Sci. Rep. Lab. Amphibian Biol., Hiroshima Univ. 13: 99–136 (1994)

The Differences in Recombination Rate between the Male and Female in *Rana nigromaculata* and *Rana brevipoda*

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ABSTRACT

The linkages among six enzyme loci, SORDH, MPI, ENO, HK, Pep-B and LDH-B, situated on chromosome No. 4 were analyzed in order to examine the differences in recombination rates between males and females. Mating experiments were conducted by using heterozygous females or males of *Rana brevipoda* and *Rana nigromaculata*, heterozygous females of interspecific hybrids between *R. nigromaculata* and *R. brevipoda* and those between *Rana plancyi chosenica* and *R. nigromaculata*.

The recombination rates among the loci controlling six enzymes located on chromosome No. 4 were found to be large in females when the frogs were pure species, while they became smaller when the frogs were hybrids. The recombination rates were almost zero in males in contrast to females. They had no relation to the existence of the sex-determining genes on the chromosomes of the species used in the present study. The differences in the recombination rates seemed to be attributable to that in the number of chiasmata. When the number of chiasmata became smaller, the recombination rates also became smaller, and in male frogs they seemed to approach zero. Thus, the present study showed that it may be better to use heterozygous males for the purpose of assuming the kinds of genes on specific chromosome. However, it may be better to use heterozygous females in order to clarify the mutual positions of loci on the same chromosome.

INTRODUCTION

Rana nigromaculata, *R. brevipoda* and *R. plancyi chosenica* are 2n=26 in chromosome number, having five pairs of large chromosomes and eight pairs of small chromosomes (NISHIOKA, 1972; NISHIOKA, OKUMOTO and RYUZAKI, 1987). MIURA (1987) has reported that there are no sexual differences in C- and late replication (LR)-bands among these three species. The females of reciprocal hybrids among them were fertile and most of the offspring produced by backcrossing of these female hybrids were diploids, while a small number of them were triploids. On the other hand, the male hybrids were almost sterile (KAWAMURA and NISHIOKA, 1977, 1978; NISHIOKA, 1983). NISHIOKA, OHTANI and SUMIDA (1980, 1987) and NISHIOKA and OHTANI (1986) were able to clarify the situations of 32 loci on

chromosomes including 25 loci controlling various enzymes and blood proteins and seven loci controlling various color mutations.

According to NISHIOKA and SUMIDA (1994), the sex chromosomes could not be identified in the Konko population of *R. brevipoda*, the Hiro and Kumano populations and five of nine male frogs of the Kaita population of *R. nigromaculata*, although these populations were male heterogametic (XX–XY type) and the LDH–B, HK, Pep–B, ENO, MPI and SORDH loci linked with the sex-determining genes on chromosome No. 4. In contrast, the sex-determining genes did not link with these six loci situated on chromosome No. 4 in the Maibara population of *R. brevipoda*, but linked with the ME-B locus situated on chromosome No. 3. In four of the nine frogs of the Kaita population of *R. nigromaculata*, the sex-determining genes did not link with any locus situated on chromosomes Nos. 3 and 4.

OKUMOTO (1980) observed the behaviors of homologous chromosomes at the diakinesis or metaphase of the first reduction divisions of spermatocytes in *R. nigromaculata* collected from the neighboring areas of Hiroshima city, *R. brevipoda* from Konko-cho, Okayama Prefecture, and reciprocal hybrids between these two species. It was found that meiotic spreads obtained from male frogs formed bivalents which were ring-shaped by conjugation of two homologous chromosomes at both ends, while the other bivalents were rod-shaped by conjugation of two homologous chromosomes at one end. While ring-shaped bivalents were far more numerous than rod-shaped ones in *R. nigromaculata* and *R. brevipoda*, rod-shaped bivalents were far more numerous than ring-shaped ones in the hybrids between them. Thus, it was quite clear that very few chiasmata were formed in the meioses of males of *R. nigromaculata*, *R. brevipoda* and reciprocal hybrids between these species.

OHTANI (1990) reported that the average number of the chiasmata found in bivalent (lampbrush) chromosome No. 4 of 50 oocytes were 4.36 in female *R. nigromaculata* from the suburbs of Hiroshima city, 4.14 in female *R. brevipoda* from Konko-cho and 4.64 in female *R. plancyi chosenica* from Korea. They were also 2.96 in female hybrids between female *R. nigromaculata* and male *R. brevipoda*, and 3.20 in female hybrids between female *R. plancyi chosenica* and male *R. nigromaculata*.

In the present study, the linkages among the six loci of SORDH, MPI, ENO, HK, Pep–B and LDH–B enzymes, situated on chromosome No. 4, were analyzed to examine the differences in recombination rates between males and females by using the Hiro, Kumano and Kaita populations of *R. nigromaculata*, the Konko and Maibara populations of *R. brevipoda*, the hybrids among the foregoing populations, the hybrids between female *R. plancyi chosenica* and male *R. nigromaculata* of the Hiro population and various backcrosses.

MATERIALS AND METHODS

The following frogs were used in the present study.

1. *Rana brevipoda* Ito

(1) Seven females and five males of the Konko population belonging to the Typical race (BB.Ko♀, Nos. 4~10 and BB.Ko♂, Nos. 7~11) and two females and five males of the Maibara population belonging to the Nagoya race (BB.Ma♀, Nos. 2 and 3, and BB.Ma♂, Nos. 1~4 and 9). (2) Three female and six male hybrids between females of the Maibara population belonging to the Nagoya race and a male of the Konko population belonging to the Typical race (BB.MK♀, Nos. 1~3 and BB.MK♂, Nos. 1~6) and three female and four male hybrids between a female of the Konko population belonging to the Typical race and a male of the Maibara population belonging to the Nagoya race (BB.KM♀, Nos. 1~3 and BB.KM♂, Nos. 1~4).

2. *Rana nigromaculata* Hallowell

(1) A female of the Ushita population (NN.Us♀, No. 1), a female of the Aomori population (NN.Ao♀, No. 1), 15 females of the Kaita population (NN.Ka♀, Nos. 1~4, 6~16), six males of the Kaita population (NN.Ka♂, Nos. 1~6) and three males of the Kumano population (NN.Km♂, Nos. 1~3). (2) Three female and three male offspring between a female and a male of the Kaita population (NN.KK♀, Nos. 1~3 and NN.KK♂, Nos. 1~3) and a female and three male offspring between a female of the Aomori population and a male of the Kaita population (NN.AK♀, No. 1 and NN.AK♂, Nos. 1~3).

3. Interspecific hybrids

Four female hybrids between a female *R. nigromaculata* of the Aomori population and a male *R. brevipoda* of the Maibara population belonging to the Nagoya race (NB♀, Nos. 1~4), and four female hybrids between a female *R. plancyi chosenica* of Suwon, Korea, and a male *R. nigromaculata* of the Hiro population (CN♀, Nos. 1~4).

All matings were made by the artificial fertilization method. Eggs were always obtained from females whose ovulation was accelerated by injection of suspension of bullfrog pituitaries into the body cavity. Tadpoles were fed on boiled spinach or chard. Frogs were fed on tropical crickets, *Gryllus bimaculatus* De Geer (Nishioka and Matsuura, 1977).

The enzymes extracted from skeletal muscles and livers of the foregoing 79 frogs and their offspring were analyzed by the method of horizontal starch-gel electrophoresis. The kinds of enzymes used in electrophoresis, abbreviations, Enzyme Commission Numbers (E. C. No.) and buffer systems are shown in Table 1. The six loci of ENO, HK, LDH-B, MPI, Pep-B and SORDH enzymes were located on chromosome No. 4 have been reported by Nishioka, Ohtani and Sumida (1980, 1987) and Nishioka and Sumida (1994).

TABLE 1
Enzymes analyzed in the present study

Enzyme	Abbreviation	E.C.No.	Sample	Buffer system
Enolase	ENO	4.2.1.11	Skeletal muscle	T-C pH 7.0
Hexokinase	HK	2.7.1.1	Liver	T-B-E pH 8.0
Lactate dehydrogenase	LDH	1.1.1.27	Skeletal muscle	T-C pH 6.0
Mannose phosphate isomerase	MPI	5.3.1.8	〃	T-C pH 7.0
Peptidase	Pep	3.4.3.1	〃	T-B-E pH 8.0
Sorbitol dehydrogenase	SORDH	1.1.1.14	Liver	T-C pH 7.0

T-C, Tris-citrate buffer T-B-E, Tris-borate-EDTA buffer

The method of electrophoresis has been shown by NISHIOKA, OHTANI and SUMIDA (1980). The detection of each enzyme was done by the methods of BREWER (1970) and HARRIS and HOPKINSON (1976) with a slight modification. The presence of linkage between the loci of enzymes was judged by calculating contingency χ^2 values at the significance level of $P<0.01$ (CAMPBELL, 1974; WRIGHT, RICHARDS and NACE, 1980).

The genotypes of the six loci in 79 frogs used in matings are shown in Table 2. The electrophoretic bands corresponding to multiple alleles at each locus were named a, b, c, ------ in the order of mobility from fast to slow, and the alleles were shown by *a*, *b*, *c*, ------.

OBSERVATION

I. Matings with heterozygous females of R. brevipoda

1. Linkage between the MPI and HK loci

a. BB.Ma♀ × NN.Ka♂

In 1988, three matings were made between a female *R. brevipoda* of the Maibara population (BB.Ma♀, No. 2) in which the genotypes of the MPI and HK loci were *cd* and *ab*, respectively, and three male *R. nigromaculata* of the Kaita population (NN.Ka♂, Nos. 1, 2 and 4) in which the genotypes of the MPI and HK loci were *gd* or *dd* and *bc* or *cc*, respectively. When the presence of linkage between the MPI and HK loci was examined in 171 offspring, 90 (52.6%) were parental and 81 were recombinants ($\chi^2=0.47$, $P>0.49$). Although the recombination rate between the two loci on chromosome No. 4 was very high, being 47.4% (Table 3, Figs. 1, 2a), in contrast to that in the offspring of the heterozygous males, it was evident that these two loci were linked with each other on chromosome No. 4 (NISHIOKA, OHTANI and SUMIDA, 1987).

2. Linkage between the MPI and LDH-B loci

a. BB.MK♀ × BB.Ko♂ or BB.Ma♂

TABLE 2

Genotypes at six loci of each individual used in the present study

Kind	Sex	No.	Year used for mating	Genotype					
				ENO	HK	LDH-B	MPI	Pep-B	SORDH
BB.Ko	♀	4*	1986	—	—	*cc*	*aa*	—	—
		5*	1986	—	—	*cc*	*aa*	—	—
		6*	1986	—	—	*cc*	*aa*	—	—
		7*	1986	—	—	*cc*	*aa*	—	—
		8*	1986	—	—	*cc*	*aa*	—	—
		9*	1988	*aa*	*aa*	*cc*	*aa*	*bb*	*ff*
		10*	1988	*aa*	—	*cc*	*ad*	*bb*	*ff*
	♂	7	1989	*aa*	*aa*	*cc*	*aa*	*bb*	*ff*
		8	1989	*aa*	*aa*	*cc*	*dd*	*bb*	*ff*
		9	1989	*aa*	*aa*	*cc*	*aa*	*bb*	*ff*
		10	1989	—	*aa*	*cc*	*aa*	—	—
		11	1989	*aa*	*aa*	*cc*	*aa*	*bb*	*ff*
BB.Ma	♀	2*	1988	*aa*	*ab*	*dd*	*cd*	*bb*	*ff*
		3*	1988	*aa*	*aa*	*dd*	*dd*	*bb*	*ff*
	♂	1*	1986	—	*ac*	*cd*	*da*	*bb*	*ff*
		2*	1986	—	*aa*	*cd*	*ae*	*bb*	*ff*
		3*	1986	—	*aa*	*cd*	*de*	*bb*	*ff*
		4*	1986	—	*aa*	*cd*	*de*	*bb*	*ff*
		9	1989	—	—	*dd*	*dd*	—	—
BB.MK	♀	1	1989	—	—	*dc*	*da*	—	—
		2	1989	—	—	*dc*	*da*	—	—
		3	1989	—	—	*dc*	*da*	—	—
	♂	1*	1988	*aa*	*ab*	*dc*	*ca*	*bb*	*ff*
		2*	1988	*aa*	*aa*	*dc*	*ca*	*bb*	*ff*
		3*	1988	*aa*	*aa*	*dc*	*ca*	*bb*	*ff*
		4*	1988	*aa*	*aa*	*dc*	*ca*	*bb*	*ff*
		5*	1990	*aa*	*aa*	*dc*	*ca*	*bb*	*ff*
		6*	1990	*aa*	*aa*	*dc*	*ca*	*bb*	*ff*
BB.KM	♀	1	1989	—	—	*cd*	*ac*	—	—
		2	1989	—	—	*cd*	*ac*	—	—
		3	1989	—	—	*cd*	*ac*	—	—
	♂	1*	1988	*aa*	*aa*	*cd*	*ae*	*bb*	*ff*
		2*	1988	*aa*	*aa*	*cd*	*ae*	*bb*	*ff*
		3*	1988	*aa*	—	*cd*	*ae*	*bb*	*ff*
		4*	1988	*aa*	—	*cd*	*ae*	*bb*	*ff*
CN	♀	1*	1985	—	—	*ab*	*jd*	*cb*	*eb*
		2*	1985	—	—	*ab*	*nd*	*cb*	*eb*
		3*	1985	—	—	*ab*	*jd*	*cb*	*eb*
		4*	1985	—	—	*ab*	*nd*	*cb*	*eb*
NN.Us	♀	1	1988	*bb*	*cc*	*bb*	*dd*	*bb*	*ab*
NN.Ao	♀	1*	1988	*bb*	*cc*	*bb*	*dd*	*cc*	*bb*
NN.Ka	♀	1*	1988	*bb*	*cd*	*bb*	*dd*	*bb*	*ab*
		2*	1988	*bb*	*cc*	*bb*	*dd*	*bb*	*bb*
		3*	1988	*bb*	*dd*	*bb*	*gd*	*bb*	*ab*
		4*	1988	*bb*	*cc*	*bb*	*dd*	*bb*	*bb*
		6*	1990	*bb*	—	*bb*	*dg*	*bb*	*bb*
		7*	1990	*bb*	—	*bb*	*dg*	*bb*	*bb*
		8*	1990	*bb*	—	*bb*	*dg*	*bb*	*bb*
		9*	1990	*bb*	*dc*	*bb*	*dg*	*bb*	*bb*
		10*	1990	*bb*	—	*bb*	*dg*	*bb*	*bb*
		11*	1990	*bb*	—	*bb*	*dg*	*bb*	*bb*
		12*	1990	*bb*	—	*bb*	*dg*	*bb*	*bb*
		13*	1990	*bb*	—	*bb*	*dg*	*bb*	*bb*
		14*	1990	*bb*	—	*bb*	*dg*	*bb*	*bb*
		15*	1990	*bb*	*dc*	*bb*	*dg*	*bb*	*bb*
		16	1989	—	—	—	*gg*	*bb*	*bb*
	♂	1*	1988	*bb*	*bc*	*bb*	*gd*	*bb*	*ab*
		2*	1988	*bb*	*cc*	*bb*	*gd*	*bb*	*ab*
		3*	1988	*ba*	*dc*	*bb*	*dd*	*bb*	*ab*
		4*	1988	*bb*	*cc*	*bb*	*dd*	*bb*	*ab*
		5*	1988	*bb*	*cc*	*bb*	*gd*	*bb*	*ab*
		6*	1988	*bb*	*cc*	*bb*	*gd*	*bb*	*db*
NN.Km	♂	1*	1985	*bb*	—	*bb*	*gd*	*bb*	*bb*
		2*	1985	*bb*	—	*bb*	*gd*	*bb*	*bb*
		3*	1985	*bb*	—	*bb*	*gd*	*bb*	*bb*
NN.KK	♀	1	1988	—	—	*bb*	*dd*	—	—
		2	1989	—	—	—	*dd*	*bb*	*ab*
		3	1990	—	—	*bb*	*dd*	—	—
	♂	1	1989	—	*cc*	—	*dd*	*bb*	*bb*
		2	1989	—	*cc*	—	*dd*	*bb*	*bb*
		3	1989	—	*cc*	—	*dd*	*bb*	*bb*
NN.AK	♀	1	1989	—	*cb*	—	*dg*	*cb*	*ba*
	♂	1	1989	—	—	—	*dg*	*cb*	*ba*
		2	1989	—	—	—	*dg*	*cb*	*ba*
		3	1989	—	—	—	*dg*	*cb*	*ba*
NB	♀	1	1989	*ba*	*ca*	*bd*	*dc*	*cb*	*bf*
		2	1989	*ba*	*ca*	*bd*	*dc*	*cb*	*bf*
		3	1989	*ba*	*ca*	*bd*	*dc*	*cb*	*bf*
		4	1989	*ba*	*ca*	*bd*	*dc*	*cb*	*bf*

—, not examined. *, These frogs are the same as those used by NISHIOKA and SUMIDA (1994).

