30.1% (Table 7).

9. Chromosome No. 11

a. Adenosine deaminase (ADA)

The genotype for ADA which is A^bA^b or A^nA^b, or A^nA^n or A^bA^n was compared

with the constitution, BB or NB, or NN or BN, of each of the 13 bivalents of each oocyte in 551 backcrosses produced from female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with males of the two parental species. The results showed that the constitution of bivalent No. XI agreed with the genotype for ADA in 407 (73.9%) and disagreed in 144 (26.1%) of the female backcrosses ($\chi^2=125.5$, $P<0.00001$). The constitutions of the other 12 bivalents, Nos. I~X, XII and XIII, agreed with the genotype for ADA in 254 (46.1%)~300 (54.4%) of the female backcrosses. Thus, the gene controlling ADA is located on chromosome No. 11 with a recombination rate of about 26.1% (Table 7).

VII. Linkage of loci for albinisms and various enzymes and blood proteins

The locations of 23 genes controlling 16 enzymes and blood proteins on the nine chromosomes were assumed as follows: the genes of Ab and ADH-A on chromosome No. 1, the genes of Pep-C, SOD-B, ME-A and α-GDH on chromosome No. 2, the genes of MDH-B and ME-B on chromosome No. 3, the genes of LDH-B, Pep-B, MPI and HK on chromosome No. 4, the gene of Pep-A on chromosome No. 5, the genes of IDH-B and Hb on chromosome No. 6, the genes of Prot-C and ALD on chromosome No. 9, the genes of Est-1, -2, -4 and -5, and Pep-D on chromosome No. 10 and the gene of ADA on chromosome No. 11 (Tables 5~7).

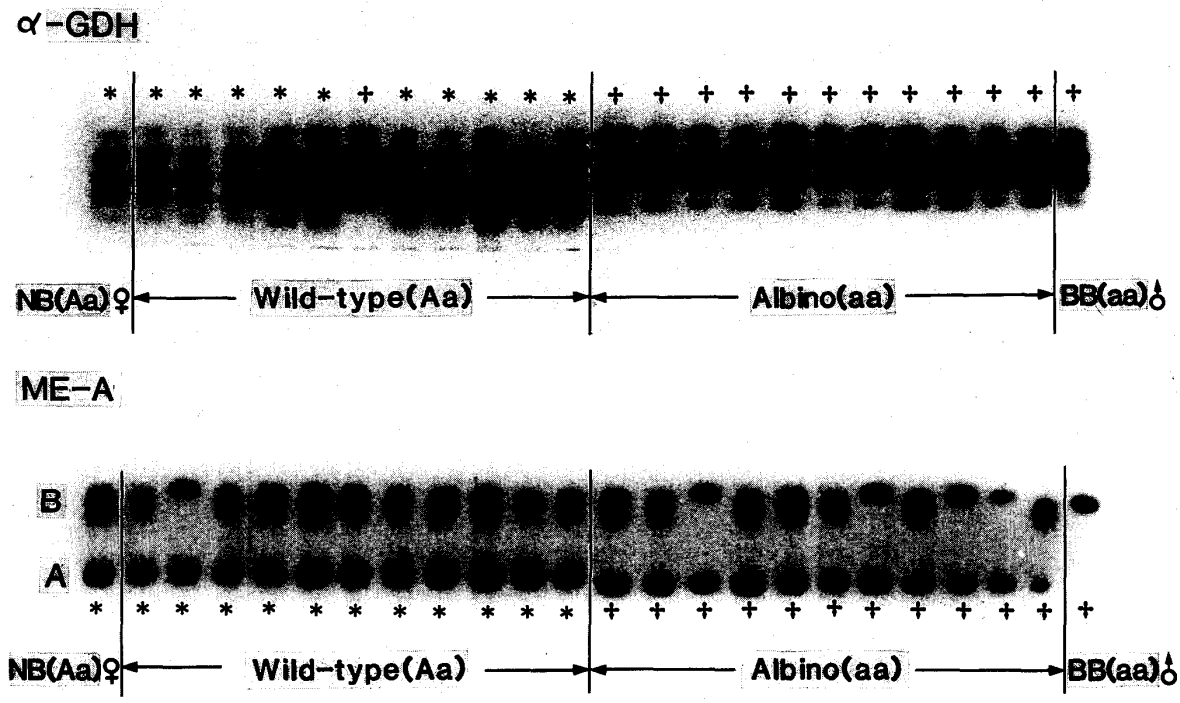


Fig. 3. Electrophoretic patterns of α-GDH and ME-A in wild-type and albinic female backcrosses between *Rana nigromaculata* and *R. brevipoda*, NB(*Aa*)♀×BB(*aa*)♂.

α-GDH: ✱ G^nG^b, + G^bG^b ME-A: ✱ A^nA^b, + A^bA^b

In order to draw a genetic map, linkage relationships were examined between albino genes and the genes controlling various enzymes and blood proteins on chromosomes Nos. 1, 2, 3 and 9.

1. Albino genes *a* and *c*

Of the four enzyme-controlling genes located on chromosome No. 2, the gene for ME-A appeared to be most closely located to albino gene *a*, as these two genes were linked with each other in all of 99 female backcrosses (Fig. 3). Albino gene *a* and the gene controlling α-GDH were secondly close together in linkage relationship, as these genes were linked in 95 (96.0%) of 99 female backcrosses and not linked in only four (4.0%), being recombination ($\chi^2=83.6$, $P<0.00001$).

TABLE 8

Tests for linkage of two loci for albino genes *a* and *c* on chromosome No. 2 to 23 loci for enzymes and blood proteins

Loci and their linkage		No. of analyzed frogs	Agree (%)	Disagree	χ^2	P	Recombination rate (%)
Chromosome 2 —	Albino gene *c*	**102**	**86 (84.3)**	**16**	**48.0**	**<0.00001**	**15.7**
〃	Albino gene *a*	**103**	**82 (79.6)**	**21**	**36.1**	**<0.00001**	**20.4**
〃	Pep-C	**524**	**480 (91.6)**	**44**	**362.8**	**<0.00001**	**8.4**
〃	SOD-B	**111**	**84 (75.7)**	**27**	**29.3**	**<0.00001**	**24.3**
〃	ME-A	**324**	**227 (70.1)**	**97**	**52.2**	**<0.00001**	**29.9**
〃	α-GDH	**551**	**377 (68.4)**	**174**	**74.8**	**<0.00001**	**31.6**
Albino gene *a* —	Albumin (Ab)	98	52 (53.1)	46	0.37	0.54	
〃	ADH-A	99	55 (55.6)	44	1.22	0.27	
〃	ME-A	**99**	**99 (100)**	**0**	**99.0**	**<0.00001**	**0**
〃	α-GDH	**99**	**95 (96.0)**	**4**	**83.6**	**<0.00001**	**4.0**
〃	Pep-C	**99**	**77 (77.8)**	**22**	**30.6**	**<0.00001**	**22.2**
〃	SOD-B	**53**	**34 (64.2)**	**19**	**4.25**	**<0.04**	**35.8**
〃	MDH-B	99	55 (55.6)	44	1.22	0.27	
〃	ME-B	99	58 (58.6)	41	2.92	0.09	
〃	LDH-B	99	43 (43.4)	56	1.71	0.19	
〃	Pep-B	99	48 (48.5)	51	0.09	0.76	
〃	MPI	99	43 (43.4)	56	1.71	0.19	
〃	Pep-A	99	55 (55.6)	44	1.22	0.27	
〃	IDH-B	99	57 (57.6)	42	2.27	0.13	
〃	Hemoglobin (Hb)	101	56 (55.4)	45	1.20	0.27	
〃	Prot-C	97	49 (50.5)	48	0.01	0.92	
〃	ALD	99	52 (52.5)	47	2.25	0.62	
〃	Est-4	99	44 (44.4)	55	1.22	0.27	
〃	Pep-D	99	47 (47.5)	52	2.25	0.62	
〃	ADA	99	53 (53.5)	46	0.49	0.48	

The gene for Pep-C was linked with albino gene *a* in 77 (77.8%) of 99 female backcrosses and not linked in 22 (22.2%), being recombination ($\chi^2=30.6$, $P<0.00001$). The gene controlling SOD-B was linked with albino gene *a* in 34 (64.2%) of 53 female backcrosses and not linked in 19 (35.8%), being recombination ($\chi^2=4.25$, $0.05>P>0.01$). This situation seems to indicate that these two genes are remotely linked. Albino gene *a* did not link with 15 other genes controlling 13 kinds of blood proteins and enzymes on chromosomes Nos. 1, 3~6 and 9~11 ($P>0.05$) (Table 8).