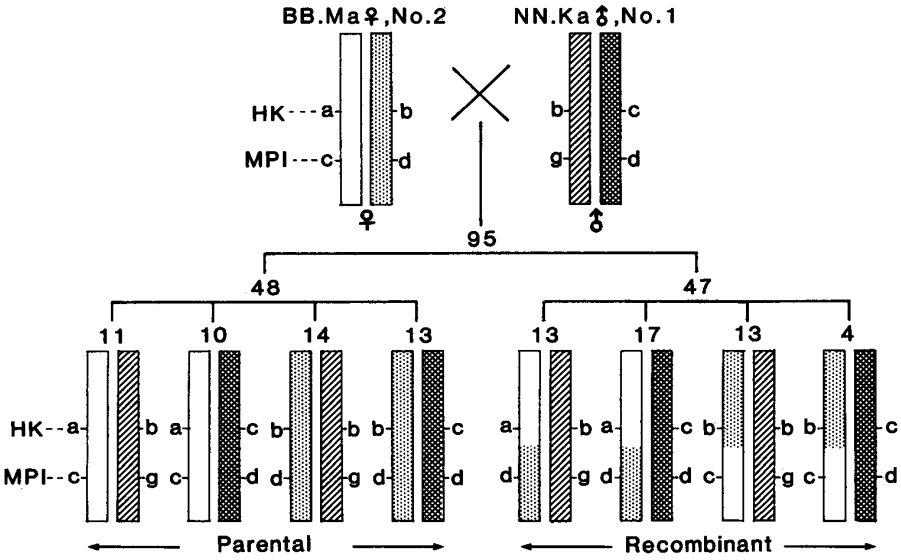

Fig. 1. Inheritance of the MPI and HK enzymes in a mating with a heterozygous female, BB.Ma♀, No. 2 × NN.Ka♂, No. 1, and linkage analysis between these two loci in *Rana brevipoda*. It was assumed that recombinants must have resulted from crossing-over between the MPI and HK loci.

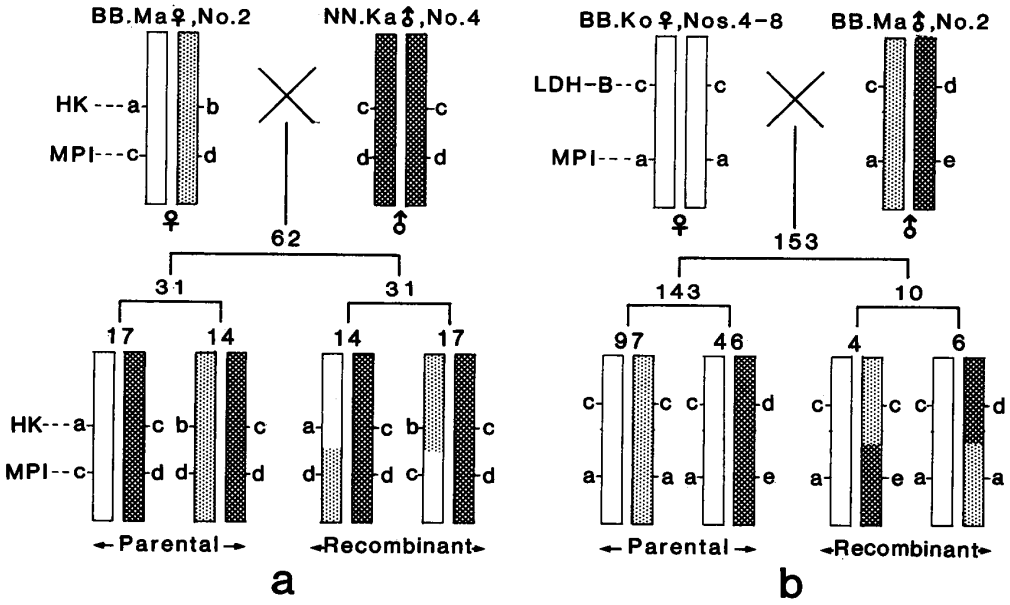

Fig. 2a. Inheritance of the MPI and HK enzymes in a mating with a heterozygous female, BB.Ma♀, No. 2 × NN.Ka♂, No. 4, and linkage analysis between these two loci in *Rana brevipoda* (Table 3).

Fig. 2b. Inheritance of the MPI and LDH-B enzymes in the five matings with a heterozygous male, BB.Ko♀, Nos. 4–8 × BB.Ma♂, No. 2, and linkage analysis between these two loci in *Rana brevipoda* (Table 5).

It was assumed that recombinants must have resulted from crossing-over between the MPI and HK loci or between the MPI and LDH-B loci.

In 1986, from a mating between a female *R. brevipoda* of the Maibara population in which the genotypes of the MPI and LDH-B loci were both *dd* and a male *R. brevipoda* of the Konko population in which the genotypes of the MPI and LDH-B loci were *aa* and *cc*, respectively, offspring (BB.MK) in which the MPI and LDH-

TABLE 3

Inheritance of the MPI and HK loci in matings with a heterozygous female of *Rana brevipoda*

Year	Parents		No. of metamorphosed frogs	No. of analyzed frogs	MPI			HK			MPI and HK linkage				
					Genotype		No. of offspring	Genotype		No. of offspring	Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
	Female	Male			Parents ♀ ♂	Offspring		Parents ♀ ♂	Offspring						
1988	BB.Ma, No. 2	NN.Ka, No. 1	109	95	*cd* *gd*	*cg* *cd* *dg* *dd*	38 57	*ab* *bc*	*ab* *ac* *bb* *bc*	51 44	48 (50.5)	47	0.01	0.92	49.5
		NN.Ka, No. 2	91	14	*cd* *gd*	*cg* *cd* *dg* *dd*	6 8	*ab* *cc*	*ac* *bc*	9 5	11 (78.6)	3	4.57	0.03	21.4
		NN.Ka, No. 4	94	62	*cd* *dd*	*cd* *dd*	34 28	*ab* *cc*	*ac* *bc*	31 31	31 (50.0)	31	0	1.00	50.0
	Total		294	171		*c-* *d-*	78 93		*a-* *b-*	91 80	90 (52.6)	81	0.47	0.49	47.4

TABLE 4

Inheritance of the MPI and LDH-B loci in matings with heterozygous females of *Rana brevipoda*

Year	Parents		No. of metamorphosed frogs	No. of analyzed frogs	MPI			LDH-B			MPI and LDH-B linkage				
					Genotype		No. of offspring	Genotype		No. of offspring	Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
	Female	Male			Parents ♀ ♂	Offspring		Parents ♀ ♂	Offspring						
1989	BB.MK, No. 1	BB.Ko, No. 10	59	57	*da* *aa*	*da* *aa*	25 32	*dc* *cc*	*dc* *cc*	30 27	30 (52.6)	27	0.16	0.69	47.4
	BB.MK, No. 1	BB.Ma, No. 9	36	34	*da* *dd*	*dd* *ad*	22 12	*dc* *dd*	*dd* *cd*	15 19	15 (44.1)	19	0.47	0.49	55.9
	BB.MK, No. 2	BB.Ko, No. 10	115	106	*da* *aa*	*da* *aa*	50 56	*dc* *cc*	*dc* *cc*	49 57	57 (53.8)	49	0.60	0.44	46.2
	BB.MK, No. 2	BB.Ma, No. 9	48	45	*da* *dd*	*dd* *ad*	23 22	*dc* *dd*	*dd* *cd*	24 21	22 (48.9)	23	0.22	0.88	51.1
	BB.MK, No. 3	BB.Ko, No. 10	46	45	*da* *aa*	*da* *aa*	25 20	*dc* *cc*	*dc* *cc*	22 23	26 (57.8)	19	1.09	0.30	42.2
	BB.MK, No. 3	BB.Ma, No. 9	29	27	*da* *dd*	*dd* *ad*	10 17	*dc* *dd*	*dd* *cd*	14 13	13 (48.1)	14	0.04	0.85	51.9
	Total		333	314		*d-* *a-*	155 159		*d-* *c-*	154 160	163 (51.9)	151	0.45	0.50	48.1
1989	BB.KM, No. 1	BB.Ko, No. 7	10	8	*ac* *aa*	*aa* *ca*	6 2	*cd* *cc*	*cc* *dc*	5 3	5 (62.5)	3	0.50	0.48	37.5
	BB.KM, No. 1	BB.Ko, No. 8	21	19	*ac* *dd*	*ad* *cd*	12 7	*cd* *cc*	*cc* *dc*	9 10	8 (42.1)	11	0.47	0.49	57.9
	BB.KM, No. 2	BB.Ko, No. 9	23	22	*ac* *aa*	*aa* *ca*	14 8	*cd* *cc*	*cc* *dc*	12 10	10 (45.5)	12	0.18	0.67	54.5
	BB.KM, No. 3	BB.Ko, No. 10	52	47	*ac* *aa*	*aa* *ca*	24 23	*cd* *cc*	*cc* *dc*	29 18	22 (46.8)	25	0.19	0.66	53.2
	BB.KM, No. 3	BB.Ma, No. 9	60	55	*ac* *dd*	*ad* *cd*	28 27	*cd* *dd*	*cd* *dd*	31 24	30 (54.5)	25	0.45	0.50	45.6
	Total		166	151		*a-* *c-*	84 67		*c-* *d-*	86 65	75 (49.7)	76	0.01	0.14	50.3

B loci were *da* and *dc*, respectively, were produced. In 1989, six backcrossings were made between three females (BB.MK♀, Nos. 1~3) and two males including a male *R. brevipoda* of the Konko population (BB.Ko♂, No. 10) in which the genotypes of the MPI and LDH-B loci were *aa* and *cc*, respectively, and a male *R. brevipoda* of the Maibara population (BB.Ma♂, No. 9) in which the genotypes of the MPI and LDH-B loci were both *dd*. When the presence of linkage between the MPI and LDH-B loci was examined in 314 backcrosses, 163 (51.9%) were parental and 151 were recombinants ($\chi^2=0.45$, $P>0.49$). Although the recombination rate between the two loci was very high, being 48.1% (Table 4, Figs. 3b, 4), in contrast to that in the offspring of the heterozygous males, it was evident that these two loci were linked with each other on chromosome No. 4 (NISHIOKA, OHTANI and SUMIDA, 1987).

b. BB.KM♀ × BB.Ko♂ or BB.Ma♂

In 1986, from a mating between a female *R. brevipoda* of the Konko population in which the genotypes of the MPI and LDH-B loci were *aa* and *cc*, respectively, and a male *R. brevipoda* of the Maibara population in which the genotypes of the MPI and LDH-B loci were *cc* and *dd*, respectively, offspring (BB.KM) whose genotypes of the MPI and LDH-B loci were *ac* and *cd*, respectively, were obtained. In 1989, five backcrossings were made between three females (BB.KM♀, Nos. 1~3) obtained from the foregoing crossing and five males including four male *R. brevipoda* of the Konko population (BB.Ko♂, Nos. 7~10) in which the genotypes of the MPI and LDH-B loci were *aa* or *dd* and *cc*, respectively, and a male *R.*

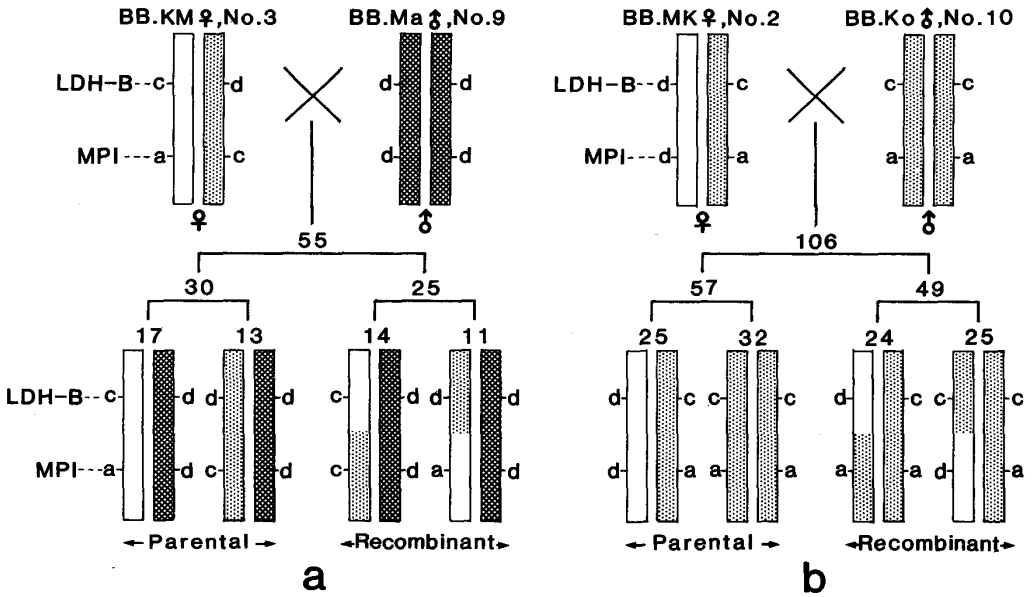

Fig. 3. Inheritance of the MPI and LDH-B enzymes in the two matings with heterozygous females and linkage analysis between these two loci in *Rana brevipoda*. It was assumed that recombinants must have resulted from crossing-over between the MPI and HK loci.

a. Using a mating of BB.KM♀, No. 3 × BB.Ma♂, No. 9 (Table 4)

b. Using a mating of BB.MK♀, No. 2 × BB.Ko♂, No. 10 (Table 4)

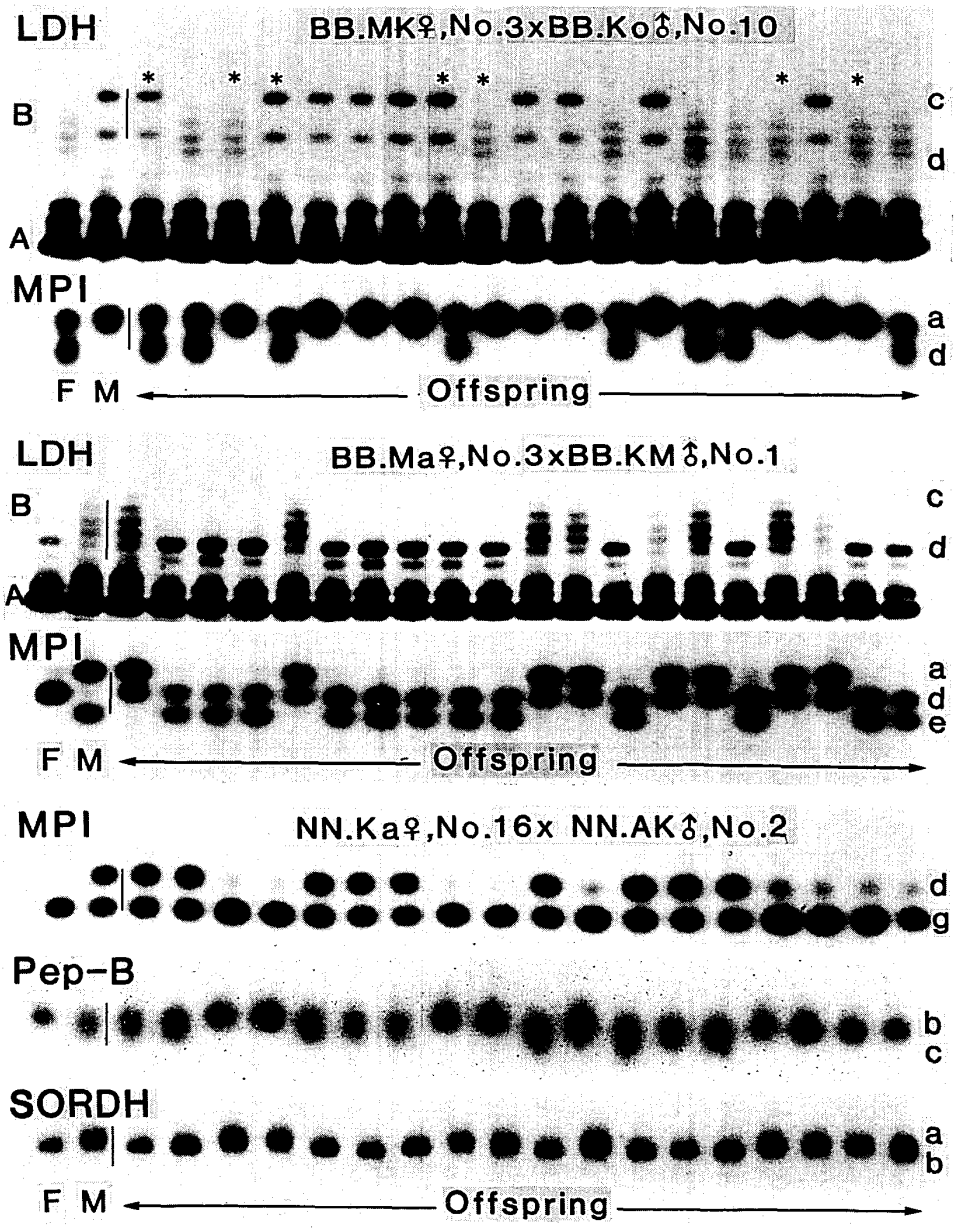

Fig. 4. Electrophoretic patterns of four enzymes in the offspring produced from a heterozygous female or males and their parents. The upper gel slice shows the LDH-B and MPI patterns of the offspring produced from BB.MK♀, No. 3 × BB.Ko♂, No. 10 (Table 4). At these two loci, the genotypes of a female (F) parent were heterozygous *dc* and *da*, respectively, and those of a male (M) parent were homozygous *cc* and *aa*, respectively. Of the 19 offspring, 12 were parental and the other seven were recombinants. The middle gel slice shows the LDH-B and MPI patterns of the offspring produced from BB.Ma♀, No. 3 × BB.KM♂, No. 1 (Table 5). At these two loci, the genotypes of a male parent were heterozygous *cd* and *ae*, respectively, and those of a female parent were both homozygous *dd*. All the 20 offspring were parental and no recombinants were found. The lower gel slice shows the MPI, Pep-B and SORDH patterns of the offspring produced from NN.Ka♀, No. 16 × NN.AK♂, No. 2 (Table 8). At these three loci, the genotypes of a male parent were heterozygous *dg*, *cb* and *ba*, respectively, and those of a female parent were homozygous *gg*, *bb* and *bb*, respectively. All the 18 offspring were parental and no recombinants were found. *, Recombinants

TABLE 5

Inheritance of the MPI and LDH-B loci in matings with heterozygous males of *Rana brevipoda*

Year	Parents		No. of metamorphosed frogs	No. of analyzed frogs	MPI			LDH-B			MPI and LDH-B linkage				
					Genotype		No. of offspring	Genotype		No. of offspring	Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
	Female	Male			Parents ♀ ♂	Offspring		Parents ♀ ♂	Offspring						
1986	BB.Ko, Nos. 6–8	BB.Ma, No. 1	70	69	*aa* *da*	*ad*	34	*cc* *cd*	*cc*	35	68 (98.6)	1	65.06	<0.00001	1.4
						aa	35		*cd*	34					
	BB.Ko, Nos. 4–8	BB.Ma, No. 2	165	153	*aa* *ae*	*aa*	103	*cc* *cd*	*cc*	101	143 (93.5)	10	115.61	<0.00001	6.5
						ae	50		*cd*	52					
	BB.Ko, Nos. 6–8	BB.Ma, No. 3	44	42	*aa* *de*	*ad*	22	*cc* *cd*	*cc*	23	41 (97.6)	1	38.10	<0.00001	2.4
						ae	20		*cd*	19					
	BB.Ko, Nos. 7, 8	BB.Ma, No. 4	41	37	*aa* *de*	*ad*	18	*cc* *cd*	*cc*	19	36 (97.3)	1	33.11	<0.00001	2.7
						ae	19		*cd*	18					
	Total		320	301		*-d -a*	177		*cc*	178	288 (95.7)	13	251.25	<0.00001	4.3
						-a -e	124		*cd*	123					
1988	BB.Ma, No. 3	BB.KM, No. 1	116	93	*dd* *ae*	*da*	42	*dd* *cd*	*dc*	42	93 (100)	0	93.00	<0.00001	0
						de	51		*dd*	51					
	BB.Ko, No. 10	BB.KM, No. 1	108	89	*ad* *ae*	*aa da*	43	*cc* *cd*	*cc*	43	89 (100)	0	89.00	<0.00001	0
						ae de	46		*cd*	46					
	NN.KK, No. 1	BB.KM, No. 1	76	70	*dd* *ae*	*da*	37	*bb* *cd*	*bc*	37	70 (100)	0	70.00	<0.00001	0
						de	33		*bd*	33					
	BB.Ma, No. 3	BB.KM, No. 2	109	87	*dd* *ae*	*da*	45	*dd* *cd*	*dc*	45	87 (100)	0	87.00	<0.00001	0
						de	42		*dd*	42					
	BB.Ko, No. 10	BB.KM, No. 2	112	88	*ad* *ae*	*aa da*	43	*cc* *cd*	*cc*	42	87 (98.9)	1	84.05	<0.00001	1.1
						ae de	45		*cd*	46					
	NN.KK, No. 1	BB.KM, No. 2	19	14	*dd* *ae*	*da*	3	*bb* *cd*	*bc*	3	14 (100)	0	14.00	<0.0002	0
						de	11		*bd*	11					
	BB.Ma, No. 3	BB.KM, No. 3	64	63	*dd* *ae*	*da*	30	*dd* *cd*	*dc*	30	63 (100)	0	63.00	<0.00001	0
						de	33		*dd*	33					
	BB.Ko, No. 10	BB.KM, No. 3	101	87	*ad* *ae*	*aa da*	37	*cc* *cd*	*cc*	37	87 (100)	0	87.00	<0.00001	0
						ae de	50		*cd*	50					
	BB.Ma, No. 3	BB.KM, No. 4	102	86	*dd* *ae*	*da*	39	*dd* *cd*	*dc*	39	86 (100)	0	86.00	<0.00001	0
						de	47		*dd*	47					
	BB.Ko, No. 10	BB.KM, No. 4	87	80	*ad* *ae*	*aa da*	41	*cc* *cd*	*cc*	41	80 (100)	0	80.00	<0.00001	0
						ae de	39		*cd*	39					
	Total		894	757		*-a*	360		*-c*	359	756 (99.9)	1	753.01	<0.00001	0.1
						-e	397		*-d*	398					
1988	BB.Ma, No. 2	BB.MK, No. 1	107	77	*cd* *ca*	*ca da*	38	*dd* *dc*	*dc*	38	77 (100)	0	77.00	<0.00001	0
						cc dc	39		*dd*	39					
	BB.Ko, No. 9	BB.MK, No. 1	74	67	*aa* *ca*	*aa*	38	*cc* *dc*	*cc*	38	67 (100)	0	67.00	<0.00001	0
						ac	29		*cd*	29					

	NN.Ao, No. 1	BB.MK, No. 1	116	104	*dd* *ca*	*da* *dc*	53 51	*bb* *dc*	*bc* *bd*	53 51	104 (100)	0	104.00	<0.00001	0
	NN.Ka, No. 1	BB.MK, No. 1	71	61	*dd* *ca*	*da* *dc*	20 41	*bb* *dc*	*bc* *bd*	20 41	61 (100)	0	61.00	<0.00001	0
	BB.Ma, No. 2	BB.MK, No. 2	108	67	*cd* *ca*	*ca* *da* *cc* *dc*	35 32	*dd* *dc*	*dc* *dd*	35 32	67 (100)	0	67.00	<0.00001	0
	BB.Ko, No. 9	BB.MK, No. 2	92	68	*aa* *ca*	*aa* *ac*	37 31	*cc* *dc*	*cc* *cd*	37 31	68 (100)	0	68.00	<0.00001	0
	NN.Ao, No. 1	BB.MK, No. 2	95	31	*dd* *ca*	*da* *dc*	14 17	*bb* *dc*	*bc* *bd*	14 17	31 (100)	0	31.00	<0.00001	0
	NN.Ka, No. 1	BB.MK, No. 2	69	53	*dd* *ca*	*da* *dc*	21 32	*bb* *dc*	*bc* *bd*	20 33	52 (98.1)	1	49.08	<0.00001	1.9
	BB.Ma, No. 3	BB.MK, No. 3	116	105	*dd* *ca*	*da* *dc*	52 53	*dd* *dc*	*dc* *dd*	52 53	105 (100)	0	105.00	<0.00001	0
	BB.Ko, No. 10	BB.MK, No. 3	6	6	*ad* *ca*	*aa* *da* *ac* *dc*	4 2	*cc* *dc*	*cc* *cd*	4 2	6 (100)	0	6.00	0.02	0
	BB.Ma, No. 3	BB.MK, No. 4	116	97	*dd* *ca*	*da* *dc*	52 45	*dd* *dc*	*dc* *dd*	52 45	97 (100)	0	97.00	<0.00001	0
	BB.Ko, No. 10	BB.MK, No. 4	110	100	*ad* *ca*	*aa* *da* *ac* *dc*	52 48	*cc* *dc*	*cc* *cd*	52 48	100 (100)	0	100.00	<0.00001	0
	Total		1080	836		*-a* *-c*	416 420		*-c* *-d*	415 421	835 (99.9)	1	832.00	<0.00001	0.1
1990	NN.Ka, No. 6	BB.MK, No. 5	180	134	*dg* *ca*	*da* *ga* *dc* *gc*	70 64	*bb* *dc*	*bc* *bd*	70 64	134 (100)	0	134.00	<0.00001	0
	NN.Ka, No. 7		150	103	*dg* *ca*	*da* *ga* *dc* *gc*	65 38	*bb* *dc*	*bc* *bd*	65 38	103 (100)	0	103.00	<0.00001	0
	NN.Ka, No. 8		172	75	*dg* *ca*	*da* *ga* *dc* *gc*	33 42	*bb* *dc*	*bc* *bd*	33 42	75 (100)	0	75.00	<0.00001	0
	NN.Ka, No. 9		104	59	*dg* *ca*	*da* *ga* *dc* *gc*	27 32	*bb* *dc*	*bc* *bd*	27 32	59 (100)	0	59.00	<0.00001	0
	NN.Ka, No. 10		160	105	*dg* *ca*	*da* *ga* *dc* *gc*	51 54	*bb* *dc*	*bc* *bd*	51 54	105 (100)	0	105.00	<0.00001	0
	NN.Ka, No. 11	BB.MK, No. 6	152	75	*dg* *ca*	*da* *ga* *dc* *gc*	32 43	*bb* *dc*	*bc* *bd*	32 43	75 (100)	0	75.00	<0.00001	0
	NN.Ka, No. 12		69	17	*dg* *ca*	*da* *ga* *dc* *gc*	8 9	*bb* *dc*	*bc* *bd*	8 9	17 (100)	0	17.00	<0.00001	0
	NN.Ka, No. 13		338	153	*dg* *ca*	*da* *ga* *dc* *gc*	88 65	*bb* *dc*	*bc* *bd*	88 65	153 (100)	0	153.00	<0.00001	0
	NN.Ka, No. 14		318	147	*dg* *ca*	*da* *ga* *dc* *gc*	74 73	*bb* *dc*	*bc* *bd*	74 73	147 (100)	0	147.00	<0.00001	0
	NN.Ka, No. 15		99	73	*dg* *ca*	*da* *ga* *dc* *gc*	33 40	*bb* *dc*	*bc* *bd*	33 40	73 (100)	0	73.00	<0.00001	0
	NN.KK, No. 3	BB.MK, No. 5	127	103	*dd* *ca*	*da* *dc*	62 41	*bb* *dc*	*bc* *bd*	62 41	103 (100)	0	103.00	<0.00001	0
	Total		1869	1044		*-a* *-c*	543 501		*bc* *bd*	543 501	1044 (100)	0	1044.00	<0.00001	0

brevipoda of the Maibara population (BB.Ma♂, No. 9) in which the genotypes of the MPI and LDH-B loci were both *dd*. When the presence of linkage between the MPI and LDH-B loci was examined in 151 backcrosses, 75 (49.7%) were parental and 76 were recombinants ($\chi^2=0.01$, $P>0.13$). Although the recombination rate between the two loci was very high, being 50.3%, in contrast to that in the offspring of the heterozygous males (Table 4, Fig. 3a), it was evident that these two loci were linked with each other on chromosome No. 4 (NISHIOKA, OHTANI and SUMIDA, 1987).

II. Matings with heterozygous males of R. brevipoda

1. Linkage between the MPI and LDH-B loci

a. BB.Ko♀ × BB.Ma♂

In 1986, 13 matings were made between five female *R. brevipoda* of the Konko population (BB.Ko♀, Nos. 4~8) in which the genotypes of the MPI and LDH-B loci were *aa* and *cc*, respectively, and four males including a male *R. brevipoda* of the Maibara population (BB.Ma♂, No. 1) in which the genotypes of the MPI and LDH-B loci were *da* and *cd*, respectively, another male *R. brevipoda* of the Maibara population (BB.Ma♂, No. 2) in which the genotypes of the MPI and LDH-B loci were *ae* and *cd*, respectively, and two more male *R. brevipoda* of the Maibara population (BB.Ma♂, Nos. 3 and 4) in which the genotypes of the MPI and LDH-B loci were *de* and *cd*, respectively. When the presence of linkage between the MPI and LDH-B loci was examined in 301 offspring, 288 (95.7%) were parental and 13 were recombinants ($\chi^2=251.25$, $P<0.00001$). Thus, it was found that the MPI and LDH-B loci were closely linked with each other and the recombination rate between these two loci was very low, being 4.3 % (Table 5; Fig. 2b).

b. BB.Ma♀, BB.Ko♀ or NN.KK♀ × BB.KM♂

In 1986, offspring (BB.KM) were produced by three matings between a female *R. brevipoda* of the Konko population in which the genotypes of the MPI and LDH-B loci were *aa* and *cc*, respectively, and three male *R. brevipoda* of the Maibara population in which the genotypes of the MPI and LDH-B loci were *ae* or *de* and *dd* or *cd*, respectively. In 1988, 10 matings were made between four males (BB.KM♂, Nos. 1~4) which were obtained by the foregoing matings and whose genotypes of the MPI and LDH-B loci were *ae* and *cd*, respectively, and three females including a female *R. brevipoda* of the Maibara population (BB.Ma♀, No. 3) in which the genotypes of the MPI and LDH-B loci were both *dd*, a female *R. brevipoda* of the Konko population (BB.Ko♀, No. 10) in which the genotypes of the MPI and LDH-B loci were *ad* and *cc*, respectively, and a female offspring of *R. nigromaculata* of the Kaita population (NN.KK♀, No. 1) in which the genotypes of the MPI and LDH-B loci were *dd* and *bb*, respectively. When the presence of linkage between the MPI and LDH-B loci was examined in 757 offspring, 756

(99.9%) were parental and only one was recombinant ($\chi^2=753.01$, $P<0.00001$). Thus, the MPI and LDH-B loci were found to be closely linked with each other and the recombination rate between these two loci was extremely low, being 0.1 %, in contrast to that in the offspring of the heterozygous females (Table 5; Fig. 4).

c. BB.Ma♀, BB.Ko♀, NN.Ao♀ or NN.Ka♀ × BB. MK♂

In 1986, offspring (BB.MK) whose genotypes of the MPI and LDH-B loci were *ca* and *dc*, respectively, were produced by a mating between a female *R. brevipoda* of the Maibara population (BB.Ma♀) in which the genotypes of the MPI and LDH-B loci were *cc* and *cd*, respectively, and a male *R. brevipoda* of the Konko population (BB.Ko♂) in which the genotypes of the MPI and LDH-B loci were *aa* and *cc*, respectively. In 1988, 12 matings were made between four males of the foregoing offspring (BB.MK♂, Nos. 1~4) and six females including two female *R. brevipoda* of the Maibara population (BB.Ma♀, Nos. 2 and 3) in which the genotypes of the MPI and LDH-B loci were *cd* or *dd* and *dd*, respectively, two female *R. brevipoda* of the Konko population (BB.Ko♀, Nos. 9 and 10) in which the genotypes of the MPI and LDH-B loci were *aa* or *ad* and *cc*, respectively, and two female *R. nigromaculata* of the Aomori and Kaita populations (NN.Ao♀, No. 1 and NN.Ka♀, No. 1) in which the genotypes of the MPI and LDH-B loci were *dd* and *bb*, respectively. When the presence of linkage between the MPI and LDH-B loci was examined in 836 offspring, 835 (99.9%) were parental and one was recombinant ($\chi^2=832.00$, $P<0.00001$). Thus, it was found that the MPI and LDH-B loci were closely linked with each other and the recombination rate between these two loci was very low, being 0.1 %, in contrast to that in the offspring of the heterozygous females (Table 5).

d. NN.Ka♀ or NN.KK♀ × BB.MK♂

In 1990, 11 matings were made between two males (BB.MK♂, Nos. 5 and 6) which were obtained by the foregoing mating and whose genotypes of the MPI and LDH-B loci were *ca* and *dc*, respectively, and 11 female *R. nigromaculata*, 10 of which were of the Kaita population (NN.Ka♀, Nos. 6~15) in which the genotypes of the MPI and LDH-B loci were *dg* and *bb*, respectively, and the remaining one was the offspring of the Kaita population (NN.KK♀, No. 3) in which the genotypes of the MPI and LDH-B loci were *dd* and *bb*, respectively. When the presence of linkage between the MPI and LDH-B loci was examined in 1044 offspring, all the offspring were parental and there was no offspring which was recombinant ($\chi^2=1044.00$, $P<0.00001$). Thus, it was found that the MPI and LDH-B loci were closely linked with each other and the recombination rate between these two loci was zero, in contrast to that in the offspring of the heterozygous females (Table 5).