Albino gene *c* seemed to be most closely linked with the gene controlling Pep-C, as these two genes were linked with each other in 86 (84.3%) of 102 female backcrosses and not linked in 16 (15.7%), being recombination ($\chi^2=48.0$,

TABLE 8 Continued

Loci and their linkage		No. of analyzed frogs	Agree (%)	Disagree	χ^2	P	Recombination rate (%)
Albino gene *c* ——	Albumin (Ab)	102	50 (49.0)	52	0.04	0.84	
〃	ADH-A	99	47 (47.5)	52	0.25	0.62	
〃	Pep-C	**102**	**86 (84.3)**	**16**	**48.0**	**<0.00001**	**15.7**
〃	ME-A	**101**	**69 (68.3)**	**32**	**13.6**	**<0.0003**	**31.2**
〃	*α*-GDH	**102**	**69 (67.6)**	**33**	**12.7**	**<0.0004**	**32.4**
〃	MDH-B	102	51 (50.0)	51	0.00	1.00	
〃	ME-B	102	52 (51.0)	50	0.04	0.84	
〃	LDH-B	102	53 (52.0)	49	0.16	0.69	
〃	Pep-B	102	56 (54.9)	46	0.98	0.32	
〃	HK	99	50 (50.5)	49	0.01	0.92	
〃	Pep-A	102	54 (52.9)	48	0.35	0.55	
〃	IDH-B	102	50 (49.0)	52	0.04	0.84	
〃	Hemoglobin (Hb)	102	47 (46.1)	55	0.63	0.43	
〃	Prot-C	102	55 (53.9)	47	0.63	0.43	
〃	ALD	101	59 (58.4)	42	2.86	0.09	
〃	Est-1	102	49 (48.0)	53	0.16	0.69	
〃	Est-2	101	49 (48.5)	52	0.09	0.77	
〃	Est-5	102	49 (48.0)	53	0.16	0.69	
〃	Pep-D	102	45 (44.1)	57	1.41	0.23	
〃	ADA	102	61 (59.8)	41	3.92	0.05	
α-GDH ——	ME-A	**324**	**297 (91.7)**	**27**	**225.0**	**<0.00001**	**8.3**
〃	Pep-C	**524**	**361 (68.9)**	**163**	**74.8**	**<0.00001**	**31.1**
〃	SOD-B	111	64 (57.7)	47	2.60	0.11	42.3
ME-A ——	Pep-C	**309**	**214 (69.3)**	**95**	**45.8**	**<0.00001**	**30.7**
〃	SOD-B	**53**	**34 (64.2)**	**19**	**4.25**	**<0.04**	**35.8**
Pep-C ——	SOD-B	**111**	**80 (72.1)**	**31**	**21.6**	**<0.00001**	**27.9**

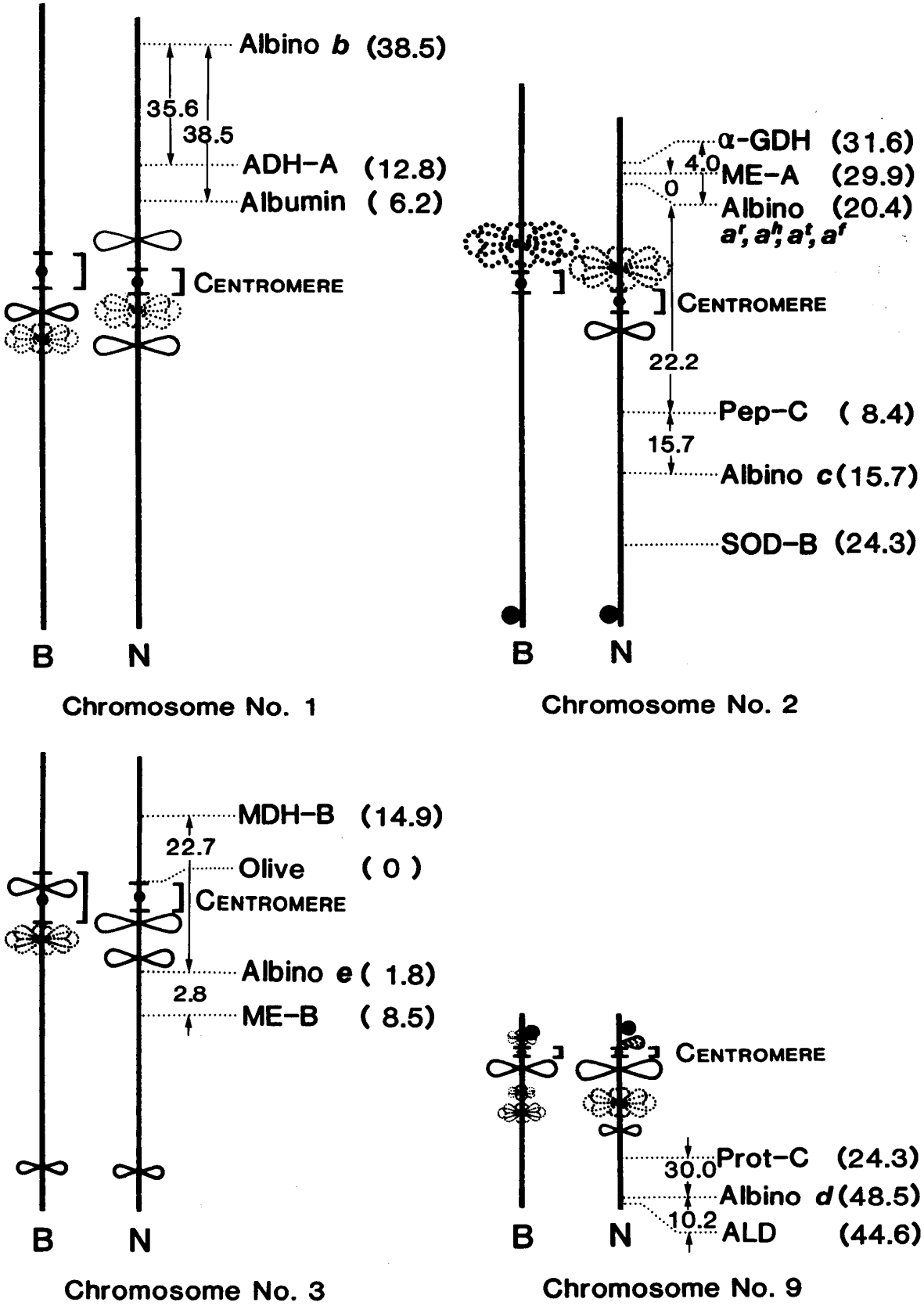


Fig. 4. Sites for five albino loci (*a*, *b*, *c*, *d* and *e*) and linked genes on lampbrush chromosomes, Nos. 1, 2, 3 and 9.

Parentheses show the recombination rate between the gene and the centromere.

$P<0.00001$). Albino gene *c* and the gene controlling ME-A were secondly close in linkage relationship, as they were linked in 69 (68.3%) of 101 female backcrosses and not linked in 32 (31.7%), being recombination ($\chi^2=13.6$, $P<0.0003$). The gene controlling α-GDH was linked with albino gene *c* in 69 (67.6%) of 102 female backcrosses and not linked in 33 (32.4%), being recombination ($\chi^2=12.7$, $P<0.0004$). No linkages were found between albino gene *c* and 17 other genes controlling 14 blood proteins and enzymes on chromosomes Nos. 1, 3~6 and 9~11 ($P>0.05$) (Table 8).

The gene controlling α-GDH was linked with the gene controlling ME-A in 297 (91.7%) of 324 female backcrosses and not linked in 27 (8.3%), being recombination ($\chi^2=225.0$, $P<0.00001$). The genes controlling α-GDH and Pep-C were linked with each other in 361 (68.9%) of 524 female backcrosses and not linked in 163 (31.1%), being recombination ($\chi^2=74.8$, $P<0.00001$). The genes controlling α-GDH and SOD-B were linked in 64 (57.7%) of 111 female backcrosses and not linked in 47 (42.3%), being recombination ($\chi^2=2.60$, $P>0.1$). Thus, these two genes are considered to be located far apart from each other, even if they are linked with each other (Table 8).

The genes controlling ME-A and Pep-C were linked with each other in 214 (69.3%) of 309 female backcrosses and not linked in 95 (30.7%), being recombination ($\chi^2=45.8$, $P<0.00001$). The genes controlling ME-A and SOD-B were linked with each other in 34 (64.2%) of 53 female backcrosses and not linked in 19 (35.8%), being recombination ($\chi^2=4.25$, $0.05>P>0.01$). Thus, these two genes are considered to be located far apart from each other, even if they are linked with each other (Table 8).

The genes controlling Pep-C and SOD-B were linked with each other in 80 (72.1%) of 111 female backcrosses, and not linked in 31 (27.9%), being recombination ($\chi^2=21.6$, $P<0.00001$).

It is assumed from the foregoing findings that albino gene *a* is located at the site of about 20.4% in recombination rate from the centromere on an arm of chromosome No. 2, the gene controlling ME-A is located at the site of about 29.9% and the gene controlling α-GDH is located at the site of about 31.6%. On the other arm, it is assumed that the gene controlling Pep-C is located at the site of about 8.4% in recombination rate from the centromere, albino gene *c* is located at the site of about 15.7%, and the gene controlling SOD-B is located at the site of about 24.3% in recombination rate (Table 8; Fig. 4).

2. Albino gene *b*

As previously described, it was somewhat difficult to assume that albino gene *b* is located on chromosome No. 1, since the agreement between the constitution of the genotype and that of the bivalent was found in only 61.5% of the female backcrosses. Then, the existence of linkages between albino gene *b* and 17 genes controlling 14 enzymes and blood proteins on chromosomes Nos. 1~6 and 9~11 was examined (Table 9). The results showed that albino gene *b* is linked with the gene controlling ADH-A which was located on chromosome No. 1 in 67 (64.4%)

TABLE 9

Tests for linkage of the locus for albino gene *b* on chromosome No. 1 to 17 loci for enzymes and blood proteins

Loci and their linkage		No. of analyzed frogs	Agree (%)	Disagree	χ^2	*P*	Recombination rate (%)
Chromosome 1 —	Albino gene *b*	**104**	**64 (61.5)**	**40**	**5.54**	**<0.02**	**38.5**
〃	Albumin (Ab)	**104**	**98 (94.2)**	**6**	**81.4**	**<0.00001**	**5.8**
〃	Albumin (Ab)	**549**	**515 (93.8)**	**34**	**421.4**	**<0.00001**	**6.2**
〃	ADH-A	**104**	**93 (89.4)**	**11**	**64.7**	**<0.00001**	**10.6**
〃	ADH-A	**548**	**478 (87.2)**	**70**	**303.8**	**<0.00001**	**12.8**
Albino gene *b* —	Albumin (Ab)	**104**	**64 (61.5)**	**40**	**5.54**	**<0.02**	**38.5**
〃	ADH-A	**104**	**67 (64.4)**	**37**	**8.66**	**<0.004**	**35.6**
〃	α-GDH	104	48 (46.2)	56	0.62	0.43	
〃	ME-A	101	48 (47.5)	53	0.25	0.62	
〃	Pep-C	104	44 (42.3)	60	2.46	0.12	
〃	MDH-B	104	46 (44.2)	58	1.38	0.24	
〃	LDH-B	104	40 (38.5)	65	—	—	
〃	HK	69	29 (42.0)	40	1.75	0.19	
〃	Pep-A	104	49 (47.1)	55	0.35	0.56	
〃	IDH-B	104	50 (48.1)	54	0.15	0.70	
〃	Hemoglobin (Hb)	104	47 (45.2)	57	0.96	0.33	
〃	Prot-C	104	58 (55.8)	46	1.38	0.24	
〃	ALD	104	57 (54.8)	47	0.96	0.33	
〃	Est-1	98	50 (51.0)	48	0.04	0.84	
〃	Est-5	104	62 (59.6)	42	3.85	0.05	
〃	Pep-D	104	60 (57.7)	44	2.46	0.12	
〃	ADA	104	48 (46.2)	56	0.62	0.43	
ADH-A —	Albumin (Ab)	**103**	**99 (96.1)**	**4**	**87.6**	**<0.00001**	**3.9**
〃	Albumin (Ab)	**540**	**496 (91.9)**	**44**	**378.3**	**<0.00001**	**8.1**