TABLE 6

Inheritance of the SORDH, MPI, HK and Pep-B loci in

Year	Parents		No. of metamorphosed frogs	No. of analyzed frogs	SORDH		
					Genotype		No. of offspring
	Female	Male			Parents ♀ ♂	Offspring	
1989	NN.AK, No. 1	NN.KK, No. 1	48	48	*ba* *bb*	*bb*	25
						ab	23
		NN.KK, No. 2	79	77	*ba* *bb*	*bb*	38
						ab	39
		NN.KK, No. 3	38	37	*ba* *bb*	*bb*	17
						ab	20
	Total		165	162	*ba* *bb*	*bb*	80
						ab	82

III. Matings with a heterozygous female of R. nigromaculata

In 1988, a mating was made between a female of the Aomori population (NN.Ao♀, No. 1) in which the genotypes of the SORDH, MPI, HK and Pep-B loci were *bb*, *dd*, *cc* and *cc*, respectively, and a male of the Kaita population (NN.Ka♂, No. 1) in which the genotypes of the SORDH, MPI, HK and Pep-B loci were *ab*, *gd*, *bc* and *bb*, respectively. As a female offspring (NN.AK♀, No. 1) was heterozygous *ba*, *dg*, *cb* and *cb* in the genotypes of SORDH, MPI, HK and Pep-B loci, respectively, three matings were made in 1989 between this female and three male offspring of the Kaita population (NN.KK♂, Nos. 1~3) in which the genotypes of the SORDH, MPI, HK and Pep-B loci were homozygous *bb*, *dd*, *cc* and *bb*, respectively. Of the offspring, 162 were analyzed on the genotypes of the foregoing four loci (Table 6) in order to clarify the presence of linkages among the four loci situated on chromosome No. 4 and to calculate the recombination rates in the female (Table 7).

SORDH—MPI loci: When the presence of linkage between the SORDH and MPI loci was examined in 162 offspring, it was found that 158 (97.5%) were parental and four were recombinants ($\chi^2=146.40$, $P<0.00001$). Thus, the SORDH and MPI loci were closely linked with each other and the recombination rate between these two loci was 2.5%.

SORDH—HK loci: When the presence of linkage between the SORDH and HK loci was examined in the foregoing 162 offspring, it was found that 100 (61.7%) were parental and 62 were recombinants ($\chi^2=8.91$, $0.002<P<0.003$). Thus, the SORDH and HK loci were linked with each other and the recombination rate between these two loci was 38.3%.

SORDH—Pep-B loci: When the presence of linkage between the SORDH and Pep-B loci was examined in the foregoing 162 offspring, it was found that 102 (63.0%) were parental and 60 were recombinants ($\chi^2=10.89$, $0.0009<P<0.001$). Thus, the SORDH and Pep-B loci were linked with each other and the recombination rate between these two loci was 37.0%.

matings with a heterozygous female of *Rana nigromaculata*

MPI				HK				Pep-B			
Genotype			No. of offspring	Genetype			No. of offspring	Genetype			No. of offspring
Parents ♀	Parents ♂	Offspring		Parents ♀	Parents ♂	Offspring		Parents ♀	Parents ♂	Off spring	
dg	*dd*	*dd*	25	*cb*	*cc*	*cc*	24	*cb*	*bb*	*cb*	27
		gd	23			*bc*	24			*bb*	21
dg	*dd*	*dd*	40	*cb*	*cc*	*cc*	38	*cb*	*bb*	*cb*	41
		gd	37			*bc*	39			*bb*	36
dg	*dd*	*dd*	15	*cb*	*cc*	*cc*	15	*cb*	*bb*	*cb*	16
		gd	22			*bc*	22			*bb*	21
dg	*dd*	*dd*	80	*cb*	*cc*	*cc*	77	*cb*	*bb*	*cb*	84
		gd	82			*bc*	85			*bb*	78

TABLE 7

Linkage analysis in a heterozygous female of *Rana nigromaculata*

Year	Locus pair	Parents		No. of analyzed frogs	Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
		Female	Male						
1989	SORDH—MPI	NN.AK, No. 1	NN.KK, No. 1	48	48 (100)	0	48.00	<0.00001	0
			NN.KK, No. 2	77	75 (97.4)	2	69.21	<0.00001	2.6
			NN.KK, No. 3	37	35 (94.6)	2	29.43	<0.00001	5.4
		Total		162	158 (97.5)	4	146.40	<0.00001	2.5
	SORDH—HK	NN.AK, No. 1	NN.KK, No. 1	48	29 (60.4)	19	2.08	0.15	39.6
			NN.KK, No. 2	77	46 (59.7)	31	2.92	0.09	40.3
			NN.KK, No. 3	37	25 (67.6)	12	4.57	0.03	32.4
		Total		162	100 (61.7)	62	8.91	<0.003	38.3
	SORDH—Pep-B	NN.AK, No. 1	NN.KK, No. 1	48	30 (62.5)	18	3.00	0.08	37.5
			NN.KK, No. 2	77	48 (62.3)	29	4.69	0.03	37.7
			NN.KK, No. 3	37	24 (64.9)	13	3.27	0.07	35.1
		Total		162	102 (63.0)	60	10.89	<0.001	37.0
	MPI—HK	NN.AK, No. 1	NN.KK, No. 1	48	29 (60.4)	19	2.08	0.15	39.6
			NN.KK, No. 2	77	46 (59.7)	31	2.92	0.09	40.3
			NN.KK, No. 3	37	23 (62.2)	14	2.19	0.14	37.8
		Total		162	98 (60.5)	64	7.14	<0.01	39.5
	MPI—Pep-B	NN.AK, No. 1	NN.KK, No. 1	48	30 (62.5)	18	3.00	0.08	37.5
			NN.KK, No. 2	77	50 (64.9)	27	6.87	<0.01	35.1
			NN.KK, No. 3	37	22 (59.5)	15	1.32	0.25	40.5
		Total		162	102 (63.0)	60	10.89	<0.001	37.0
	HK—Pep-B	NN.AK, No. 1	NN.KK, No. 1	48	43 (89.6)	5	30.08	<0.00001	10.4
			NN.KK, No. 2	77	68 (88.3)	9	45.21	<0.00001	11.7
			NN.KK, No. 3	37	36 (97.3)	1	33.11	<0.00001	2.7
		Total		162	147 (90.7)	15	107.56	<0.00001	9.3

MPI—HK loci: When the presence of linkage between the MPI and HK loci was examined in the foregoing 162 offspring, it was found that 98 (60.5%) were parental and 64 were recombinants ($\chi^2=7.14$, $0.009<P<0.01$). Thus, the MPI and HK loci were linked with each other and the recombination rate between

these two loci was 39.5%.

MPI—Pep-B loci: When the presence of linkage between the MPI and Pep-B loci was examined in the foregoing 162 offspring, it was found that 102 (63.0%) were parental and 60 were recombinants ($\chi^2=10.89$, $0.0009<P<0.001$). Thus, the MPI and Pep-B loci were linked with each other and the recombination rate between these two loci was 37.0%.

HK—Pep-B loci: When the presence of linkage between the HK and Pep-B loci was examined in the foregoing 162 offspring, it was found that 147 (90.7%) were parental and 15 were recombinants ($\chi^2=107.56$, $P<0.00001$). Thus, the HK and Pep-B loci were closely linked with each other and the recombination rate between these two loci was 9.3%.

IV. Matings with heterozygous males of R. nigromaculata

1. Linkage between the SORDH and MPI loci

In 1988 and 1989, a total of 13 matings was made between seven males including three male *R. nigromaculata* of the Kaita population (NN.Ka♂, Nos. 1, 2 and 5) in which the genotypes of the SORDH and MPI loci were *ab* and *gd*, respectively, three male offspring (NN.AK♂, Nos.1~3) produced from a mating, NN.Ao♀, No. 1×NN.Ka♂, No. 2, in which the genotypes of the SORDH and MPI loci were *ba* and *dg*, respectively, and a male *R. nigromaculata* of the Kaita population (NN.Ka♂, No. 6) in which the genotypes of the SORDH and MPI loci were *db* and *gd*, respectively, and six females including three female *R. nigromaculata* of the Aomori and Kaita populations (NN.Ao♀, No. 1 and NN.Ka♀, Nos. 2 and 4) in which the genotypes of the SORDH and MPI loci were *bb* and *dd*, respectively, a female *R. nigromaculata* of the Kaita population (NN.Ka♀, No. 16) in which the genotypes of the SORDH and MPI loci were *bb* and *gg*, respectively, and two female *R. brevipoda* of the Maibara and Konko populations (BB.Ma♀, No. 2 and BB.Ko♀, No. 9) in which the genotypes of the SORDH and MPI loci were *ff* and *cd* or *aa*, respectively. When the genotypes of the SORDH and MPI loci were examined in 845 offspring obtained from the 13 matings (Fig. 4), all the offspring were parental and there was no offspring which was recombinant ($\chi^2=845.00$, $P<0.00001$). Thus, it was found that these two loci were closely linked with each other and the recombination rate between the two loci was zero, in contrast to that in the offspring of the heterozygous females (Tables 8, 9).

2. Linkage between the SORDH and ENO loci

In 1988, a mating was made between a male *R. nigromaculata* of the Kaita population (NN.Ka♂, No. 3) in which the genotypes of the SORDH and ENO loci were *ab* and *ba*, respectively, and a female *R. nigromaculata* of the Kaita population (NN.Ka♀, No. 2) in which the genotypes of the SORDH and ENO loci were both *bb*. When the genotypes of the SORDH and ENO loci were analyzed in 70 offspring from the mating (Table 8), in order to confirm the

presence of linkage between the two loci, it was found that all the 70 offspring were parental and there was no offspring which was recombinant ($\chi^2=70.00$, $P<0.00001$). Thus, these two loci were closely linked with each other on chromosome No. 4 and the recombination rate between the two loci was zero, in contrast to that in the offspring of the heterozygous females (Table 9).

3. Linkage between the SORDH and HK loci

In 1988, four matings were made between two male *R. nigromaculata* of the Kaita population (NN.Ka♂, Nos. 1 and 3) in which the genotypes of the SORDH and HK loci were *ab* and *bc* or *dc*, respectively, and four females including two female *R. nigromaculata* of the Aomori and Kaita populations (NN.Ao♀, No. 1 and NN.Ka♀, No. 2) in which the genotypes of the SORDH and HK loci were *bb* and *cc*, respectively, and two female *R. brevipoda* of the Maibara and Konko populations (BB.Ma♀, No. 2 and BB.Ko♀, No. 9) in which the genotypes of the SORDH and HK loci were *ff* and *ab* or *aa*, respectively. When the genotypes of the SORDH and HK loci were analyzed in 286 offspring produced from the four matings in order to confirm the presence of linkage between the two loci, it was found that 285 (99.7%) were parental and only one was recombinant ($\chi^2=282.01$, $P<0.00001$). Thus, these two loci were closely linked with each other on chromosome No. 4 and the recombination rate between the two loci was very low, being 0.3%, in contrast to that in the offspring of the heterozygous females (Tables 8, 9).

4. Linkage between the SORDH and Pep-B loci

In 1989, three matings were made between three male offspring (NN.AK♂, Nos. 1~3) produced from a mating, NN.Ao♀, No. 1 × NN.Ka♂, No. 2, of *R. nigromaculata* in which the genotypes of the SORDH and Pep-B loci were *ba* and *cb*, respectively, and a female *R. nigromaculata* of the Kaita population (NN.Ka♀, No. 16) in which the genotypes of the SORDH and Pep-B loci were both *bb*. The genotypes of the SORDH and Pep-B loci were analyzed by the electrophoresis in 330 offspring produced from the three matings, in order to confirm the presence of linkage between the two loci (Fig. 4). It was found that all the 330 offspring were parental and there was no recombinant ($\chi^2=330.00$, $P<0.00001$). Thus, these two loci were closely linked with each other and the recombination rate between the two loci was zero, in contrast to that in the offspring of the heterozygous females (Tables 8, 9).

5. Linkage between the MPI and HK loci

In 1988, four matings were made between a male *R. nigromaculata* of the Kaita population (NN.Ka♂, No. 1) in which the genotypes of the MPI and HK loci were *gd* and *bc*, respectively, and four females including two female *R. nigromaculata* of the Aomori and Ushita populations (NN.Ao♀, No. 1 and NN.Us♀, No. 1) in which the genotypes of the MPI and HK loci were *dd* and *cc*, respectively, a female *R. brevipoda* of the Maibara population (BB.Ma♀, No. 2) in which the genotypes of the MPI and HK loci were *cd* and *ab*, respectively, and a female *R. brevipoda* of the

TABLE 8

Inheritance of the SORDH, MPI, ENO, HK and Pep-B loci in

Year	Parents		No. of metamorphosed frogs	No. of analyzed frogs	SORDH		
					Genotype		No. of offspring
	Female	Male			Parents ♀	Parents ♂	Offspring
1988	NN.Ao, No. 1	NN.Ka, No. 1	111	70	*bb*	*ab*	*ba* 33 *bb* 37
	NN.Us, No. 1		102	20	—	—	— — — —
	BB.Ma, No. 2		109	95	*ff*	*ab*	*fa* 51 *fb* 44
	BB.Ko, No. 9		65	51	*ff*	*ab*	*fa* 26 *fb* 25
	Total		387	236			*-a* 110 *-b* 106
1988	NN.Ao, No. 1	NN.Ka, No. 2	113	25	*bb*	*ab*	*ba* 11 *bb* 14
	BB.Ma, No. 2		91	62	*ff*	*ab*	*fa* 28 *fb* 34
	BB.Ko, No. 9		94	61	*ff*	*ab*	*fa* 31 *fb* 30
	Total		298	148			*-a* 70 *-b* 78
1988	NN.Ka, No. 2	NN.Ka, No. 3	122	70	*bb*	*ab*	*ba* 32 *bb* 38
	NN.Ka, No. 3		6	4	—	—	— — — —
	Total		128	74			*ba* 32 *bb* 38
1988	NN.Ka, No. 2	NN.Ka, No. 5	102	19	*bb*	*ab*	*ba* 9 *bb* 10
	NN.Ka, No. 4		111	9	*bb*	*ab*	*ba* 6 *bb* 3
	Total		213	28			*ba* 15 *bb* 13
1988	NN.Ka, No. 2	NN.Ka, No. 6	202	70	*bb*	*db*	*bd* 37 *bb* 33
	NN.Ka, No. 4		83	53	*bb*	*db*	*bd* 27 *bb* 26
	Total		285	123			*bd* 64 *bb* 59
1989	NN.KK, No. 2	NN.AK, No. 1	27	25	—	—	— — — —
	NN.Ka, No. 16		107	105	*bb*	*ba*	*ba* 51 *bb* 54
	Total		134	130			*ba* 51 *bb* 54
1989	NN.KK, No. 2	NN.AK, No. 2	38	20	—	—	— — — —
	NN.Ka, No. 16		126	106	*bb*	*ba*	*ba* 44 *bb* 62
	Total		164	126			*ba* 44 *bb* 62
1989	NN.KK, No. 2	NN.AK, No. 3	26	25	—	—	— — — —
	NN.Ka, No. 16		119	119	*bb*	*ba*	*ba* 59 *bb* 60
	Total		145	144			*ba* 59 *bb* 60