Fig. 5. Microphotographs of bivalent No. I in oocytes of four female backcrosess between *Rana nigromaculata* and *R. brevipoda*, BN♀×NN♂. ×400

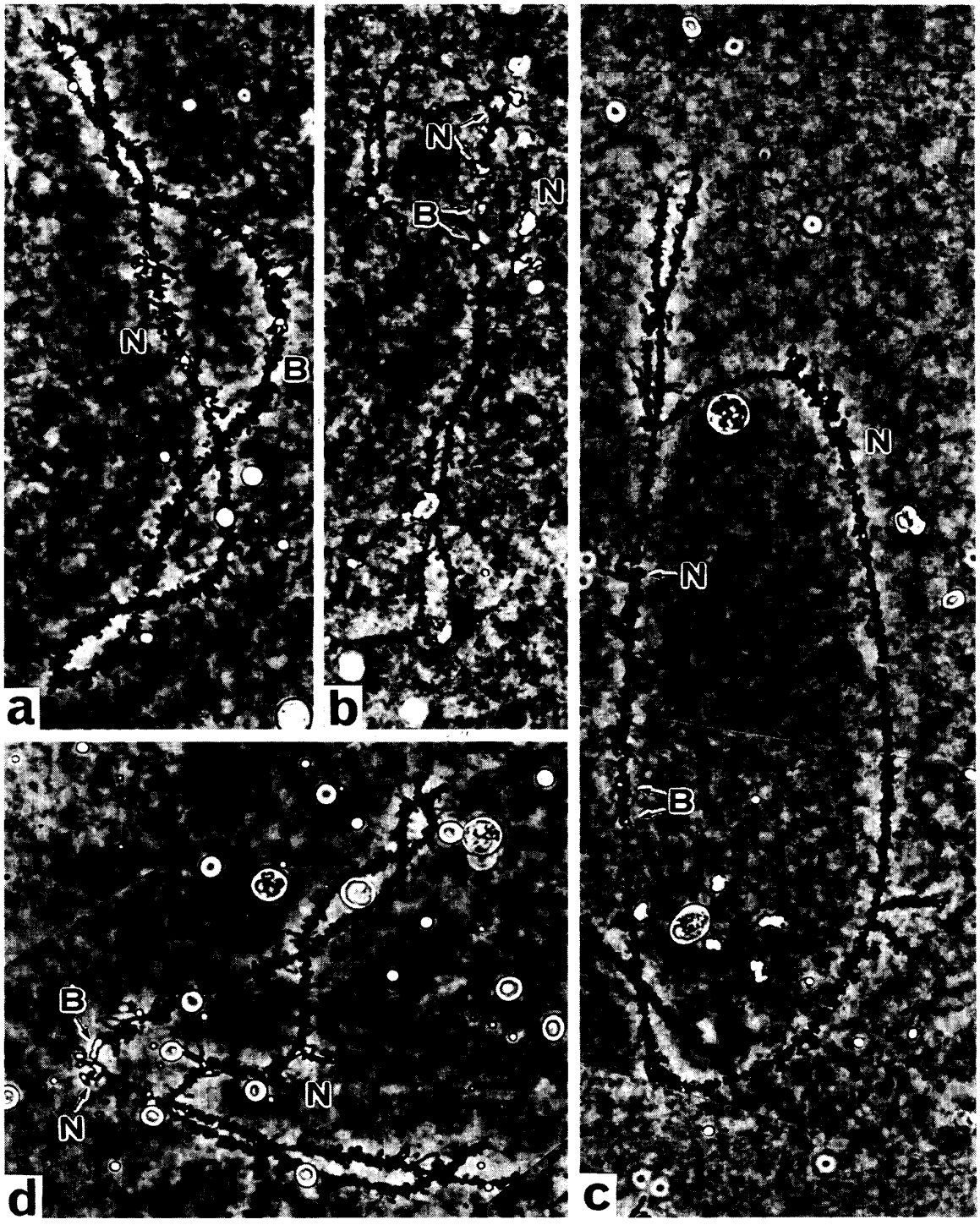
a. A pair (NB) of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome in an oocyte of female No. 68, BN(*Bb*)♀×NN(*bb*)♂.

b. A pair $\left(\frac{N}{B}N\right)$ of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome having a translocation from a *R. nigromaculata* chromosome in an oocyte of female No. 69, BN(*Dd*)♀×NN(*dd*)♂.

c. A pair $\left(\frac{N}{B}N\right)$ of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome having a translocation from a *R. nigromaculata* chromosome in an oocyte of female No. 87, BN(*Cc*)♀×NN(*cc*)♂.

d. A pair $\left(\frac{B}{N}N\right)$ of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome having a translocation from a *R. brevipoda* chromosome in an oocyte of female No. 65, BN(*Ee*)♀×NN(*ee*)♂.

of 104 female backcrosses and not linked in 37 (35.6%), being recombination (χ^2 =8.66, $P<0.004$). Albino gene *b* was also linked with the gene controlling Ab in 64 (61.5%) of the 104 female backcrosses and not linked in 40 (38.5%), being recombination ($\chi^2=5.54$, $0.05>P>0.01$). Albino gene *b* was linked with each of the other 15 genes located on chromosomes Nos. 2~6 and 9~11 in 42.0~59.6% of the female backcrosses and not linked in 40.4~58.0%, being recombination ($P>0.05$). Thus, it is assumed that albino gene *b* is linked with the genes



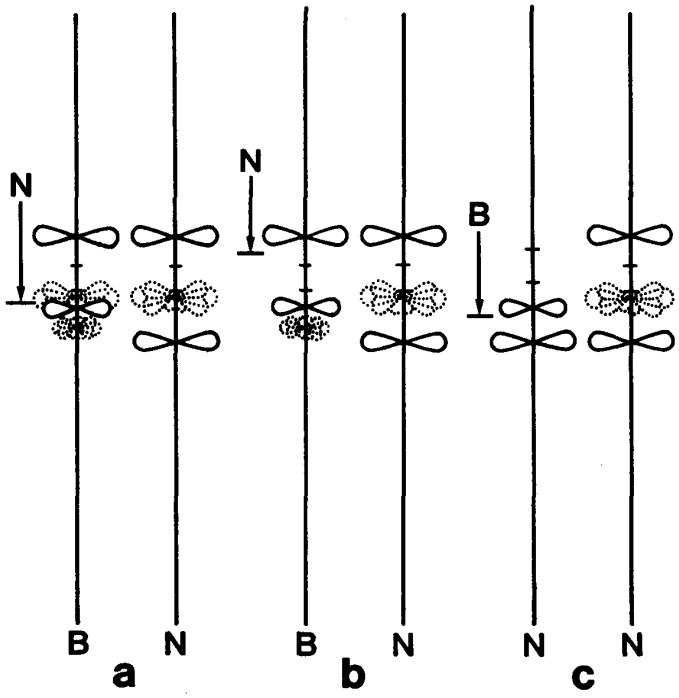


Fig. 6. Diagrams showing the constitutions of bivalent No. I in oocytes of three female backcrosses between *Rana nigromaculata* and *R. brevipoda*, BN♀×NN♂.

a. A pair $\left(\frac{N}{B}N\right)$ of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome having a translocation from a *R. nigromaculata* chromosome in an oocyte of female No. 69.

b. A pair $\left(\frac{N}{B}N\right)$ of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome having a translocation from a *R. nigromaculata* chromosome in an oocyte of female No. 87.

c. A pair $\left(\frac{B}{N}N\right)$ of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome having a translocation from a *R. brevipoda* chromosome in an oocyte of female No. 65.

controlling ADH-A and Ab. In this case, the genes of ADH-A and Ab were linked with each other in 99 (96.1%) of 103 female backcrosses, and not linked in only four (3.9%), being recombination ($\chi^2=87.6$, $P<0.00001$) (Table 9).

There were two kinds of female backcrosses, in which a translocation had occurred on bivalent No. I.

i) In a female backcross (No. 69) obtained from BN(*Dd*)♀×NN(*dd*)♂, one chromosome of bivalent No. I in the oocytes was N, while the other was $\frac{N}{B}$, in which the short arm and the proximal portion of the long arm including the centromere were derived from N, and the distal portion of the long arm was from B by translocation (Figs. 5b, 6a). In another female backcross (No. 87) obtained from BN(*Cc*)♀×NN(*cc*)♂, one chromosome of bivalent No. I in the oocytes was N, while the other was $\frac{N}{B}$, in which a part of N was translocated to the short arm of B (Figs. 5c, 6b). These two female backcrosses (Nos. 69 and 87) were A^nA^n derived from *Rana nigromaculata* in genotypes for Ab and ADH-A.

ii) In a female backcross (No. 65) produced from a mating, BN(*Ee*) ♀ ×NN(*ee*) ♂, one chromosome of bivalent No. I in the oocytes was N, while the other was $\frac{B}{N}$, in which the short arm and the proximal portion of the long arm including the centromere were derived from B, and the distal portion of the long arm was from N by translocation (Figs. 5d, 6c). The genes for Ab and ADH-A were A^nA^b consisting of A^n derived from *Rana nigromaculata* and A^b derived from *Rana brevipoda*.