matings with heterozygous males of *Rana nigromaculata*

MPI				ENO			HK			
Genotype			No. of off-spring	Genotype		No. of off-spring	Genotype			No. of off-spring
Parents ♀	Parents ♂	Off-spring		Parents ♀	Parents ♂		Parents ♀	Parents ♂	Off-spring	
dd	*gd*	*dg*	33	—	—	—	—	*cc*	*bc*	*cb*	33
		dd	37			—	—			*cc*	37
dd	*gd*	*dg*	11	—	—	—	—	*cc*	*bc*	*cb*	11
		dd	9			—	—			*cc*	9
cd	*gd*	*cg dg*	51	—	—	—	—	*ab*	*bc*	*ab bb*	52
		cd dd	44			—	—			*ac bc*	43
aa	*gd*	*ag*	26	—	—	—	—	*aa*	*bc*	*ab*	26
		ad	25			—	—			*ac*	25
		-g	121							*-b*	122
		-d	115							*-c*	114
dd	*gd*	*dg*	11	—	—	—	—	—	—	—	—
		dd	14			—	—			—	—
cd	*gd*	*cg dg*	28	—	—	—	—	—	—	—	—
		cd dd	34			—	—			—	—
aa	*gd*	*ag*	31	—	—	—	—	—	—	—	—
		ad	30			—	—			—	—
		-g	70								
		-d	78								
—	—	—	—	*bb*	*ba*	*bb*	32	*cc*	*dc*	*cd*	32
		—	—			*ba*	38			*cc*	38
—	—	—	—	*bb*	*ba*	*bb*	2	*dd*	*dc*	*dd*	2
		—	—			*ba*	2			*dc*	2
						bb	34			*ba*	34
						ba	40			*-c*	40
dd	*gd*	*dg*	9	—	—	—	—	—	—	—	—
		dd	10			—	—			—	—
dd	*gd*	*dg*	6	—	—	—	—	—	—	—	—
		dd	3			—	—			—	—
		-g	15								
		-d	13								
dd	*gd*	*dg*	37	—	—	—	—	Pep-B			
		dd	33			—	—				
dd	*gd*	*dg*	27	—	—	—	—	Genotype			No. of off-spring
		dd	26			—	—				
		dg	64					Parents ♀	Parents ♂	Off-spring	
		dd	59								
dd	*dg*	*dg*	15	—	—	—	—	*bb*	*cb*	*bb*	15
		dd	10			—	—			*bc*	10
gg	*dg*	*gg*	51	—	—	—	—	*bb*	*cb*	*bb*	51
		gd	54			—	—			*bc*	54
		dg	66							*bb*	66
		dd	64							*bc*	64
dd	*dg*	*dg*	12	—	—	—	—	*bb*	*cb*	*bb*	12
		dd	8			—	—			*bc*	8
gg	*dg*	*gg*	44	—	—	—	—	*bb*	*cb*	*bb*	44
		gd	62			—	—			*bc*	62
		-g	56							*bb*	56
		-d	70							*bc*	70
dd	*dg*	*dg*	16	—	—	—	—	*bb*	*cb*	*bb*	16
		dd	9			—	—			*bc*	9
gg	*dg*	*gg*	59	—	—	—	—	*bb*	*cb*	*bb*	59
		gd	60			—	—			*bc*	60
		-g	75							*bb*	75
		-d	69							*bc*	69

TABLE 9

Linkage analysis in heterozygous males of *Rana nigromaculata*

Year	Locus pair	Parents		No. of analyzed frogs	Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
		Female	Male						
1988	SORDH—MPI	NN.Ao, No. 1	NN.Ka, No. 1	70	70 (100)	0	70.00	<0.00001	0
		BB.Ma, No. 2		95	95 (100)	0	95.00	<0.00001	0
		BB.Ko, No. 9		51	51 (100)	0	51.00	<0.00001	0
		NN.Ao, No. 1	NN.Ka, No. 2	25	25 (100)	0	25.00	<0.00001	0
		BB.Ma, No. 2		62	62 (100)	0	62.00	<0.00001	0
		BB.Ko, No. 9		61	61 (100)	0	61.00	<0.00001	0
		NN.Ka, No. 2	NN.Ka, No. 5	19	19 (100)	0	19.00	<0.0001	0
		NN.Ka, No. 4		9	9 (100)	0	9.00	<0.003	0
		NN.Ka, No. 2	NN.Ka, No. 6	70	70 (100)	0	70.00	<0.00001	0
		NN.Ka, No. 4		53	53 (100)	0	53.00	<0.00001	0
1989		NN.Ka, No. 16	NN.AK, No. 1	105	105 (100)	0	105.00	<0.00001	0
			NN.AK, No. 2	106	106 (100)	0	106.00	<0.00001	0
			NN.AK, No. 3	119	119 (100)	0	119.00	<0.00001	0
		Total		845	845 (100)	0	845.00	<0.00001	0
1988	SORDH—ENO	NN.Ka, No. 2	NN.Ka, No. 3	70	70 (100)	0	70.00	<0.00001	0
	SORDH—HK	NN.Ao, No. 1	NN.Ka, No. 1	70	70 (100)	0	70.00	<0.00001	0
		BB.Ma, No. 2		95	94 (98.9)	1	91.04	<0.00001	1.1
		BB.Ko, No. 9		51	51 (100)	0	51.00	<0.00001	0
		NN.Ka, No. 2	NN.Ka, No. 3	70	70 (100)	0	70.00	<0.00001	0
		Total		286	285 (99.7)	1	282.01	<0.00001	0.3
1989	SORDH—Pep-B	NN.Ka, No. 16	NN.AK, No. 1	105	105 (100)	0	105.00	<0.00001	0
			NN.AK, No. 2	106	106 (100)	0	106.00	<0.00001	0
			NN.AK, No. 3	119	119 (100)	0	119.00	<0.00001	0
		Total		330	330 (100)	0	330.00	<0.00001	0
1988	MPI—HK	NN.Ao, No. 1	NN.Ka, No. 1	70	70 (100)	0	70.00	<0.00001	0
		NN.Us, No. 1		20	20 (100)	0	20.00	<0.00001	0
		BB.Ma, No. 2		95	94 (98.9)	1	91.04	<0.00001	1.1
		BB.Ko, No. 9		51	51 (100)	0	51.00	<0.00001	0
		Total		236	235 (99.6)	1	232.02	<0.00001	0.4
1989	MPI—Pep-B	NN.KK, No. 2	NN.AK, No. 1	25	25 (100)	0	25.00	<0.00001	0
		NN.Ka, No. 16		105	105 (100)	0	105.00	<0.00001	0
		NN.KK, No. 2	NN.AK, No. 2	20	20 (100)	0	20.00	<0.00001	0
		NN.Ka, No. 16		106	106 (100)	0	106.00	<0.00001	0
		NN.KK, No. 2	NN.AK, No. 3	25	25 (100)	0	25.00	<0.00001	0
		NN.Ka, No. 16		119	119 (100)	0	119.00	<0.00001	0
		Total		400	400 (100)	0	400.00	<0.00001	0
1988	ENO—HK	NN.Ka, No. 2	NN.Ka, No. 3	70	70 (100)	0	70.00	<0.00001	0
		NN.Ka, No. 3		4	4 (100)	0	4.00	0.05	0
		Total		74	74 (100)	0	74.00	<0.00001	0

Konko population (BB.Ko♀, No. 9) in which the genotypes of the MPI and HK loci were both *aa*. In 236 offspring produced from these four matings, the genotypes of the MPI and HK loci were analyzed (Table 8). When linkage was examined, it was found that 235 (99.6%) offspring were parental and only one was recombinant ($\chi^2=232.02$, $P<0.00001$). Thus, these two loci were closely linked with each other and the recombination rate between the MPI and HK loci was

very low, being 0.4%. This was quite in contrast with that in the offspring of the heterozygous females (Table 9).

6. Linkage between the MPI and Pep-B loci

In 1989, six matings were made between three male offspring (NN.AK♂, Nos. 1~3) produced from a mating, NN.Ao♀, No. 1 × NN.Ka♂, No. 2, of *R. nigromaculata* in which the genotypes of MPI and Pep-B loci were *dg* and *cb*, respectively, and two female *R. nigromaculata*, one of which was of the Kaita population (NN.Ka♀, No. 16) in which the genotypes of the MPI and Pep-B loci were *gg* and *bb*, respectively, and the other was the offspring of the Kaita population (NN.KK♀, No. 2) in which the genotypes of the MPI and Pep-B loci were *dd* and *bb*, respectively. The genotypes of the MPI and Pep-B loci were analyzed in 400 offspring produced from the six matings (Table 8) to confirm the presence of linkage between the MPI and Pep-B loci (Fig. 4). It was found that all the 400 offspring were parental and no offspring was recombinant ($\chi^2=400.00$, $P<0.00001$). Thus, these two loci were closely linked with each other and the recombination rate was zero, in contrast to that in the offspring of the heterozygous females (Table 9).

7. Linkage between the ENO and HK loci

In 1988, two matings were made between a male *R. nigromaculata* of the Kaita population (NN.Ka♂, No. 3) in which the genotypes of the ENO and HK loci were *ba* and *dc*, respectively, and two female *R. nigromaculata* of the Kaita population (NN.Ka♀, Nos. 2 and 3) in which the genotypes of the ENO and HK loci were *bb* and *cc* or *dd*, respectively. The genotypes of the ENO and HK loci were analyzed in 74 offspring produced from the two matings (Table 8). It was found that all the 74 offspring were parental and there was no recombinant ($\chi^2=74.00$, $P<0.00001$). Thus, these two loci were closely linked with each other and the recombination rate between the two loci was zero, in contrast to that in the offspring of the heterozygous females (Table 9).

V. Matings with heterozygous females of interspecific hybrids

1. Hybrids (NB) between *R. nigromaculata* and *R. brevipoda*

In 1986, four female hybrids (NB♀, Nos. 1~4) in which the genotypes of the six loci, the SORDH, MPI, ENO, HK, LDH-B and Pep-B loci, were *bf*, *dc*, *ba*, *ca*, *bd* and *cb*, respectively, were obtained from a mating between a female *R. nigromaculata* of the Aomori population and a male *R. brevipoda* of the Maibara population. In 1989, they were backcrossed by six matings with four male *R. brevipoda* of the Konko population including three males (BB.Ko♂, Nos. 7, 9 and 11) in which the genotypes of the SORDH, MPI, ENO, HK, LDH-B and Pep-B loci were *ff*, *aa*, *aa*, *aa*, *cc* and *bb*, respectively, and a male (BB.Ko♂, No. 8) in which the genotypes of the same six loci were *ff*, *dd*, *aa*, *aa*, *cc* and *bb*, respectively. As 141 backcrosses

TABLE 10
Inheritance of the SORDH, MPI, ENO, HK, Pep-B and LDH-B

Year	Parents		No. of metamorphosed frogs	No. of analyzed frogs	SORDH			MPI		
					Genotype		No. of offspring	Genotype		No. of offspring
	Female	Male			Parents ♀ ♂	Offspring		Parents ♀ ♂	Offspring	
1989	NB, No. 1	BB.Ko, No. 7	14	13	*bf* *ff*	*bf*	4	*dc* *aa*	*da*	4
						ff	9		*ca*	9
	NB, No. 2	BB.Ko, No. 9	2	2	*bf* *ff*	*bf*	2	*dc* *aa*	*da*	2
						ff	0		*ca*	0
	NB, No. 3	BB.Ko, No. 7	26	24	*bf* *ff*	*bf*	13	*dc* *aa*	*da*	11
						ff	11		*ca*	13
	NB, No. 3	BB.Ko, No. 8	56	51	*bf* *ff*	*bf*	26	*dc* *dd*	*dd*	27
						ff	25		*cd*	24
	NB, No. 4	BB.Ko, No. 9	22	22	*bf* *ff*	*bf*	11	*dc* *aa*	*da*	10
						ff	11		*ca*	12
	NB, No. 4	BB.Ko, No. 11	35	29	*bf* *ff*	*bf*	16	*dc* *aa*	*da*	16
						ff	13		*ca*	13
	Total		155	141		*bf*	72		*d-*	70
						ff	69		*c-*	71
1985	CN, No. 1	NN.Km, No. 1	103	20	*eb* *bb*	*eb*	10	*jd* *gd*	*jg jd*	10
						bb	10		*dg dd*	10
	CN, No. 1	NN.Km, No. 2	123	79	*eb* *bb*	*eb*	39	*jd* *gd*	*jg jd*	33
						bb	40		*dg dd*	46
	CN, No. 1	NN.Km, No. 3	91	32	*eb* *bb*	*eb*	22	*jd* *gd*	*jg jd*	21
						bb	10		*dg dd*	11
	CN, No. 2	NN.Km, No. 1	118	19	*eb* *bb*	*eb*	12	*nd* *gd*	*ng nd*	14
						bb	7		*dg dd*	5
	CN, No. 2	NN.Km, No. 3	90	14	*eb* *bb*	*eb*	7	*nd* *gd*	*ng nd*	7
						bb	7		*dg dd*	7
	CN, No. 3	NN.Km, No. 1	118	23	*eb* *bb*	*eb*	13	*jd* *gd*	*jg jd*	12
						bb	10		*dg dd*	11
	CN, No. 4	NN.Km, No. 2	90	48	*eb* *bb*	*eb*	17	*nd* *gd*	*ng nd*	17
						bb	31		*dg dd*	31
	Total		733	235		*eb*	120		*j-, n-*	114
						bb	115		*d-*	121

were produced from the six matings, their genotypes were analyzed (Table 10). The presence of linkages among the six loci situated on chromosome No. 4 was confirmed and the recombination rates among the six loci in the female hybrids were calculated (Table 11).

a. Linkage between the SORDH and each of five other loci

SORDH—MPI loci: When the presence of linkage between the SORDH and MPI loci was examined in 141 backcrosses, it was found that 137 (97.2%) were parental and four were recombinants ($\chi^2=125.45$, $P<0.00001$). Thus, the SORDH and MPI loci were closely linked with each other and the recombination rate between these two loci was 2.8%.

SORDH—ENO loci: When the presence of linkage between the SORDH and ENO loci was examined in the foregoing 141 backcrosses, it was found that 131

loci in matings with heterozygous females of hybrids

ENO				HK				Pep-B				LDH-B			
Genotype			No. of off-spring	Genotype			No. of off-spring	Genotype			No. of off-spring	Genotype			No. of off-spring
Parents ♀	Parents ♂	Off-spring		Parents ♀	Parents ♂	Off-spring		Parents ♀	Parents ♂	Off-spring		Parents ♀	Parents ♂	Off-spring	
ba	*aa*	*ba*	3	*ca*	*aa*	*ca*	5	*cb*	*bb*	*cb*	6	*bd*	*cc*	*bc*	7
		aa	10			*aa*	8			*bb*	7			*dc*	6
ba	*aa*	*ba*	2	*ca*	*aa*	*ca*	1	*cb*	*bb*	*cb*	1	*bd*	*cc*	*bc*	1
		aa	0			*aa*	1			*bb*	1			*dc*	1
ba	*aa*	*ba*	11	*ca*	*aa*	*ca*	8	*cb*	*bb*	*cb*	8	*bd*	*cc*	*bc*	9
		aa	13			*aa*	16			*bb*	16			*dc*	15
ba	*aa*	*ba*	26	*ca*	*aa*	*ca*	28	*cb*	*bb*	*cb*	29	*bd*	*cc*	*bc*	31
		aa	25			*aa*	23			*bb*	22			*dc*	20
ba	*aa*	*ba*	11	*ca*	*aa*	*ca*	6	*cb*	*bb*	*cb*	7	*bd*	*cc*	*bc*	8
		aa	11			*aa*	16			*bb*	15			*dc*	14
ba	*aa*	*ba*	15	*ca*	*aa*	*ca*	12	*cb*	*bb*	*cb*	11	*bd*	*cc*	*bc*	13
		aa	14			*aa*	17			*bb*	18			*dc*	16
		ba	68			*ca*	60			*cb*	62			*bc*	69
		aa	73			*aa*	81			*bb*	79			*dc*	72
—	—	—	—	—	—	—	—	*cb*	*bb*	*cb*	10	*ab*	*bb*	*ab*	8
—	—	—	—	—	—	—	—			*bb*	10			*bb*	12
—	—	—	—	—	—	—	—	*cb*	*bb*	*cb*	29	*ab*	*bb*	*ab*	36
—	—	—	—	—	—	—	—			*bb*	50			*bb*	43
—	—	—	—	—	—	—	—	*cb*	*bb*	*cb*	19	*ab*	*bb*	*ab*	20
—	—	—	—	—	—	—	—			*bb*	13			*bb*	12
—	—	—	—	—	—	—	—	*cb*	*bb*	*cb*	12	*ab*	*bb*	*ab*	12
—	—	—	—	—	—	—	—			*bb*	7			*bb*	7
—	—	—	—	—	—	—	—	*cb*	*bb*	*cb*	8	*ab*	*bb*	*ab*	7
—	—	—	—	—	—	—	—			*bb*	6			*bb*	7
—	—	—	—	—	—	—	—	*cb*	*bb*	*cb*	11	*ab*	*bb*	*ab*	12
—	—	—	—	—	—	—	—			*bb*	12			*bb*	11
—	—	—	—	—	—	—	—	*cb*	*bb*	*cb*	23	*ab*	*bb*	*ab*	20
—	—	—	—	—	—	—	—			*bb*	25			*bb*	28
										cb	112			*ab*	115
										bb	123			*bb*	120

(92.9%) were parental and 10 were recombinants ($\chi^2=103.84$, $P<0.00001$). Thus, the SORDH and ENO loci were linked with each other and the recombination rate between these two loci was 7.1%.