From the foregoing two kinds of female backcrosses, in which a translocation had occurred in bivalent No. I, it seemed evident that the genes controlling Ab and ADH-A are located very close to the centromere on the short arm of chromosome No. 1, being 5.8% and 10.6% in recombination rate, respectively, and that albino gene *b* is located at the site of about 35.6% in recombination rate from the locus for ADH-A on the short arm (Table 9; Fig. 4).

3. Albino gene *d*

a. Detection of the chromosome bearing albino gene *d*

As described earlier (Tables 3, 4), the chromosome bearing albino gene *d* of the fourth group could not be assumed to a certainty, since the highest agreement between the constitution of the genotype and that of the bivalent No. VII was found in 58 (57.4%) of 101 female backcrosses and disagreement (recombination) was observed in 43 (42.6%) ($\chi^2=2.2$, $P>0.1$). Then, linkages were examined between albino gene *d* and 17 genes controlling 13 enzymes and blood proteins on

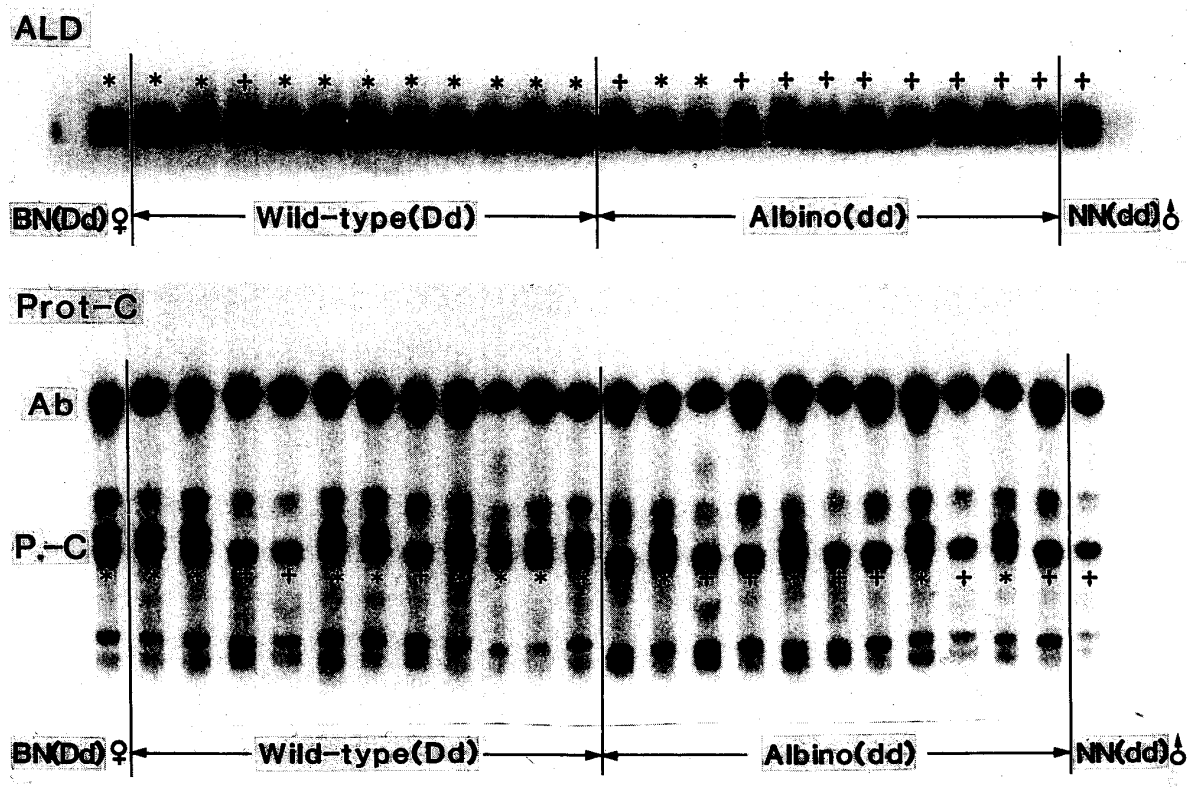


Fig. 7. Electrophoretic patterns of ALD and Protein-C in wild-type and albinic female backcrosses between *Rana nigromaculata* and *R. brevipoda*, BN(*Dd*) ♀ ×NN(*dd*) ♂.

ALD: * A^bA^n, + A^nA^n Prot-C: * C^bC^n, + C^nC^n

TABLE 10

Tests for linkage of the locus for albino gene *d* on chromosome No. 9 to 17 loci for enzymes and blood proteins

Loci and their linkage		No. of analyzed frogs	Agree (%)	Disagree	χ^2	*P*	Recombination rate (%)
Chromosome 9 —	Albino gene *d*	**101**	**52 (51.5)**	**49**	**0.09**	**0.77**	**48.5**
〃	Prot-C	**100**	**73 (73.0)**	**27**	**21.2**	**<0.00001**	**27.0**
〃	Prot-C	**547**	**414 (75.7)**	**133**	**144.4**	**<0.00001**	**24.3**
〃	ALD	**98**	**52 (53.1)**	**46**	**0.37**	**0.54**	**46.9**
〃	ALD	**547**	**302 (55.2)**	**245**	**5.94**	**<0.02**	**44.6**
Albino gene *d* —	Albumin (Ab)	100	40 (40.0)	60	—	—	
〃	ADH-A	101	40 (39.6)	61	—	—	
〃	α-GDH	101	49 (48.5)	52	0.09	0.77	
〃	Pep-C	101	49 (48.5)	52	0.09	0.77	
〃	MDH-B	101	57 (56.4)	44	1.67	0.20	
〃	ME-B	101	56 (55.4)	45	1.20	0.27	
〃	LDH-B	101	54 (53.5)	47	0.49	0.49	
〃	Pep-B	101	48 (47.5)	53	0.25	0.62	
〃	Pep-A	101	43 (42.6)	58	2.23	0.14	
〃	IDH-B	101	40 (39.6)	61	—	—	
〃	Hemoglobin (Hb)	101	43 (42.6)	58	2.23	0.14	
〃	Prot-C	**100**	**70 (70.0)**	**30**	**16.0**	**<0.0001**	**30.0**
〃	ALD	**98**	**88 (89.8)**	**10**	**62.1**	**<0.00001**	**10.2**
〃	Est-1	101	55 (54.5)	46	0.80	0.37	
〃	Est-5	93	50 (53.8)	43	0.53	0.47	
〃	Pep-D	101	54 (53.5)	47	0.49	0.49	
〃	ADA	101	52 (51.5)	49	0.09	0.77	
Protein-C —	ALD	**97**	**67 (69.1)**	**30**	**14.1**	**<0.0002**	**30.9**
〃	ALD	**537**	**357 (66.5)**	**180**	**58.3**	**<0.00001**	**33.5**

chromosomes Nos. 1~6 and 9~11 (Table 10; Fig. 7).

The results showed that albino gene *d* was linked with the gene controlling ALD in 88 (89.8%) of 98 female backcrosses and not linked in 10 (10.2%), being recombination ($\chi^2=62.1$, $P<0.00001$). It was also linked with the gene controlling Prot-C in 70 (70.0%) of 100 female backcrosses and not linked in 30 (30.0%), being recombination ($\chi^2=16.0$, $P<0.0001$). Albino gene *d* was linked with the other 15 genes located on chromosomes Nos. 1~6, 10 and 11 in 39.6~56.4% ($P>0.05$). As the genes controlling ALD and Prot-C are probably located on chromosome No. 9, albino gene *d* is also assumed to be located on this chromosome.

The linkage between the two genes controlling ALD and Prot-C was confirmed

from the observations that they were linked with each other in 67 (69.1%) of 97 female backcrosses produced from BN(*Dd*)♀×NN(*dd*)♂ and not linked in 30 (30.9%), being recombination ($\chi^2=14.1$, $P<0.0002$). They were also linked in 357 (66.5%) of 537 female backcrosses produced from NB(*Aa*)♀×BB(*aa*)♂, BN(*Bb*)♀×NN(*bb*)♂, BN(*Cc*)♀×NN(*cc*)♂, BN(*Dd*)♀×NN(*dd*)♂ and BN(*Ee*)♀×NN(*ee* or *Ee*)♂, and not linked in 180 (33.5%), being recombination ($\chi^2=58.3$, $P<0.00001$). On the other hand, the location of the gene for Prot-C on chromosome No. 9 was confirmed from comparison between the genotype and the constitution of each of the 13 bivalents in the oocytes of female backcrosses (Tables 7, 10). It was found that the genotype agreed with the constitution of bivalent No. IX in 73 (73.0%) of 100 female backcrosses produced from BN(*Dd*)♀×NN

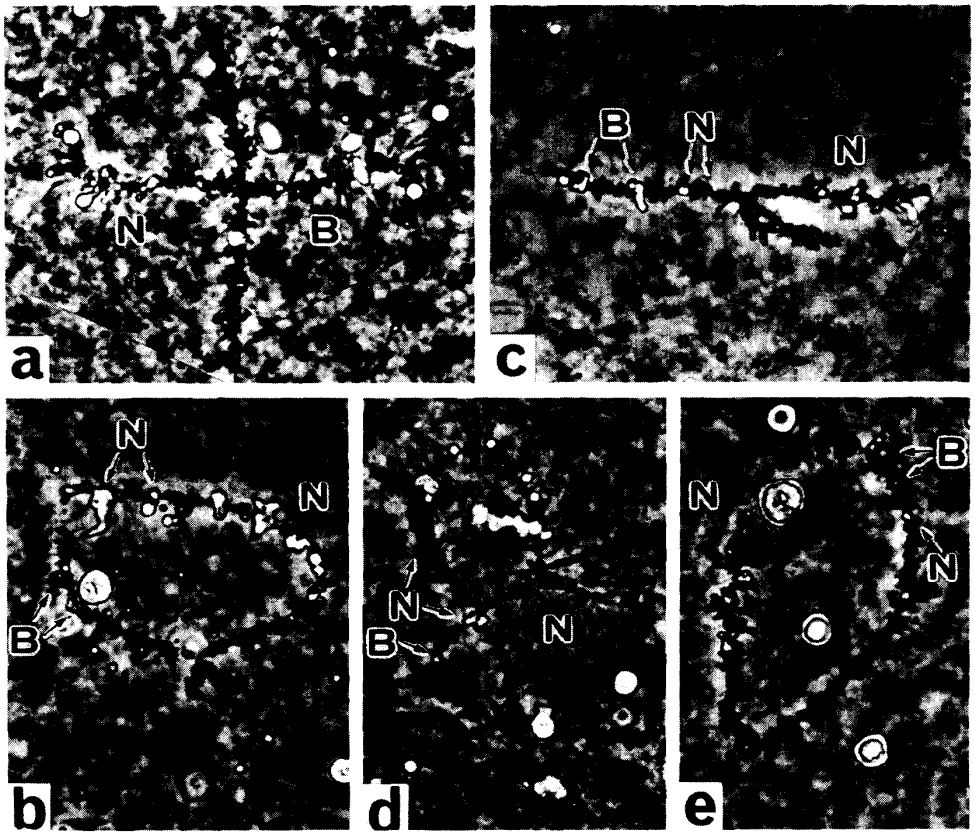


Fig. 8. Microphotographs of bivalent No. IX in oocytes of five female backcrosses between *Rana nigromaculata* and *R. brevipoda*, BN♀×NN♂. ×400

a. A pair (NB) of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome in an oocyte of female No. 66.

b. A pair $\left(\frac{N}{B}N\right)$ of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome having a translocation from a *R. nigromaculata* chromosome in an oocyte of female No. 44.

c. A pair $\left(\frac{B}{N}N\right)$ of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome having a translocation from a *R. brevipoda* chromosome in an oocyte of female No. 61.

d. A pair $\left(\frac{N}{B}N\right)$ of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome having a translocation from a *R. nigromaculata* chromosome in an oocyte of female No. 81.

e. A pair $\left(\frac{B}{N}N\right)$ of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome having a translocation from a *R. brevipoda* chromosome in an oocyte of female No. 33.

(*dd*)♂, and disagreed in 27 (27.0%), being recombination ($\chi^2=21.2$, $P<0.00001$). Similar agreements were also found in backcrosses produced from NB(*Aa*)♀×BB(*aa*)♂, BN(*Bb*)♀×NN(*bb*)♂, BN(*Cc*)♀×NN(*cc*)♂ and BN(*Ee*)♀×NN(*ee* or *Ee*)♂. Thus, it seemed evident that the gene controlling Prot-C is located on chromosome No. 9 (Tables 7, 10).

b. Assumption of the site of albino gene *d* on chromosome No. 9

The site of albino gene *d* as well as those of the genes controlling Prot-C and

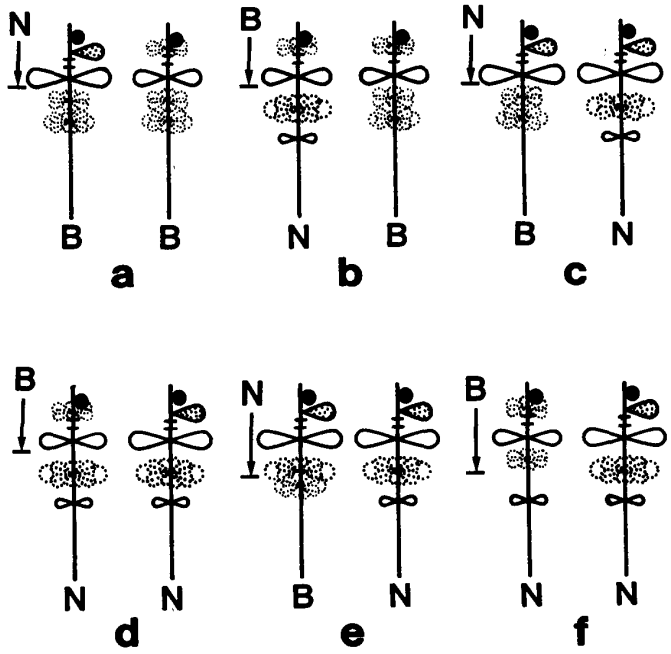


Fig. 9. Diagrams showing the constitutions of bivalent No. IX in oocytes of 18 female backcrosses between *Rana nigromaculata* and *R. brevipoda*, NB♀×BB♂ and BN♀×NN♂.

a. A pair $\left(\frac{N}{B}B\right)$ of a *Rana brevipoda* chromosome and a *R. brevipoda* chromosome having a translocation from a *R. nigromaculata* chromosome in oocytes of two females, Nos. 18 and 26.

b. A pair $\left(\frac{B}{N}B\right)$ of a *Rana brevipoda* chromosome and a *R. nigromaculata* chromosome having a translocation from a *R. brevipoda* chromosome in oocytes of three females, Nos. 34, 40 and 52.

c. A pair $\left(\frac{N}{B}N\right)$ of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome having a translocation from a *R. nigromaculata* chromosome in oocytes of five females, Nos. 32, 44, 97, 60 and 110.

d. A pair $\left(\frac{B}{N}N\right)$ of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome having a translocation from a *R. brevipoda* chromosome in oocytes of three females, Nos. 61, 93 and 42.

e. A pair $\left(\frac{N}{B}N\right)$ of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome having a translocation from a *R. nigromaculata* chromosome in oocytes of three females, Nos. 20, 81 and 28.

f. A pair $\left(\frac{B}{N}N\right)$ of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome having a translocation from a *R. brevipoda* chromosome in oocytes of two females, Nos. 24 and 33.

ALD was assumed by using translocations found in bivalent No. IX in the oocytes of 18 female backcrosses. These backcrosses were divided into four types on the basis of the constitution of this bivalent.

i) Constitution of bivalent No. IX

$\frac{N}{B}$ B-type. In two female backcrosses (Nos. 18 and 26) produced from NB(*Aa*) ♀ × BB(*aa*) ♂, one chromosome of bivalent No. IX was B, and the other was $\frac{N}{B}$, in which the short arm and the proximal portion of the long arm including

TABLE 11

Phenotypes and genotypes for albino gene *d*, Protein-C and ALD in 18 backcrosses, whose chromosome No. 9 has a translocation

Backcrossing	Individual no.	Constitution of bivalent chromosome	Albino gene *d*		Protein-C		ALD	
			Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype
NB(*Aa*) ♀ × BB(*aa*) ♂	18	$\frac{N}{B}$ B	—	—	NB	C^nC^b	NB	A^nA^b
	26	$\frac{N}{B}$ B	—	—	BB	C^bC^b	BB	A^bA^b
	34	$\frac{B}{N}$ B	—	—	NB	C^nC^b	NB	A^nA^b
	40	$\frac{B}{N}$ B	—	—	NB	C^nC^b	NB	A^nA^b
	52	$\frac{B}{N}$ B	—	—	NB	C^nC^b	NB	A^nA^b
BN(*Bb*) ♀ × NN(*bb*) ♂	32	$\frac{N}{B}$ N	—	—	BN	C^bC^n	NN	A^nA^n
	44	$\frac{N}{B}$ N	—	—	BN	C^bC^n	BN	A^bA^n
BN(*Cc*) ♀ × NN(*cc*) ♂	20	$\frac{N}{B}$ N	—	—	NN	C^nC^n	NN	A^nA^n
	61	$\frac{B}{N}$ N	—	—	NN	C^nC^n	BN	A^bA^n
	81	$\frac{N}{B}$ N	—	—	BN	C^bC^n	BN	A^bA^n
	97	$\frac{N}{B}$ N	—	—	BN	C^bC^n	BN	A^bA^n
BN(*Dd*) ♀ × NN(*dd*) ♂	93	$\frac{B}{N}$ N	Albino (NN)	*dd*	NN	C^nC^n	NN	A^nA^n
BN(*Ee*) ♀ × NN(*ee*) ♂	24	$\frac{B}{N}$ N	—	—	NN	C^nC^n	BN	A^bA^n
	28	$\frac{N}{B}$ N	—	—	BN	C^bC^n	BN	A^bA^n
	33	$\frac{B}{N}$ N	—	—	BN	C^bC^n	NN	A^nA^n
	42	$\frac{B}{N}$ N	—	—	NN	C^nC^n	NN	A^nA^n
	60	$\frac{N}{B}$ N	—	—	BN	C^bC^n	BN	A^bA^n
	110	$\frac{N}{B}$ N	—	—	BN	C^bC^n	NN	A^nA^n

the centromere were derived from N, while the distal portion of the long arm was derived from B by translocation (Table 11; Fig. 9a).

$\frac{B}{N}$ B-type. In three female backcrosses (Nos. 34, 40 and 52) produced from NB(*Aa*)♀×BB(*aa*)♂, one chromosome was B, and the other was $\frac{B}{N}$, in which the short arm and the proximal portion of the long arm including the centromere were derived from B, while the distal portion of the long arm was derived from N by translocation (Table 11; Fig. 9b).

$\frac{N}{B}$ N-type. In a total of eight female backcrosses, including two (Nos. 32 and 44) produced from BN(*Bb*)♀×NN(*bb*)♂, three (Nos. 20, 81 and 97) from BN(*Cc*)♀×NN(*cc*)♂ and three (Nos. 28, 60 and 110) from BN(*Ee*)♀×NN(*ee*)♂, one chromosome was N, and the other was $\frac{N}{B}$, in which the short arm and the proximal portion of the long arm including the centromere were derived from N, while the distal portion of the long arm was derived from B by translocation (Table 11; Figs. 8b, 8d, 9c, 9e).