SORDH—HK loci: When the presence of linkage between the SORDH and HK loci was examined in the foregoing 141 backcrosses, it was found that 103 (73.0%) were parental and 38 were recombinants ($\chi^2=29.96$, $P<0.00001$). Thus, the SORDH and HK loci were linked with each other and the recombination rate between these two loci was 27.0%.

SORDH—Pep-B loci: When the presence of linkage between the SORDH and Pep-B loci was examined in the foregoing 141 backcrosses, it was found that 103 (73.0%) were parental and 38 were recombinants ($\chi^2=29.96$, $P<0.00001$). Thus, the SORDH and Pep-B loci were linked with each other and the recombination rate between these two loci was 27.0%.

TABLE 11

Linkage analysis in female hybrids between *Rana nigromaculata* and *Rana brevipoda*

Year	Locus pair	Parents		No. of analyzed frogs	Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
		Female	Male						
1989	SORDH—MPI	NB, No. 1	BB.Ko, No. 7	13	13 (100)	0	13.00	<0.0004	0
		NB, No. 2	BB.Ko, No. 9	2	2 (100)	0	2.00	0.16	0
		NB, No. 3	BB.Ko, No. 7	24	22 (91.7)	2	16.67	<0.0001	8.3
		NB, No. 3	BB.Ko, No. 8	51	50 (98.0)	1	47.08	<0.00001	2.0
		NB, No. 4	BB.Ko, No. 9	22	21 (95.5)	1	18.18	<0.0001	4.5
		NB, No. 4	BB.Ko, No. 11	29	29 (100)	0	29.00	<0.00001	0
		Total		141	137 (97.2)	4	125.45	<0.00001	2.8
	SORDH—ENO	NB, No. 1	BB.Ko, No. 7	13	12 (92.3)	1	9.31	<0.003	7.7
		NB, No. 2	BB.Ko, No. 9	2	2 (100)	0	2.00	0.16	0
		NB, No. 3	BB.Ko, No. 7	24	22 (91.7)	2	16.67	<0.0001	8.3
		NB, No. 3	BB.Ko, No. 8	51	49 (96.1)	2	43.31	<0.00001	3.9
		NB, No. 4	BB.Ko, No. 9	22	20 (90.9)	2	14.73	<0.0002	9.1
		NB, No. 4	BB.Ko, No. 11	29	26 (89.7)	3	18.24	<0.0001	10.3
		Total		141	131 (92.9)	10	103.84	<0.00001	7.1
	SORDH—HK	NB, No. 1	BB.Ko, No. 7	13	8 (61.5)	5	0.69	0.41	38.5
		NB, No. 2	BB.Ko, No. 9	2	1 (50.0)	1	0	1.00	50.0
		NB, No. 3	BB.Ko, No. 7	24	19 (79.2)	5	8.17	<0.005	20.8
		NB, No. 3	BB.Ko, No. 8	51	43 (84.3)	8	21.02	<0.00001	15.7
		NB, No. 4	BB.Ko, No. 9	22	13 (59.1)	9	0.73	0.39	40.9
		NB, No. 4	BB.Ko, No. 11	29	19 (65.5)	10	2.79	0.09	34.5
		Total		141	103 (73.0)	38	29.96	<0.00001	27.0
	SORDH—Pep-B	NB, No. 1	BB.Ko, No. 7	13	7 (53.8)	6	0.08	0.78	46.2
		NB, No. 2	BB.Ko, No. 9	2	1 (50.0)	1	0	1.00	50.0
		NB, No. 3	BB.Ko, No. 7	24	19 (79.2)	5	8.17	<0.005	20.8
		NB, No. 3	BB.Ko, No. 8	51	44 (86.3)	7	26.84	<0.00001	13.7
		NB, No. 4	BB.Ko, No. 9	22	14 (63.6)	8	1.64	0.20	36.4
		NB, No. 4	BB.Ko, No. 11	29	18 (62.1)	11	1.69	0.19	37.9
		Total		141	103 (73.0)	38	29.96	<0.00001	27.0
	SORDH—LDH-B	NB, No. 1	BB.Ko, No. 7	13	8 (61.5)	5	0.69	0.41	38.5
		NB, No. 2	BB.Ko, No. 9	2	1 (50.0)	1	0	1.00	50.0
		NB, No. 3	BB.Ko, No. 7	24	16 (66.7)	8	2.67	0.10	33.3
		NB, No. 3	BB.Ko, No. 8	51	40 (78.4)	11	16.49	<0.0001	21.6
		NB, No. 4	BB.Ko, No. 9	22	11 (50.0)	11	0	1.00	50.0
		NB, No. 4	BB.Ko, No. 11	29	14 (48.3)	15	0.03	0.85	51.7
		Total		141	90 (63.8)	51	10.79	<0.002	36.2
	MPI—ENO	NB, No. 1	BB.Ko, No. 7	13	12 (92.3)	1	9.31	<0.003	7.7
		NB, No. 2	BB.Ko, No. 9	2	2 (100)	0	2.00	0.16	0
		NB, No. 3	BB.Ko, No. 7	24	24 (100)	0	24.00	<0.00001	0
		NB, No. 3	BB.Ko, No. 8	51	50 (98.0)	1	47.08	<0.00001	2.0
		NB, No. 4	BB.Ko, No. 9	22	21 (95.5)	1	18.18	<0.0001	4.5
		NB, No. 4	BB.Ko, No. 11	29	26 (89.7)	3	18.24	<0.0001	10.3
		Total		141	135 (95.7)	6	118.02	<0.00001	4.3
	MPI—HK	NB, No. 1	BB.Ko, No. 7	13	8 (61.5)	5	0.69	0.41	38.5
		NB, No. 2	BB.Ko, No. 9	2	1 (50.0)	1	0	1.00	50.0
		NB, No. 3	BB.Ko, No. 7	24	21 (87.5)	3	13.50	<0.0003	12.5
		NB, No. 3	BB.Ko, No. 8	51	44 (86.3)	7	26.84	<0.00001	13.7
		NB, No. 4	BB.Ko, No. 9	22	14 (63.6)	8	1.64	0.20	36.4
		NB, No. 4	BB.Ko, No. 11	29	19 (65.5)	10	2.79	0.09	34.5
		Total		141	107 (75.9)	34	37.79	<0.00001	24.1
	MPI—Pep-B	NB, No. 1	BB.Ko, No. 7	13	7 (53.8)	6	0.08	0.78	46.2
		NB, No. 2	BB.Ko, No. 9	2	1 (50.0)	1	0	1.00	50.0
		NB, No. 3	BB.Ko, No. 7	24	21 (87.5)	3	13.50	<0.0003	12.5
		NB, No. 3	BB.Ko, No. 8	51	45 (88.2)	6	29.82	<0.00001	11.8
		NB, No. 4	BB.Ko, No. 9	22	15 (68.2)	7	2.91	0.09	31.8
		NB, No. 4	BB.Ko, No. 11	29	18 (62.1)	11	1.69	0.19	37.9
		Total		141	107 (75.9)	34	37.79	<0.00001	24.1

TABLE 11 Continued

Linkage analysis in female hybrids between *Rana nigromaculata* and *Rana brevipoda*

Year	Locus pair	Parents		No. of analyzed frogs	Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
		Female	Male						
1989	MPI—LDH-B	NB, No. 1	BB.Ko, No. 7	13	8 (61.5)	5	0.69	0.41	38.5
		NB, No. 2	BB.Ko, No. 9	2	1 (50.0)	1	0	1.00	50.0
		NB, No. 3	BB.Ko, No. 7	24	16 (66.7)	8	2.67	0.10	33.3
		NB, No. 3	BB.Ko, No. 8	51	41 (80.4)	10	18.84	<0.0001	19.6
		NB, No. 4	BB.Ko, No. 9	22	12 (54.5)	10	0.18	0.67	45.5
		NB, No. 4	BB.Ko, No. 11	29	14 (48.3)	15	0.03	0.85	51.7
		Total		141	92 (65.2)	49	13.11	<0.0003	34.8
	ENO—HK	NB, No. 1	BB.Ko, No. 7	13	9 (69.2)	4	1.92	0.17	30.8
		NB, No. 2	BB.Ko, No. 9	2	1 (50.0)	1	0	1.00	50.0
		NB, No. 3	BB.Ko, No. 7	24	23 (95.8)	1	20.17	<0.00001	4.2
		NB, No. 3	BB.Ko, No. 8	51	45 (88.2)	6	29.82	<0.00001	11.8
		NB, No. 4	BB.Ko, No. 9	22	15 (68.2)	7	2.91	0.09	31.8
		NB, No. 4	BB.Ko, No. 11	29	22 (75.9)	7	7.76	<0.006	24.1
		Total		141	115 (81.6)	26	56.18	<0.00001	18.4
	ENO—Pep-B	NB, No. 1	BB.Ko, No. 7	13	8 (61.5)	5	0.69	0.41	38.5
		NB, No. 2	BB.Ko, No. 9	2	1 (50.0)	1	0	1.00	50.0
		NB, No. 3	BB.Ko, No. 7	24	21 (87.5)	3	13.50	<0.0003	12.5
		NB, No. 3	BB.Ko, No. 8	51	46 (90.2)	5	32.96	<0.00001	9.8
		NB, No. 4	BB.Ko, No. 9	22	16 (72.7)	6	4.55	0.03	27.3
		NB, No. 4	BB.Ko, No. 11	29	21 (72.4)	8	5.83	0.02	27.6
		Total		141	113 (80.1)	28	51.24	<0.00001	19.9
	ENO—LDH-B	NB, No. 1	BB.Ko, No. 7	13	9 (69.2)	4	1.92	0.17	30.8
		NB, No. 2	BB.Ko, No. 9	2	1 (50.0)	1	0	1.00	50.0
		NB, No. 3	BB.Ko, No. 7	24	16 (66.7)	8	2.67	0.10	33.3
		NB, No. 3	BB.Ko, No. 8	51	42 (82.4)	9	21.35	<0.00001	17.6
		NB, No. 4	BB.Ko, No. 9	22	13 (59.1)	9	0.73	0.39	40.9
		NB, No. 4	BB.Ko, No. 11	29	17 (58.6)	12	0.86	0.35	41.4
		Total		141	98 (69.5)	43	21.45	<0.00001	30.5
	HK—Pep-B	NB, No. 1	BB.Ko, No. 7	13	12 (92.3)	1	9.31	<0.003	7.7
		NB, No. 2	BB.Ko, No. 9	2	2 (100)	0	2.00	0.16	0
		NB, No. 3	BB.Ko, No. 7	24	24 (100)	0	24.00	<0.00001	0
		NB, No. 3	BB.Ko, No. 8	51	50 (98.0)	1	47.08	<0.00001	2.0
		NB, No. 4	BB.Ko, No. 9	22	21 (95.5)	1	18.18	<0.0001	4.5
		NB, No. 4	BB.Ko, No. 11	29	28 (96.6)	1	25.14	<0.00001	3.4
		Total		141	137 (97.2)	4	125.45	<0.00001	2.8
	HK—LDH-B	NB, No. 1	BB.Ko, No. 7	13	9 (69.2)	4	1.92	0.17	30.8
		NB, No. 2	BB.Ko, No. 9	2	2 (100)	0	2.00	0.16	0
		NB, No. 3	BB.Ko, No. 7	24	19 (79.2)	5	8.17	<0.005	20.8
		NB, No. 3	BB.Ko, No. 8	51	46 (90.2)	5	32.96	<0.00001	9.8
		NB, No. 4	BB.Ko, No. 9	22	18 (81.8)	4	8.91	<0.003	18.2
		NB, No. 4	BB.Ko, No. 11	29	22 (75.9)	7	7.76	<0.006	24.1
		Total		141	116 (82.3)	25	58.73	<0.00001	17.7
	Pep-B—LDH-B	NB, No. 1	BB.Ko, No. 7	13	8 (61.5)	5	0.69	0.41	38.5
		NB, No. 2	BB.Ko, No. 9	2	2 (100)	0	2.00	0.16	0
		NB, No. 3	BB.Ko, No. 7	24	19 (79.2)	5	8.17	<0.005	20.8
		NB, No. 3	BB.Ko, No. 8	51	47 (92.2)	4	36.25	<0.00001	7.8
		NB, No. 4	BB.Ko, No. 9	22	19 (86.4)	3	11.64	<0.0007	13.6
		NB, No. 4	BB.Ko, No. 11	29	23 (79.3)	6	9.97	<0.002	20.7
		Total		141	118 (83.7)	23	64.01	<0.00001	16.3

SORDH—LDH-B loci: When the presence of linkage between the SORDH and LDH-B loci was examined in the foregoing 141 backcrosses, it was found that 90 (63.8%) were parental and 51 were recombinants ($\chi^2=10.79$, $0.001<P<0.002$). Thus, the SORDH and LDH-B loci were linked with each other and the

recombination rate between these two loci was 36.2% (Table 11).

b. Linkage between the MPI and each of four other loci

MPI—ENO loci: When the presence of linkage between the MPI and ENO loci was examined in the foregoing 141 backcrosses, it was found that 135 (95.7%) were parental and six were recombinants ($\chi^2=118.02$, $P<0.00001$). Thus, the MPI and ENO loci were linked with each other and the recombination rate between these two loci was 4.3%.

MPI—HK loci: When the presence of linkage between the MPI and HK loci was examined in the foregoing 141 backcrosses, it was found that 107 (75.9%) were parental and 34 were recombinants ($\chi^2=37.79$, $P<0.00001$). Thus, the MPI and HK loci were linked with each other and the recombination rate between these two loci was 24.1%.

MPI—Pep-B loci: When the presence of linkage between the MPI and Pep-B loci was examined in the foregoing 141 backcrosses, it was found that 107 (75.9%) were parental and 34 were recombinants ($\chi^2=37.79$, $P<0.00001$). Thus, the MPI and Pep-B loci were linked with each other and the recombination rate between these two loci was 24.1%.

MPI—LDH-B loci: When the presence of linkage between the MPI and LDH-B loci was examined in the foregoing 141 backcrosses, it was found that 92 (65.2%) were parental and 49 were recombinants ($\chi^2=13.11$, $P<0.0003$). Thus, the MPI and LDH-B loci were linked with each other and the recombination rate between these two loci was 34.8% (Table 11).

c. Linkage between the ENO and each of three other loci

ENO—HK loci: When the presence of linkage between the ENO and HK loci was examined in the foregoing 141 backcrosses, it was found that 115 (81.6%) were parental and 26 were recombinants ($\chi^2=56.18$, $P<0.00001$). Thus, the ENO and HK loci were linked with each other and the recombination rate between these two loci was 18.4%.

ENO—Pep-B loci: When the presence of linkage between the ENO and Pep-B loci was examined in the foregoing 141 backcrosses, it was found that 113 (80.1%) were parental and 28 were recombinants ($\chi^2=51.24$, $P<0.00001$). Thus, the ENO and Pep-B loci were linked with each other and the recombination rate between these two loci was 19.9%.

ENO—LDH-B loci: When the presence of linkage between the ENO and LDH-B loci was examined in the foregoing 141 backcrosses, 98 (69.5%) were parental and 43 were recombinants ($\chi^2=21.45$, $P<0.00001$). Thus, the ENO and LDH-B loci were linked with each other and the recombination rate between these two loci was 30.5% (Table 11).

d. Linkage between the HK and each of two other loci

HK—Pep-B loci: When the presence of linkage between the HK and Pep-B loci was examined in the foregoing 141 backcrosses, it was found that 137 (97.2%)

were parental and four were recombinants ($\chi^2=125.45$, $P<0.00001$). Thus, the HK and Pep-B loci were closely linked with each other and the recombination rate between these two loci was 2.8%.