$\frac{B}{N}$ N-type. In a total of five female backcrosses, including one (No. 61) produced from BN(*Cc*)♀×NN(*cc*)♂, one albino (No. 93) from BN(*Dd*)♀× NN(*dd*)♂ and three (Nos. 24, 33 and 42) from BN(*Ee*)♀×NN(*ee*)♂, one chromosome was N, while the other was $\frac{B}{N}$, in which the short arm and the proximal portion of the long arm including the centromere were derived from B, and the distal portion of the long arm was from N by translocation (Table 11; Figs. 8c, 8e, 9d, 9f).

ii) Genotype (electrophoretic pattern)

The genotypes for Prot–C and ALD in a female backcross No. 26 belonging to the $\frac{N}{B}$B-type were C^bC^b and A^bA^b derived from *Rana brevipoda,* respectively, while those in a female backcross No. 18 belonging to the $\frac{N}{B}$B-type were C^nC^b and A^nA^b, respectively. The genotypes for Prot–C and ALD in three female backcrosses (Nos. 34, 40 and 52) belonging to the $\frac{B}{N}$B-type were C^nC^b and A^nA^b, respectively. In five (Nos. 44, 81, 97, 28 and 60) of eight female backcrosses belonging to the $\frac{N}{B}$N-type, the genotypes for Prot–C and ALD were C^bC^n and A^bA^n, respectively, while the other three (Nos. 32, 20 and 110) were not the same as these five female backcrosses in genotype. In two (Nos. 32 and 110) of them, the genotypes for Prot–C and ALD were C^bC^n and A^nA^n, respectively. In contrast, they were C^nC^n and A^nA^n in the remainder (No. 20), respectively. The genotypes for Prot–C and ALD in two (Nos. 93 and 42) of five female backcrosses belonging to the $\frac{B}{N}$N-type were C^nC^n and A^nA^n derived from *Rana nigromaculata,* respectively, while those in two (Nos. 61 and 24) of the other three were C^nC^n *and* A^bA^n, respectively. In the remaining one (No. 33), the genotypes for Prot–C and ALD were C^bC^n and A^nA^n, respectively (Table 11).

iii) Comparison in constitution between bivalent No. IX and the genotype

It was found that the genotypes for Prot–C and ALD agreed in constitution with the distal portion of the long arm of bivalent No. IX in 11 of the 18 female

backcrosses, including Nos. 26, 34, 40 and 52 produced from NB(*Aa*) ♀ ×BB(*aa*) ♂, No. 44 from BN(*Bb*) ♀ ×NN(*bb*) ♂, Nos. 81 and 97 from BN(*Cc*) ♀ ×NN(*cc*) ♂, albino No. 93 from BN(*Dd*) ♀ ×NN(*dd*) ♂ and Nos. 28, 42 and 60 from BN(*Ee*) ♀ ×NN(*ee*) ♂. These findings seem to indicate that the genes controlling Prot-C and ALD together with albino gene *d* are located on the distal portion of the long arm of chromosome No. 9.

However, it was noteworthy that there were seven female backcrosses, in which the genotypes did not agree in constitution with the distal portion of the long arm of bivalent No. IX. These female backcrosses included No. 18 produced from NB (*Aa*) ♀ ×BB(*aa*) ♂, No. 32 from BN(*Bb*) ♀ ×NN(*bb*) ♂, Nos. 20 and 61 from BN (*Cc*) ♀ ×NN(*cc*) ♂ and Nos. 24, 33 and 110 from BN(*Ee*) ♀ ×NN(*ee*) ♂. In four (Nos. 32, 61, 24 and 110) of these seven female backcrosses, the genotype for Prot-C agreed in constitution with the translocated part of bivalent No. IX, while the genotype for ALD did not agree with the latter. In two (Nos. 18 and 20) of the other three female backcrosses, the genotypes for both Prot-C and ALD did not agree in constitution with the translocated portion of the long arm of bivalent No. IX. In the remainder (No. 33), the genotype for ALD agreed in constitution with the translocated portion, while that for Prot-C did not agree with the latter. These findings seem to show that a short or long distal part of the translocated portion was double translocated and that the gene of ALD is more distantly located than that of Prot-C on chromosome No. 9. They also seem to correspond to the findings that the genotype for Prot-C agreed in constitution with bivalent No. IX in 75.7% ($\chi^2=144.4$, $P<0.00001$) of the female backcrosses, while the genotype for ALD agreed in 55.2% ($\chi^2=5.94$, $P<0.02$), as shown in Tables 7 and 10.

4. Albino gene *e*

a. Linkage group including albino gene *e*

It is assumed that albino gene *e* is located on chromosome No. 3 at the site of 1.8% in recombination rate from the centromere, and that the genes controlling MDH-B and ME-B are located on chromosome No. 3 at the sites of about 14.9% and 8.5% in recombination rate from the centromere, respectively (Table 5). Then, the linkage between these two genes for the enzymes and albino gene *e* on chromosome No. 3 was examined together with the relationships between albino gene *e* and 16 genes controlling 11 kinds of enzymes and blood proteins located on chromosomes Nos. 1, 2, 4~6 and 9~11 (Table 12).

Albino gene *e* was linked with the gene of MDH-B in 85 (77.3%) of 110 female backcrosses and not linked in 25 (22.7%), being recombination ($\chi^2=32.7$, $P<0.00001$). It was linked with the gene of ME-B in 105 (97.2%) of 108 female backcrosses and not linked in three (2.8%), being recombination ($\chi^2=96.3$, $P<0.00001$) (Fig. 10). Albino gene *e* was not linked with any of the 16 genes controlling 11 blood proteins and enzymes on the other chromosomes, Nos. 1, 2, 4~6 and 9~11 ($P>0.05$).

The gene of MDH-B was linked with that of ME-B in 363 (81.6%) of 445

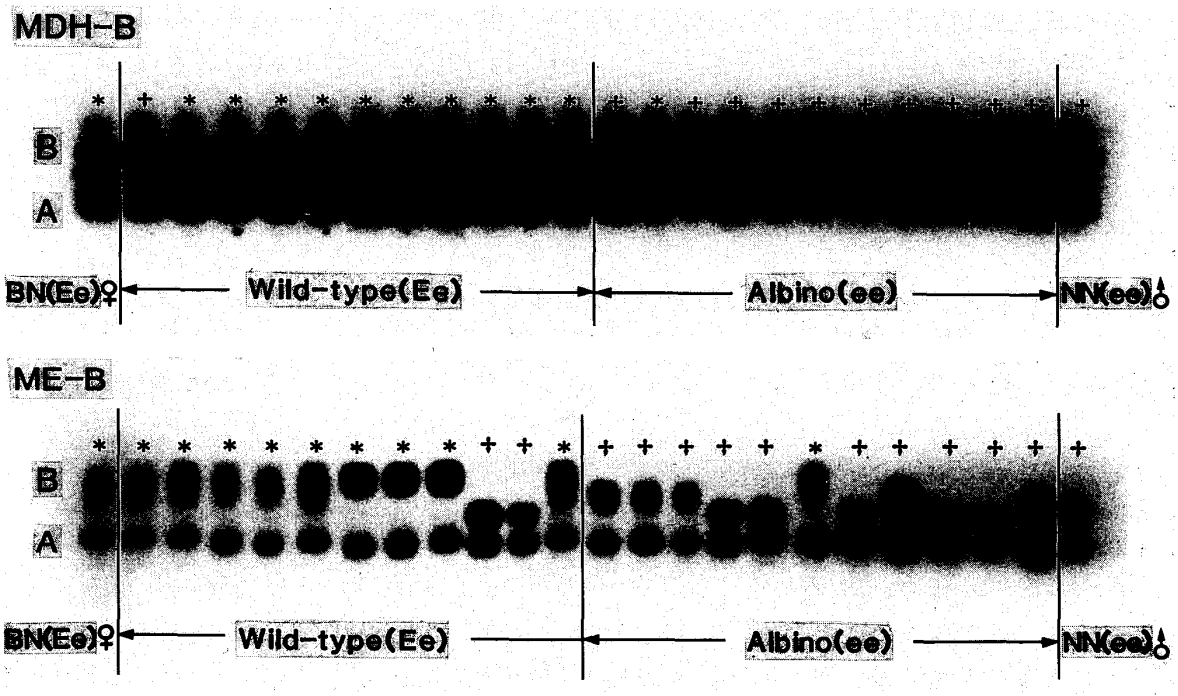


Fig. 10. Electrophoretic patterns of MDH-B and ME-B in wild-type and albinic female backcrosses between *Rana nigromaculata* and *R. brevipoda*, BN(*Ee*) ♀×NN(*ee*) ♂.
MDH-B: * B^bB^n, + B^nB^n ME-B: * B^bB^n, + B^nB^n

female backcrosses and not linked in 82 (18.4%), being recombination (χ^2= 177.4, $P<0.00001$) (Table 12).

Olive mutant gene *i* was linked with the genes controlling MDH-B or ME-B in 87 (87.0%) or 88 (88.0%) of 100 female backcrosses and not linked in 13 (13.0%) or 12 (12.0%), being recombination (χ^2=54.8, $P<0.00001$ or χ^2=57.76, $P<0.00001$), as described by NISHIOKA and OHTANI (1986).

b. Assumption of the site of albino gene *e* on chromosome No. 3

A translocation was found on bivalent No. III in the oocytes of four (Nos. 40, 62, 83 and 112) of 123 female backcrosses obtained from a mating, BN(*Ee*) ♀×NN(*ee*) ♂ (Table 13). In two (Nos. 40 and 83) of them which were both albinos (*ee*) of the TY-type, one chromosome of bivalent No. III was N derived from *Rana nigromaculata*, while the other was $\frac{B}{N}$ in which a portion of B derived from *Rana brevipoda* was translocated to the long arm of N (Tables 4, 13; Figs. 11b, 12a). In these two albinic females, the genotype for ME-B was B^nB^n derived from *Rana nigromaculata*, while the genotype for MDH-B was B^bB^n derived from the two species.

In the remaining two female backcrosses (Nos. 62 and 112) which were of the wild-type (*Ee*), one chromosome of bivalent No. III was N, while the other was $\frac{N}{B}$ in which a segment of N derived from *Rana nigromaculata* was translocated to the long arm of B derived from *Rana brevipoda* (Tables 4, 13; Figs. 11c, 12b). Thus, the genotype for albinism in these two wild-type females showed heterozygous *Ee* consisting of *E* derived from *Rana brevipoda* and *e* derived from *Rana nigromaculata*.

TABLE 12
Tests for linkage of the locus for albino gene *e* on chromosome No. 3 to 18 loci for enzymes and blood proteins

Loci and their linkage	No. of analyzed frogs	Agree (%)	Disagree	χ^2	*P*	Recombination rate (%)
Chromosome 3 — Albino gene *e*	**112**	**110 (98.2)**	**2**	**104.1**	**<0.00001**	**1.8**
〃 ME-B	**445**	**407 (91.5)**	**38**	**306.0**	**<0.00001**	**8.5**
〃 MDH-B	**551**	**469 (85.1)**	**82**	**271.8**	**<0.00001**	**14.9**
Albino gene *e* — Albumin (Ab)	110	56 (50.9)	54	0.04	0.85	
〃 ADH-A	110	52 (47.3)	58	0.33	0.57	
〃 α-GDH	110	55 (50.0)	55	0.00	1.00	
〃 Pep-C	103	50 (48.5)	53	0.09	0.77	
〃 ME-B	**108**	**105 (97.2)**	**3**	**96.3**	**<0.00001**	**2.8**
〃 MDH-B	**110**	**85 (77.3)**	**25**	**32.7**	**<0.00001**	**22.7**
〃 LDH-B	110	47 (42.7)	63	2.33	0.13	
〃 Pep-B	110	45 (40.9)	65	3.64	0.06	
〃 Pep-A	110	54 (49.1)	56	0.04	0.85	
〃 Hemoglobin (Hb)	110	47 (42.7)	63	2.33	0.13	
〃 IDH-B	110	40 (36.4)	70	—	—	
〃 Prot-C	110	56 (50.9)	54	0.04	0.85	
〃 ALD	110	51 (46.4)	59	0.58	0.45	
〃 Est-1	110	49 (44.5)	61	1.31	0.25	
〃 Est-2	110	49 (44.5)	61	1.31	0.25	
〃 Est-5	110	49 (44.5)	61	1.31	0.25	
〃 Pep-D	110	40 (36.4)	70	—	—	
〃 ADA	110	49 (44.5)	61	1.31	0.25	
MDH-B — ME-B	**445**	**363 (81.6)**	**82**	**177.4**	**<0.00001**	**18.4**

The genotype for ME-B was also B^bB^n, consisting of B^b derived from *Rana brevipoda* and B^n derived from *Rana nigromaculata*. In contrast, the genotype for MDH-B was B^nB^n derived from *Rana nigromaculata* (Tables 4, 13).

The foregoing findings seem to show that albino gene *e* is located on the long arm of chromosome No. 3 at the site of about 1.8% in recombination rate from the centromere, as shown in Fig. 4. The gene controlling ME-B is also assumed to be located on the long arm at the site of about 8.5% in recombination rate from the centromere, while the gene controlling MDH-B is assumed to be located on the short arm at the site of about 14.9%. The distances of albino gene *e* from the genes of MDH-B and ME-B are assumed to be about 22.7% and 2.8% in recombination rate, respectively. The distance between the genes controlling ME-B and MDH-B was assumed to be about 18.4% in recombination rate (Table 12).

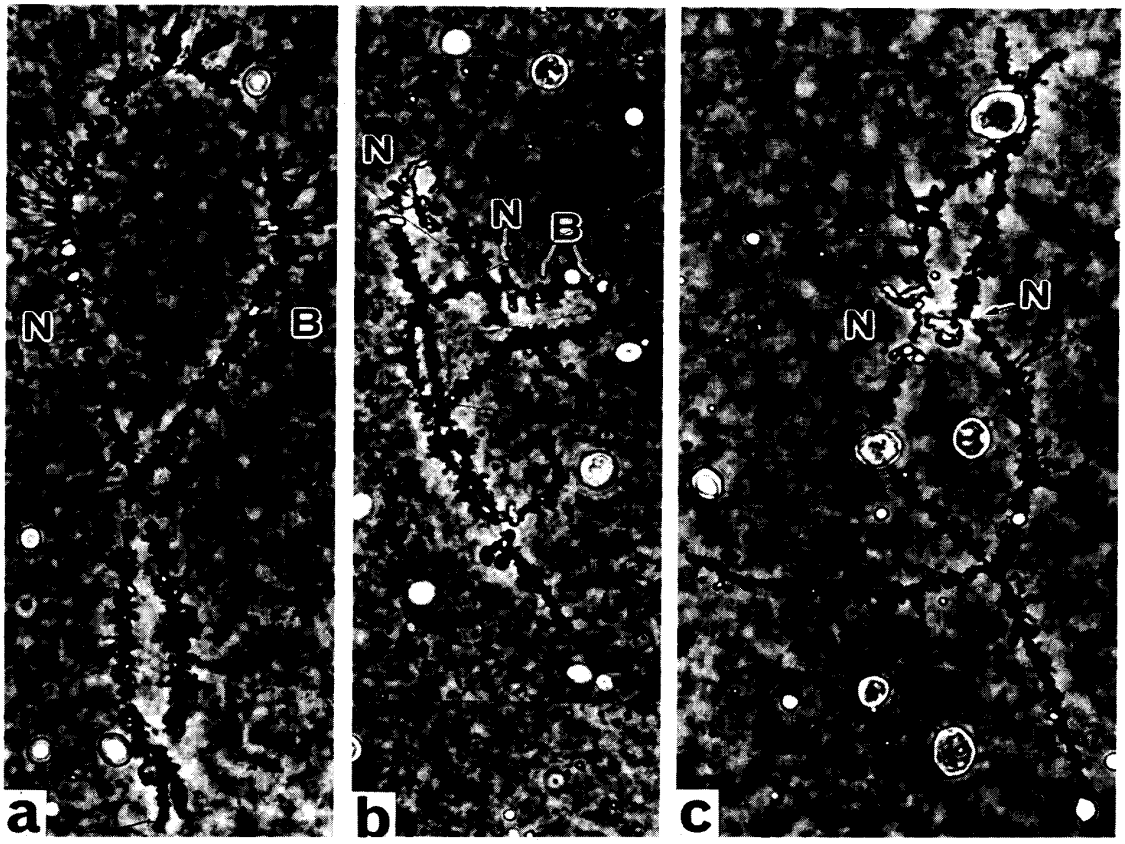


Fig. 11. Microphotographs of bivalent No. III in oocytes of three female backcrosses between *Rana nigromaculata* and *R. brevipoda*, BN(*Ee*) ♀×NN(*ee*) ♂. ×400

a. A pair (NB) of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome in an oocyte of female No. 52.

b. A pair $\left(\frac{B}{N}N\right)$ of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome having a translocation from a *R. brevipoda* chromosome in an oocyte of female No. 40.

c. A pair $\left(\frac{N}{B}N\right)$ of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome having a translocation from a *R. nigromaculata* chromosome in an oocyte of female No. 112.

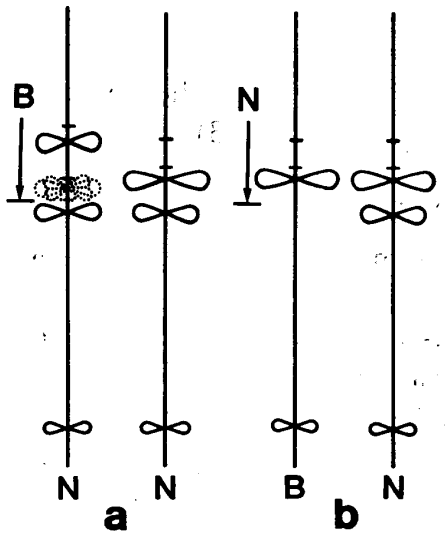


Fig. 12. Diagrams showing the constitutions of bivalent No. III in oocytes of four female backcrosses between *Rana nigromaculata* and *R. brevipoda*, BN(*Ee*) ♀×NN(*ee*) ♂.

a. A pair $\left(\frac{B}{N}N\right)$ of a *Rana nigromaculata* chromosome and a *R. nigromaculata* chromosome having a translocation from a *R. brevipoda* chromosome in oocytes of females, Nos. 40 and 83.

b. A pair $\left(\frac{N}{B}N\right)$ of a *Rana nigromaculata* chromosome and a *R. brevipoda* chromosome having a translocation from a *R. nigromaculata* chromosome in oocytes of females, Nos. 62 and 112.

TABLE 13

Phenotypes and genotypes for albino gene *e*, MDH-B and ME-B in four backcrosses, whose chromosome No. 3 has a translocation

Backcrossing	Individual no.	Constitution of bivalent chromosome	Albino gene *e*		MDH-B		ME-B	
			Phenotype	Genotype	Phenotype	Genotype	Phenotype	Genotype
BN (*Ee*) ♀ × NN (*ee*) ♂	40	$\frac{B}{N}$ N	Albino (NN)	*ee*	BN	B^bB^n	NN	B^nB^n
	62	$\frac{N}{B}$ N	Wild (BN)	*Ee*	NN	B^nB^n	BN	B^bB^n
	83	$\frac{B}{N}$ N	Albino (NN)	*ee*	BN	B^bB^n	NN	B^nB^n
	112	$\frac{N}{B}$ N	Wild (BN)	*Ee*	NN	B^nB^n	BN	B^bB^n

DISCUSSION

It has been well-known in many vertebrates that albinism is due to a recessive gene in the homozygous condition. In amphibians, this has been reported in several species; in *Rana temporaria* by EALES (1933) and SMALLCOMBE (1949), in parthenogenetically developed *Rana nigromaculata* by TOKUNAGA (1949), in *Rana pipiens* by BROWDER (1968, 1972) and SMITH-GILL, RICHARDS and NACE (1970, 1972), in *Hyla arborea japonica* by DAITO (1968), in *Xenopus laevis* by HOPERSKAYA (1975) and BLUEMINK and HOPERSKAYA (1975) and in the axolotl by HUMPHREY (1967) and BENJAMIN (1970).