HK—LDH-B loci: When the presence of linkage between the HK and LDH-B loci was examined in the foregoing 141 backcrosses, it was found that 116 (82.3%) were parental and 25 were recombinants ($\chi^2=58.73$, $P<0.00001$). Thus, the HK and LDH-B loci were linked with each other and the recombination rate between these two loci was 17.7% (Table 11).

e. Linkage between the Pep-B and LDH-B loci

When the presence of linkage between the Pep-B and LDH-B loci was examined in the foregoing 141 backcrosses, 118 (83.7%) were parental and 23 were recombinants ($\chi^2=64.01$, $P<0.00001$). Thus, the Pep-B and LDH-B loci were linked with each other and the recombination rate between these two loci was 16.3% (Table 11).

2. Hybrids (CN) between *R. plancyi chosenica* and *R. nigromaculata*

In 1985, seven backcrossings of four female hybrids (CN♀, Nos. 1~4) between a female *R. plancyi chosenica* and a male *R. nigromaculata* of the Hiro population in which the genotypes of the SORDH, MPI, Pep-B and LDH-B loci were *eb*, *jd* or *nd*, *cb* and *ab*, respectively, were made with three male *R. nigromaculata* of the Kumano population (NN.Km♂, Nos.1~3) in which the genotypes of the SORDH, MPI, Pep-B and LDH-B loci were *bb*, *gd*, *bb* and *bb*, respectively. As 235 offspring were produced from the seven backcrossings, the genotypes of the foregoing four loci were analyzed (Table 10; Fig. 5). In addition, the presence of linkages among the four loci situated on chromosome No. 4 was examined and the recombination rates in female hybrids were calculated (Table 12).

a. Linkage between the SORDH and each of three other loci

SORDH—MPI loci: When the presence of linkage between the SORDH and MPI loci was examined in the 235 backcrosses, it was found that 223 (94.9%) were parental and 12 were recombinants ($\chi^2=189.45$, $P<0.00001$). Thus, the SORDH and MPI loci were linked with each other and the recombination rate between these two loci was 5.1%.

SORDH—Pep-B loci: When the presence of linkage between the SORDH and Pep-B loci was examined in the foregoing 235 backcrosses, it was found that 173 (73.6%) were parental and 62 were recombinants ($\chi^2=52.43$, $P<0.00001$). Thus, the SORDH and Pep-B loci were linked with each other and the recombination rate between these two loci was 26.4% (Table 12).

SORDH—LDH-B loci: When the presence of linkage between the SORDH and LDH-B loci was examined in the foregoing 235 backcrosses, it was found that 169 (71.9%) were parental and 66 were recombinants ($\chi^2=45.14$, $P<0.00001$). Thus, the SORDH and LDH-B loci were linked with each other and the recombination rate between these two loci was 28.1% (Table 12).

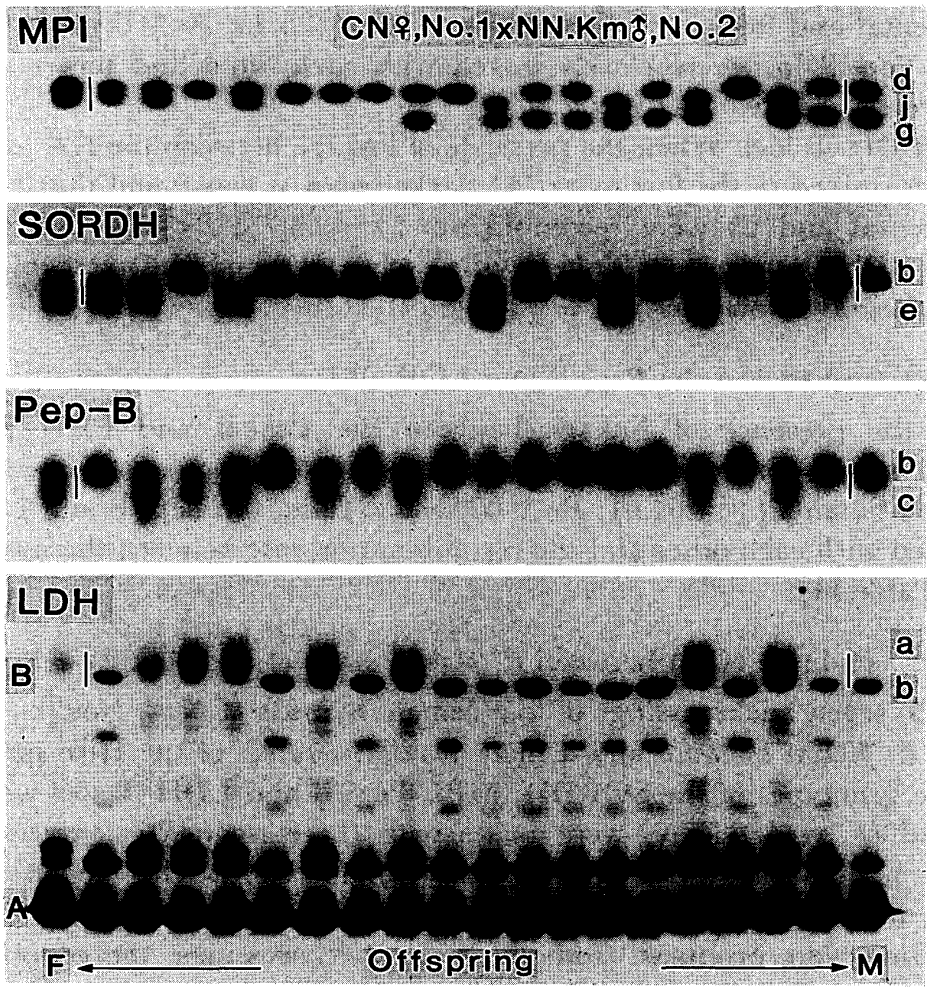

Fig. 5. Electrophoretic patterns of four enzymes, MPI, SORDH, Pep-B and LDH-B, in the offspring produced from a mating, CN♀, No. 1 × NN.Km♂, No. 2, and their parents. At these four loci, the genotypes of a female parent (F) were heterozygous *jd*, *eb*, *cb* and *ab*, respectively, and those of a male parent (M) were heterozygous *gd*, and homozygous *bb*, *bb* and *bb*, respectively. All the 18 offspring were parental and no recombinants were found.

b. Linkage between the MPI and each of two other loci

MPI—Pep-B loci: When the presence of linkage between the MPI and Pep-B loci was examined in the foregoing 235 backcrosses, it was found that 179 (76.2%) were parental and 56 were recombinants ($\chi^2=64.38$, $P<0.00001$). Thus, the MPI and Pep-B loci were linked with each other and the recombination rate between these two loci was 23.8% (Table 12).

MPI—LDH-B loci: When the presence of linkage between the MPI and LDH-B loci was examined in the foregoing 235 backcrosses, it was found that 173 (73.6%) were parental and 62 were recombinants ($\chi^2=52.43$, $P<0.00001$). Thus, the MPI and LDH-B loci were linked with each other and the recombination rate between these two loci was 26.4% (Table 12).

TABLE 12

Linkage analysis in female hybrids between *Rana plancyi chosenica* and *Rana nigromaculata*

Year	Locus pair	Parents		No. of analyzed frogs	Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
		Female	Male						
1985	SORDH—MPI	CN, No. 1	NN.Km, No. 1	20	20 (100)	0	20.00	<0.00001	0
		CN, No. 1	NN.Km, No. 2	79	74 (93.7)	5	60.27	<0.00001	6.3
		CN, No. 1	NN.Km, No. 3	32	31 (96.9)	1	28.13	<0.00001	3.1
		CN, No. 2	NN.Km, No. 1	19	17 (89.5)	2	11.84	<0.0006	10.5
		CN, No. 2	NN.Km, No. 3	14	13 (92.9)	1	10.28	<0.002	7.1
		CN, No. 3	NN.Km, No. 1	23	22 (95.7)	1	19.17	<0.00001	4.3
		CN, No. 4	NN.Km, No. 2	48	46 (95.8)	2	40.33	<0.00001	4.2
		Total		235	223 (94.9)	12	189.45	<0.00001	5.1
	SORDH—Pep-B	CN, No. 1	NN.Km, No. 1	20	16 (80.0)	4	7.20	<0.008	20.0
		CN, No. 1	NN.Km, No. 2	79	52 (65.8)	27	7.91	<0.005	34.2
		CN, No. 1	NN.Km, No. 3	32	27 (84.4)	5	15.13	<0.0002	15.6
		CN, No. 2	NN.Km, No. 1	19	11 (57.9)	8	0.47	0.49	42.1
		CN, No. 2	NN.Km, No. 3	14	13 (92.9)	1	10.28	<0.002	7.1
		CN, No. 3	NN.Km, No. 1	23	18 (78.3)	5	7.35	<0.007	21.7
		CN, No. 4	NN.Km, No. 2	48	36 (75.0)	12	12.00	<0.0006	25.0
		Total		235	173 (73.6)	62	52.43	<0.00001	26.4
	SORDH—LDH-B	CN, No. 1	NN.Km, No. 1	20	14 (70.0)	6	3.20	0.07	30.0
		CN, No. 1	NN.Km, No. 2	79	49 (62.0)	30	4.57	0.03	38.0
		CN, No. 1	NN.Km, No. 3	32	28 (87.5)	4	18.00	<0.0001	12.5
		CN, No. 2	NN.Km, No. 1	19	11 (57.9)	8	0.47	0.49	42.1
		CN, No. 2	NN.Km, No. 3	14	12 (85.7)	2	7.14	<0.008	14.3
		CN, No. 3	NN.Km, No. 1	23	18 (78.3)	5	7.35	<0.007	21.7
		CN, No. 4	NN.Km, No. 2	48	37 (77.1)	11	14.08	<0.0002	22.9
		Total		235	169 (71.9)	66	45.14	<0.00001	28.1
	MPI—Pep-B	CN, No. 1	NN.Km, No. 1	20	16 (80.0)	4	7.20	<0.008	20.0
		CN, No. 1	NN.Km, No. 2	79	55 (69.6)	24	12.16	<0.0005	30.4
		CN, No. 1	NN.Km, No. 3	32	26 (81.3)	6	12.50	<0.0005	18.7
		CN, No. 2	NN.Km, No. 1	19	13 (68.4)	6	2.58	0.11	31.6
		CN, No. 2	NN.Km, No. 3	14	12 (85.7)	2	7.14	<0.008	14.3
		CN, No. 3	NN.Km, No. 1	23	19 (82.6)	4	9.78	<0.002	17.4
		CN, No. 4	NN.Km, No. 2	48	38 (79.2)	10	16.33	<0.0001	20.8
		Total		235	179 (76.2)	56	64.38	<0.00001	23.8
	MPI—LDH-B	CN, No. 1	NN.Km, No. 1	20	14 (70.0)	6	3.20	0.07	30.0
		CN, No. 1	NN.Km, No. 2	79	50 (63.3)	29	5.58	0.02	36.7
		CN, No. 1	NN.Km, No. 3	32	27 (84.4)	5	15.13	<0.0002	15.6
		CN, No. 2	NN.Km, No. 1	19	13 (68.4)	6	2.58	0.11	31.6
		CN, No. 2	NN.Km, No. 3	14	11 (78.6)	3	4.57	0.03	21.4
		CN, No. 3	NN.Km, No. 1	23	19 (82.6)	4	9.78	<0.002	17.4
		CN, No. 4	NN.Km, No. 2	48	39 (81.3)	9	18.75	<0.0001	18.7
		Total		235	173 (73.6)	62	52.43	<0.00001	26.4
	Pep-B—LDH-B	CN, No. 1	NN.Km, No. 1	20	18 (90.0)	2	12.80	<0.0004	10.0
		CN, No. 1	NN.Km, No. 2	79	72 (91.1)	7	53.48	<0.00001	8.9
		CN, No. 1	NN.Km, No. 3	32	31 (96.9)	1	28.13	<0.00001	3.1
		CN, No. 2	NN.Km, No. 1	19	17 (89.5)	2	11.84	<0.0006	10.5
		CN, No. 2	NN.Km, No. 3	14	13 (92.9)	1	10.28	<0.002	7.1
		CN, No. 3	NN.Km, No. 1	23	23 (100)	0	23.00	<0.00001	0
		CN, No. 4	NN.Km, No. 2	48	45 (93.8)	3	36.75	<0.00001	6.2
		Total		235	219 (93.2)	16	175.36	<0.00001	6.8

c. Linkage between the Pep-B and LDH-B loci

When the presence of linkage between the Pep-B and LDH-B loci was examined in the foregoing 235 backcrosses, 219 (93.2%) were parental and 16 were recombinants ($\chi^2=175.36$, $P<0.00001$). Thus, the Pep-B and LDH-B loci were linked with each other and the recombination rate between these two loci was 6.8% (Table 12).

VI. Sexual differences in the recombination rates among six loci on chromosome No. 4

1. Recombination rate between the SORDH and each of five other loci

a. SORDH and MPI loci

When 16 matings were made with nine females whose genotypes of the SORDH and MPI loci on chromosome No. 4 were both heterozygous, it was found that 518 (96.3%) of 538 offspring were parental and the other 20 were recombinants ($\chi^2=460.97$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 3.7%. In contrast, when 13 matings were made with seven males whose genotypes of the two loci were both heterozygous, it was found that 909 offspring analyzed were all parental and there was no recombinant ($\chi^2=909.00$, $P<0.00001$), that is, the recombination rate between the two loci in the offspring of the heterozygous males was zero (Table 13).

b. SORDH and ENO loci

When six matings were made with four females whose genotypes of the SORDH and ENO loci on chromosome No. 4 were both heterozygous, it was found that 131 (92.9%) of 141 offspring analyzed were parental and the other 10 were recombinants ($\chi^2=103.84$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 7.1%. In contrast, when a mating was made with a male whose genotypes of the two loci were both heterozygous, it was found that 70 offspring analyzed were all parental and there was no recombinant ($\chi^2=70.00$, $P<0.00001$), that is, the recombination rate between the two loci in the offspring of the heterozygous male was zero (Table 13).

c. SORDH and HK loci

When nine matings were made with five females whose genotypes of the SORDH and HK loci on chromosome No. 4 were both heterozygous, it was found that 203 (67.0%) of 303 offspring analyzed were parental and the other 100 were recombinants ($\chi^2=35.01$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 33.0%. In contrast, when four matings were made with two males whose genotypes of the two loci were both heterozygous, 292 (99.7%) of 293 offspring analyzed were parental and only one was recombinant ($\chi^2=289.01$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous males was 0.3% (Table 13).

TABLE 13
Sexual differences in the recombination rates among six loci on chromosome No. 4

Female

Locus pair	No. of matings	No. of analyzed frogs	Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
SORDH—MPI	16	538	518 (96.3)	20	460.97	<0.00001	3.7
SORDH—ENO	6	141	131 (92.9)	10	103.84	<0.00001	7.1
SORDH—HK	9	303	203 (67.0)	100	35.01	<0.00001	33.0
SORDH—Pep-B	16	538	378 (70.3)	160	88.33	<0.00001	29.7
SORDH—LDH-B	13	376	259 (68.9)	117	53.63	<0.00001	31.1
MPI—ENO	6	141	135 (95.7)	6	118.02	<0.00001	4.3
MPI—HK	12	474	295 (62.2)	179	28.39	<0.00001	37.8
MPI—Pep-B	16	538	388 (72.1)	150	105.29	<0.00001	27.9
MPI—LDH-B	24	841	503 (59.8)	338	32.37	<0.00001	40.2
ENO—HK	6	141	115 (81.6)	26	56.18	<0.00001	18.4
ENO—Pep-B	6	141	113 (80.1)	28	51.24	<0.00001	19.9
ENO—LDH-B	6	141	98 (69.5)	43	21.45	<0.00001	30.5
HK—Pep-B	9	303	284 (93.7)	19	231.77	<0.00001	6.3
HK—LDH-B	6	141	116 (82.3)	25	58.73	<0.00001	17.7
Pep-B—LDH-B	13	376	337 (89.6)	39	236.18	<0.00001	10.4

Male

Locus pair	No. of matings	No. of analyzed frogs	Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
SORDH—MPI	13	909	909 (100)	0	909.00	<0.00001	0
SORDH—ENO	1	70	70 (100)	0	70.00	<0.00001	0
SORDH—HK	4	293	292 (99.7)	1	289.01	<0.00001	0.3
SORDH—Pep-B	3	358	358 (100)	0	358.00	<0.00001	0
MPI—HK	4	236	235 (99.6)	1	232.02	<0.00001	0.4
MPI—Pep-B	6	400	400 (100)	0	400.00	<0.00001	0
MPI—LDH-B	46	2938	2924 (99.5)	14	2882.27	<0.00001	0.5
ENO—HK	2	74	74 (100)	0	74.00	<0.00001	0

d. SORDH and Pep-B loci

When 16 matings were made with nine females whose genotypes of the SORDH and Pep-B loci situated on chromosome No. 4 were both heterozygous, 378 (70.3%) of 538 offspring produced from these matings were parental and the other 160 were recombinants ($\chi^2=88.33$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 29.7%. In contrast, when three matings were made with three males whose genotypes of the two loci were both heterozygous, it was found that 358 offspring analyzed were all parental and there was no recombinant ($\chi^2=358.00$, $P<0.00001$), that is, the

recombination rate between the two loci in the offspring of the heterozygous males was zero (Table 13).

e. SORDH and LDH-B loci

When 13 matings were made with eight females whose genotypes of the SORDH and LDH-B loci situated on chromosome No. 4 were both heterozygous, 259 (68.9%) of 376 offspring produced from these matings were parental, and the other 117 were recombinants ($\chi^2=53.63$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 31.1% (Table 13).