NISHIOKA and UEDA (1977) have reported that ten stocks of albinic *Hyla arborea japonica* collected from an area of about 6000 square kilometers surrounding Hiroshima, Japan, can be divided into three groups on the basis of the results of hybridization experiments. Each group has an independent locus for albinism in an autosome. The hybrids between albinos of different groups are of the wild-type in color and pattern. The albinos of the first, second and third groups are due to the presence of recessive genes, *f*, *s* and *t*, in the homozygous condition, respectively. The albinos of these three groups as well as of three stocks of the third group distinctly differ from one another in the number, size, shape or minute structure of premelanosomes contained in dermal melanophores and pigment cells of the eye. In the first group, the albinos of one stock remarkably differ from those of the other stocks in scarcity and smallness of premelanosomes. On the other hand, a dominant gene, black-eyed (*B*) or colored (*C*), is linked with gene *f* in the albinos of two stocks of the first group.

NISHIOKA and UEDA (1985) have also found in *Rana nigromaculata* group that 13 albino stocks collected from the field or obtained by irradiation of gametes are divided into five groups, each of which has a different locus, *a*, *b*, *c*, *d* or *e*. The hybrids between albinos of different groups are of the wild-type in color and pattern. The first group including eight albino stocks is genetically divided into four strains a^r, a^h, a^t and a^f. The first strain consists of three radiation-induced

stocks (NISHIOKA, 1977), while each of the second, third and fourth strains consists of three, one and one albino stock collected from the field, respectively. Albino genes a^t and a^r, a^h or a^f of the first group are codominant alleles. While each of the second, third and fourth groups consists of one albino stock, the fifth group includes two albino stocks. The albinos of different groups or strains differ from one another in development, size, number and structure of premelanosomes contained in dermal melanophores.

In the amphibians, it has become possible for the first time to detect the chromosomes bearing albino genes by utilizing lampbrush chromosomes in oocytes. NISHIOKA, OHTANI and SUMIDA (1980) detected chromosomes bearing the loci for seven kinds of enzymes and blood proteins, LDH-B, MDH-B, α-GDH, IDH-B, hemoglobin, albumin and serum protein-C, by comparing the constitutions of the genotypes at the loci for these components with those of bivalents in oocytes of female backcrosses between female reciprocal hybrids of *Rana nigromaculata* and *Rana brevipoda* and males of these parental species. According to them, the loci for these seven kinds of enzymes and blood proteins were assumed to be located on chromosomes Nos. 4, 3, 2, 6, 6, 1 and 9, respectively. High percentages of female backcrosses whose genotypes of LDH, hemoglobin and albumin agreed in constitution with definite bivalents seemed to show that the loci for these components are situated near the centromeres.

The chromosomes bearing the loci for blue and olive mutations were detected by NISHIOKA and OHTANI (1986) in *Rana nigromaculata* by comparing the constitution of the loci for the color mutations with that of the bivalents in the oocytes of female offspring produced from heterozygous female hybrids between a wild-type female *Rana brevipoda* and a male *Rana nigromaculata* homozygous for blue and olive genes by backcrossing with the foregoing male *Rana nigromaculata* or by diploid gynogenesis. According to them, the location of the olive mutant gene on chromosome No. 3 is evident, while the location of the blue mutant gene on chromosome No. 8 is very probable.

Although the site of a locus on a chromosome can be roughly surmised on the basis of recombination, it becomes more evident by examining the linkage group. Linkage of two mutant genes in amphibians has been detected for the first time by HUMPHREY (1959) in the axolotl. The fluid-imbalance syndrome in the axolotl embryo has been demonstrated to be determined by two mutant genes occupying loci close together in the same chromosome. This syndrome was first interpreted by himself (1948) as a lethal character inherited as a simple recessive. VOLPE (1970) and NACE, RICHARDS and ASHER (1970) have utilized the method of diploid gynogenesis for detecting linkage relationship and mapped the distance between pigmentation genes kandiyohi (*K*) and burnsi (*B*) and the centromere in *Rana pipiens*. BROWDER (1972) has postulated the existence of a dominant subvital gene linked to the burnsi (*B*) locus. WRIGHT, RICHARDS and NACE (1980) have established three linkage groups from among 72 loci for enzymes and blood proteins analyzed electrophoretically in *Rana pipiens*. These linkage groups were elucidated by testing linkage or independent assortment for 75 locus pairs in the

offspring of heterozygous parents. According to these authors, linkage groups 1, 2 and 3 include two, four and four loci, respectively. Thereafter, WRIGHT and RICHARDS (1982) have added two linkage groups to the foregoing three. Each of these two linkage groups includes two loci, Pep-C and SOD-1, or Pep-B and MPI. They (1983) also have demonstrated that the male is the heterogametic sex in *Rana pipiens* and the sex-determining region is linked to the loci for Pep-C and SOD-1. ELINSON (1983) has elucidated that the male is heterogametic in *Rana clamitans* and the gene for aconitase-1 is sex-linked. NISHIOKA and OHTANI (1986) have demonstrated that the olive mutant gene constitutes a linkage group with genes for MDH-B and ME-B in *Rana nigromaculata*. The olive mutant gene has been assumed to be situated near the centromere on the short arm of chromosome No. 3, while the two genes for MDH-B and ME-B are situated on the short and long arms, respectively.

In the present study, the chromosomes bearing the loci for five groups of albino genes as well as the position of each locus on the respective chromosome were detected. The detection of the chromosomes bearing these albino genes was made by comparison between the constitution of the genotype (homozygous or heterozygous) at each albino locus and that of each of the 13 bivalents in the oocytes of female backcrosses produced from heterozygous female hybrids between *Rana nigromaculata* and *Rana brevipoda* by mating with albinic males of one of these species. When the genotype agreed in constitution with a definite bivalent in all or most individuals of the female backcrosses, this bivalent was considered to bear the locus for the albino gene. The results of these comparisons showed that albino genes *a, b, c* and *e* are definitely or probably located on chromosomes Nos. 2, 1, 2 and 3, respectively. Only the chromosome bearing albino gene *d* was not identified by such a comparison, as the highest agreement was 57.4% of 101 female backcrosses which was found between the constitution of the genotype and that of bivalent No. VII. This percentage rather seemed to indicate that albino gene *d* is located on the end portion of one of the 13 chromosomes.

On the other hand, it was assumed that albino gene *d* is located on chromosome No. 9, as this gene is evidently linked with two genes located on this chromosome. The genes for Prot-C and ALD were linked with albino gene *d* in 70.0% and 89.8% of female backcrosses, respectively. As the constitution of the genotype for Prot-C agreed with that of bivalent No. IX in 75.7% of the female backcrosses examined, the locus for Prot-C is assumed to be located on chromosome No. 9, as NISHIOKA, OHTANI and SUMIDA (1980) have previously reported. Although the constitution of the genotype for ALD which agreed with that of bivalent No. IX is only 55.2% of the female backcrosses examined, the gene for ALD was assumed to be located on chromosome No. 9, as this gene was linked with that for Prot-C in 66.5% of the female backcrosses examined. Thus, albino gene *d* was assumed to be linked with the loci for Prot-C and ALD on chromosome No. 9. By comparing the constitution of bivalent No. IX having a translocation with those of the genotypes controlling Prot-C and ALD, it was assumed that these loci are located together with albino gene *d* on the distal portion of the long arm of

chromosome No. 9.

Albino gene *a* is assumed to be located at the site of about 20.4% in recombination rate from the centromere on chromosome No. 2. Although albino gene *a* is linked with albino gene *c* and four genes for Pep-C, SOD-B, ME-A and *α*-GDH on chromosome No. 2, albino gene *a* seems to be closely linked with the genes for ME-A and *α*-GDH on the same arm, as these two genes were situated at the sites of about 0% and 4.0% from albino gene *a*, respectively, and also at a distance of 8.3% in recombination rate from each other (Table 8). On the other hand, albino gene *c* is assumed to be located at the site of about 15.7% in recombination rate from the centromere on chromosome No. 2 and to be linked with gene for Pep-C at a distance of about 15.7%. The gene for Pep-C is more closely linked with that for SOD-B than those for *α*-GDH and ME-A (Table 8). Thus, it is believed that albino gene *a* is located together with the genes for ME-A and *α*-GDH on an arm of chromosome No. 2, while albino gene *c* is located together with the genes for Pep-C and SOD-B on the other arm of the same chromosome.

Albino gene *b* is assumed to be located at the site of about 38.5% in recombination rate from the centromere on chromosome No. 1. This gene is linked with the loci for Ab and ADH-A at distances of about 38.5% and 35.6% in recombination rate, respectively, while these loci are located at a distance of 8.1% from each other. The genotypes for Ab and ADH-A in backcrosses which have a translocation in the short arm of chromosome No. 1 showed that the loci for Ab and ADH-A are located on the short arm. Thus, albino gene *b* is assumed also to be located at the distal portion on the short arm of chromosome No. 1, while the loci for Ab and ADH-A are believed to be located at the sites of about 6.2% and 12.8% in recombination rate from the centromere, respectively.

Albino gene *e* is assumed to be located on chromosome No. 3. NISHIOKA and OHTANI (1986) have reported that olive mutant gene is located on the short arm of this chromosome near the centromere and is linked with genes for MDH-B and ME-B. The gene for MDH-B was situated on the short arm at the site of 13% in recombination rate from the centromere, while the gene for ME-B was situated on the long arm at the site of 12%. In the present study, the situation of the genes for MDH-B and ME-B was reexamined on the basis of more numerous female backcrosses. The results showed that the gene for ME-B is located on the long arm at the site of about 8.5% in recombination rate, while the gene for MDH-B is located on the short arm at the site of about 14.9%. Albino gene *e* is linked with genes for MDH-B and ME-B at distances of about 22.7% and 2.8% in recombination rate, respectively. By observing the genotypes for MDH-B and ME-B as well as the existence of albinism in four female backcrosses which have a translocation in bivalent No. III, it was found that albino gene *e* is probably located on the long arm of chromosome No. 3 near the centromere.

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