2. Recombination rate between the MPI and each of four other loci

a. MPI and ENO loci

When six matings were made with four females whose genotypes of the MPI and ENO loci situated on chromosome No. 4 were both heterozygous, 135 (95.7%) of 141 offspring were parental, and the other six were recombinants ($\chi^2=118.02$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 4.3% (Table 13).

b. MPI and HK loci

When 12 matings were made with six females whose genotypes of the MPI and HK loci situated on chromosome No. 4 were both heterozygous, 295 (62.2%) of 474 offspring were parental, and the other 179 were recombinants ($\chi^2=28.39$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 37.8%. In contrast, when four matings were made with a male whose genotypes of the two loci were both heterozygous, 235 (99.6%) of 236 offspring analyzed were parental, and the remainder was recombinant ($\chi^2=232.02$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous male was 0.4% (Table 13).

c. MPI and Pep-B loci

When 16 matings were made with nine females whose genotypes of the MPI and Pep-B loci situated on chromosome No. 4 were both heterozygous, 388 (72.1%) of 538 offspring analyzed were parental, and the other 150 were recombinants ($\chi^2=105.29$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 27.9%. In contrast, when six matings were made with three males whose genotypes of the two loci were both heterozygous, all the 400 offspring analyzed were parental and there was no recombinant ($\chi^2=400.00$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous males was zero (Table 13).

d. MPI and LDH-B loci

When 24 matings were made with 14 females whose genotypes of the MPI and LDH-B loci situated on chromosome No. 4 were both heterozygous, 503 (59.8%)

of 841 offspring were parental and the other 338 were recombinants ($\chi^2=32.37$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 40.2%. In contrast, when 46 matings were made with 14 males whose genotypes of the two loci were both heterozygous, 2924 (99.5%) of 2938 offspring obtained from these matings were parental, and the other 14 were recombinants ($\chi^2=2882.27$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous males was 0.5% (Table 13).

3. Recombination rate between the ENO and each of three other loci

a. ENO and HK loci

When six matings were made with four females whose genotypes of the ENO and HK loci situated on chromosome No. 4 were both heterozygous, 115 (81.6%) of 141 offspring were parental, and the other 26 were recombinants ($\chi^2=56.18$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 18.4%. In contrast, when two matings were made with a male whose genotypes of the two loci were both heterozygous, all the 74 offspring analyzed were parental and there was no recombinant ($\chi^2=74.00$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous male was zero (Table 13).

b. ENO and Pep-B loci

When the foregoing six matings were made, 113 (80.1%) of the 141 offspring were parental, and the other 28 were recombinants ($\chi^2=51.24$, $P<0.00001$). Thus, the recombination rate between the ENO and Pep-B loci in the offspring of the heterozygous females was 19.9% (Table 13).

c. ENO and LDH-B loci

When the foregoing six matings were made, 98 (69.5%) of the 141 offspring were parental, and the other 43 were recombinants ($\chi^2=21.45$, $P<0.00001$). Thus, the recombination rate between the ENO and LDH-B loci in the offspring of the heterozygous females was 30.5% (Table 13).

4. Recombination rate between the HK and each of two other loci

a. HK and Pep-B loci

When nine matings were made with five females whose genotypes of the HK and Pep-B loci situated on chromosome No. 4 were both heterozygous, 284 (93.7%) of 303 offspring analyzed were parental, and the other 19 were recombinants ($\chi^2=231.77$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 6.3% (Table 13).

b. HK and LDH-B loci

When six matings were made with four females whose genotypes of the HK and LDH-B loci situated on chromosome No. 4 were both heterozygous, 116 (82.3%)

of 141 offspring analyzed were parental, and the other 25 were recombinants ($\chi^2=58.73$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 17.7% (Table 13).

5. Recombination rate between the Pep-B and LDH-B loci

When 13 matings were made with eight females whose genotypes of the Pep-B and LDH-B loci situated on chromosome No. 4 were both heterozygous, 337 (89.6%) of 376 offspring analyzed were parental, and the other 39 were recombinants ($\chi^2=236.18$, $P<0.00001$). Thus, the recombination rate between the two loci in the offspring of the heterozygous females was 10.4% (Table 13).

DISCUSSION

WRIGHT, RICHARDS and NACE (1980), WRIGHT and RICHARDS (1982, 1983) and WRIGHT, RICHARDS, FROST, CAMOZZI and KUNZ (1983) clarified the existence of the following eight linkage groups by using two kinds of females, *Rana pipiens* and *R. sphenocephala*, and five kinds of males, *R. pipiens*, *R. sphenocephala*, *R. palustris*♀ × *R. pipiens*♂ (*pal-pip*), *R. sphenocephala*♀ × *R. berlandieri*♂ (*sph-ber*), *R. sphenocephala*♀ × *R. blairi*♂ (*sph-bla*). It was found that linkage group 1 consisted of β-GUS, Acon-1, Ab, F16DP, PGM-1, ADH-2 and β-GLU loci, linkage group 2 consisted of Gly, AP-1, AP-2 and Est-5 loci, linkage group 3 consisted of Est-1, Est-6, Est-10 and Est-4 loci, linkage group 4 consisted of MDH-2, Pep-C and SOD-1 loci, linkage group 5 consisted of Pep-B, LDH-B, MPI and HK-2 loci, linkage group 6 consisted of PGM and β-GLU loci (=linkage group 1), linkage group 7 consisted of IDH-B and Hb loci, and linkage group 8 consisted of GPI, GOT and TPI loci. They also reported that in *R. pipiens* and *R. blairi*, the sex-determining genes were linked with SOD-1 locus belonging to group 4, that in *R. berlandieri* and *R. sphenocephala*, the sex-determining genes were linked with various enzyme loci belonging to group 1, and that the foregoing four species were all of male heterogamety. The recombination rate between the Pep-C and SOD-1 loci was 6.8% in the offspring of male *pipiens*, 7.1% in the offspring of male *pal-pip* hybrids, and zero in the offspring of male *sph-ber* hybrids. The recombination rate between the LDH-B and MPI loci was zero in the backcrosses of male *pal-pip* hybrids and 2% in the backcrosses of male *sph-ber* hybrids. The recombination rate between the MPI and HK-2 loci was 3% in the offspring of male *pipiens* and 6% in the backcrosses of male *sph-ber* hybrids. The recombination rate between the MPI and Pep-B loci was 30.3% in the offspring of female *pipiens*, while it was 3% in the backcrosses of male *sph-ber* hybrids. The recombination rate between the Ab and PGM-1 loci was 40.8% in the offspring of female *pipiens*, while it was 11% in the backcrosses of male *pal-pip* hybrids, zero in the backcrosses of male *sph-ber* hybrids, and also zero in the backcrosses of male *sph-bla* hybrids. The recombination rate between the Ab and F16DP loci was 7.5% in the backcrosses of male *pal-pip* hybrids, and was very small in the backcrosses of male *sph-ber* hybrids. The recombination rate between the F16DP and PGM-1 loci was

4% in the backcrosses of male *pal-pip* hybrids, and was very small in the backcrosses of male *sph-ber* hybrids. The recombination rate between the PGM-1 and β-GLU loci was 17% in the offspring of male *pipiens* and 10% in the backcrosses of male *sph-ber* hybrids. The results of the experiments performed by WRIGHT and others seemed to show that there was no intimate relationship between the recombination rates and the existence of sex-determining genes. Furthermore, their experiments almost dealt with the recombination rates in males. Thus, the recombination rates in females were mostly dubious, even though they were larger than those in males.

The present authors examined the recombination rates among six loci, LDH-B, Pep-B, HK, ENO, MPI and SORDH, in order to clarify the sexual differences in recombination rates at the time when germ cells are formed in male and female *R. brevipoda* and *R. nigromaculata*. They used the following females and males as materials. (1) An intraspecific female hybrid, NN.AK♀, produced from the Aomori population of *R. nigromaculata* (NN.Ao)♀ × the Kaita population of *R. nigromaculata* (NN.Ka)♂. (2) Interspecific female hybrids, NB♀, produced from the Aomori population of *R. nigromaculata* (NN.Ao)♀ × the Maibara population of *R. brevipoda* (BB.Ma)♂. (3) Interspecific female hybrids, CN♀, produced from *R. plancyi chosenica* (CC)♀ × the Hiro population of *R. nigromaculata* (NN.Hr)♂. (4) Intraspecific female hybrids, BB.KM♀, produced from the Konko population of *R. brevipoda* (BB.Ko)♀ × the Maibara population of *R. brevipoda* (BB.Ma)♂. (5) Intraspecific female hybrids, BB.MK♀, produced from the Maibara population♀ × the Konko population of *R. brevipoda*♂. (6) Males of the Kaita population of *R. nigromaculata*, NN.Ka♂, some of which had sex-determining genes on chromosome No. 4, while the others had none. (7) Males of intraspecific hybrids, NN.AK♂, produced from the Aomori population♀ × the Kaita population of *R. nigromaculata*♂. (8) Males of the Maibara population of *R. brevipoda*, BB.Ma♂, which had no sex-determining genes on chromosome No. 4. (9) Males of intraspecific hybrids, BB.KM♂, produced from the Konko population♀ × the Maibara population of *R. brevipoda*♂. (10) Males of the Konko population of *R. brevipoda*, BB.Ko♂. (11) Males of intraspecific hybrids, BB.MK♂, produced from the Maibara population♀ × the Konko population of *R. brevipoda*♂.

Sexual differences in recombination rates between various loci were examined by using the foregoing five kinds of females and six kinds of males. The recombination rate between the SORDH and MPI loci was 2.5% in the offspring of a female NN.AK hybrid, 2.8% in the offspring of female NB hybrids, and 5.1% in the offspring of female CN hybrids. In contrast, the recombination rates between the SORDH and MPI loci in the offspring of a NN.Ka male in which the sex-determining genes linked with the SORDH and MPI loci on chromosome No. 4 and in the offspring of NN.Ka or NN.AK males in which they did not link with the two loci were zero. The recombination rate between the SORDH and ENO loci in the offspring of female NB hybrids was 7.1%, while it was zero in the offspring of heterozygous males of NN.Ka. The recombination rate between the SORDH and HK loci in the offspring of a female NN.AK hybrid was high, being

38.3% and that in those of female NB hybrids was 27.0%. In contrast, the recombination rate in the offspring of a male NN.Ka was extremely low, being 0.3%. The recombination rate between the SORDH and Pep-B loci was 37.0% in the offspring of a heterozygous female of NN.AK, 27.0% in the offspring of female NB hybrids and 26.4% in the offspring of female CN hybrids. In contrast, the recombination rate in the offspring of heterozygous males of NN.AK was zero.

The recombination rate between the MPI and HK loci in the offspring of a female BB.Ma in which the genotypes of the two loci were heterozygous was very high, being 47.4%, that in the offspring of a female NN.AK was 39.5%, and that in the offspring of female NB hybrids was 24.1%, on the average, the recombination rate in the offspring of these three kinds of females was 37.8%, while that in the offspring of a heterozygous male of NN.Ka was 0.4%. The recombination rate between the MPI and Pep-B loci in the offspring of a female NN.AK was 37.0%, that in the offspring of female NB hybrids was 24.1%, and that in the offspring of female CN hybrids was 23.8%. In contrast, the recombination rate in the offspring of three heterozygous males of NN.AK was zero. The recombination rate between the MPI and LDH-B loci in the offspring of three female BB.KM hybrids was 50.3%, that in the offspring of three female BB.MK hybrids was 48.1%, that in the offspring of four female NB hybrids was 34.8%, and that in the offspring of four female CN hybrids was 26.4%. In contrast, the recombination rate in the offspring of male BB.KM hybrids was 0.1%, that in the offspring of male BB.MK hybrids was 0.1%, and that in the offspring of four heterozygous males of BB.Ma was 4.3%.

The recombination rate between the ENO and HK loci in the offspring of four female NB hybrids was 18.4%. In contrast, that in the offspring of a heterozygous male of NN.Ka was zero. The recombination rate between the ENO and Pep-B loci, and that between the ENO and LDH-B loci in the offspring of female NB hybrids were 19.9% and 30.5%, respectively. The recombination rates between the HK and Pep-B loci in the offspring of a female NN.AK hybrid and four female NB hybrids were 9.3% and 2.8%, respectively. The recombination rate between the HK and LDH-B loci, and that between the Pep-B and LDH-B loci in the offspring of female NB hybrids were 17.7% and 16.3%, respectively. The recombination rate between the Pep-B and LDH-B loci in the offspring of female CN hybrids was 6.8%.

The recombination rates in the offspring of these four kinds of females agreed well with chiasma frequencies in their bivalent (lampbrush) chromosome No. 4 according to OHTANI (1990). He reported that the average chiasma numbers found in bivalent (lampbrush) chromosome No. 4 of oocytes were 4.36 in 10 females of *R. nigromaculata*, NN, collected from Hiroshima, 4.14 in 11 females of *R. brevipoda*, BB, collected from Konko, 4.64 in eight females of *R. plancyi chosenica*, CC, collected from Korea, 2.96 in nine females of NB hybrids, and 3.20 in nine females of CN hybrids.

OKUMOTO (1980) observed meiotic spreads contained in the testes of 16 males of *R. nigromaculata*, NN, collected from Hiroshima, in those of 13 males of *R. brevipoda*,

BB, collected from Konko and in those of 10 male NB hybrids between these two species. It was found that in 1905 meiotic spreads obtained from 16 males of NN, 0.7% of large chromosomes Nos. 1~5 were univalents, while the other 99.3% were bivalents. Of the bivalents, 89.4% were ring-shaped, while the other 10.6% were rod-shaped. On the other hand, in 1067 meiotic spreads obtained from 13 males of BB, 4.4% of large chromosomes Nos. 1~5 were univalents, while the other 95.6% were bivalents. Of the bivalents, 72.9% were ring-shaped, while the remaining 27.1% were bivalents. In 1110 meiotic spreads obtained from 10 male NB hybrids, 16.8% of large chromosomes Nos. 1~5 were univalents, while the other 83.2% were rod-shaped. Of the bivalents, 15.2% were ring-shaped, while the other 84.8% were rod-shaped. Thus, it was quite clear that chiasmata were scarcely formed in the meioses of the foregoing three kinds of males, NN, BB and NB.

The recombination rates among the loci controlling six enzymes located on chromosome No. 4 were large in females when the frogs were pure species, while they became smaller when the frogs were hybrids. They were almost zero in males in contrast to females. It was found that the recombination rates among the six loci had no relation to the existence of the sex-determining genes on the chromosomes of population of the species used in the present study. The differences in the recombination rates seemed to be attributable to that in the number of chiasmata. When the number of chiasmata became smaller, the recombination rates became also smaller, and in male frogs, they seemed to near zero. Thus, it was found in the present study that it may be better to use heterozygous males for the purpose of assuming the kinds of genes on specific chromosome, although it was difficult to assume the position of the loci on the chromosome on the basis of the recombination rates in males. However, it may be better to use heterozygous females in order to clarify the mutual positions of loci on the same chromosome.

ACKNOWLEDGMENTS

The authors are especially indebted to Emeritus Professor Toshijiro KAWAMURA for his encouragement and guidance during the course of this work and for his critical review of the manuscript.

This work was supported by a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Science and Culture.

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