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The Position of Sex-Determining Genes in the Chromosomes of *Rana nigromaculata* and *Rana brevipoda*

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ABSTRACT

The present authors performed crossing experiments to examine the presence of linkages between the sex-determining genes and the seven loci controlling seven enzymes located on chromosomes Nos. 3 and 4 in *Rana brevipoda* and *Rana nigromaculata*. In the crossings, male frogs whose genotypes of some enzymes were always heterozygous were used and the genotypes of the same enzymes in the offspring were compared with those of the male parents. When the genotypes of the offspring were almost the same in constitution as those of the male parents, the loci of these enzymes were considered to be linked with the sex-determining genes and the frogs were considered to be male heterogametic.

When heterozygous males of the Konko population of *R. brevipoda* were used, the sex-determining genes were found to be linked with the MPI, LDH-B and Pep-B loci on chromosome No. 4, and their recombination rates were assumed to be 11.1%, 10.5% and 5.1%, respectively, while the sex-determining genes were not linked with the ME-B locus situated on chromosome No. 3. On the other hand, when heterozygous males of the Maibara population of *R. brevipoda* were used, the sex-determining genes were found to be linked with the ME-B locus on chromosome No. 3 and the recombination rate was assumed to be 2.2%. The sex-determining genes were not linked with the MPI, LDH-B and Pep-B loci situated on chromosome No. 4. When heterozygous males of the Hiro and Kumano populations of *R. nigromaculata* were used, the sex-determining genes were found to be linked with the MPI locus on chromosome No. 4, and the recombination rates were assumed to be 8.9% and 8.6%, respectively. These results suggested that the above stated frogs were male heterogametic. In contrast, in five of the nine male frogs of the Kaita population of *R. nigromaculata*, the sex-determining genes were linked with the MPI, SORDH, ENO and HK loci on chromosome No. 4. The recombination rates of these loci were assumed to be 7.9%, 6.2%, 11.4% and 8.7%, respectively. In the remaining four male frogs, the sex-determining genes were not linked with the MPI and SORDH loci on chromosome No. 4 as well as the ME-B locus on chromosome No. 3.

In the analyses of the offspring of female frogs whose genotypes were heterozygous in any of the seven loci situated on chromosomes Nos. 3 and 4, no locus linked with the sex-determining genes was discovered.

INTRODUCTION

In both *Rana nigromaculata* and *R. brevipoda*, the chromosomes were 26 in diploid number and consisted of five pairs of large ones and eight pairs of small ones, and there were no sexual differences in the karyotype, the C-banding pattern and the late replication (LR) banding pattern (NISHIOKA, 1972; NISHIOKA, OKUMOTO and RYUZAKI, 1987; MIURA, 1987). KAWAMURA and NISHIOKA (1977) reported that these species were of the XY type in sex-determining mechanism on the basis of the studies of diploid gynogenesis and sex-reversed females obtained by hormone treatment.

NISHIOKA, OHTANI and SUMIDA (1980) made the following assumptions from the comparison of the structures of 13 bivalents (lampbrush chromosomes) in the oocytes of female backcrosses obtained from female reciprocal hybrids between *R. nigromaculata* and *R. brevipoda* with the genotypes of seven loci controlling four kinds of enzymes, α-GDH, MDH-B, LDH-B and IDH-B, and three kinds of blood proteins, Hb, Ab and Prot-C. The Ab, α-GDH, MDH-B and LDH-B loci were located on chromosomes Nos. 1, 2, 3 and 4, respectively. The Hb and IDH-B loci were located on chromosome No. 6 and the Prot-C locus was located on chromosome No. 9. They (1987) also made the following assumptions on the location of various loci from the comparison of the structures of the lampbrush chromosomes in the oocytes of female backcrosses between the foregoing two species with the genotypes of five albino loci and 23 loci controlling various enzymes and blood proteins, and from the linkage relationships between various loci. The three loci of Ab, ADH-A and albino gene *b* were on chromosome No. 1, the six loci of Pep-C, albino gene *c*, SOD-B, albino gene *a*, ME-A and α-GDH were on chromosome No. 2, the three loci of MDH-B, albino gene *e* and ME-B were on chromosome No. 3, the four loci of LDH-B, HK, Pep-B and MPI were on chromosome No. 4, the Pep-A locus was on chromosome No. 5, the two loci of Hb and IDH-B were on chromosome No. 6, the three loci of Prot-C, ALD and albino gene *d* were on chromosome No. 9, the five loci of Est-1, Est-2, Est-4, Est-5 and Pep-D were on chromosome No. 10 and the ADA locus was on chromosome No. 11. NISHIOKA and OHTANI (1986) reported that the gene controlling the olive mutation which was characterized by a small number of thin reflecting platelets was located between the ME-B and MDH-B loci on chromosome No. 3. It was assumed that the olive gene was located close to a landmark near the centromere of the lampbrush chromosome. They also reported that the gene controlling the blue mutation which had no carotenoid vesicles in xanthophores was assumed to be located on chromosome No. 8. NISHIOKA and SUMIDA (1994) recently reported that the ENO and SORDH loci were located on chromosome No. 4 and linked with the LDH-B, HK, MPI and Pep-B loci.

The studies on sex-linked genes in amphibians have been made in some European urodeles and American anurans. FERRIER, JAYLET, CAYROL, GASSER and BUISAN (1980), FERRIER, GASSER, JAYLET and CAYROL (1983), and DOURNON, GUILLET, BOUCHER and LACROIX (1984) reported that in *Pleurodeles waltl* and *P.*

poireti, the peptidase 1 (Pep-1) locus was sex-linked and these urodeles were female heterogametic. In anurans, ELINSON (1981) reported that the LDH-B locus was sex-linked and the male was heterogametic in *Rana catesbeiana*, and that the MPI locus was linked with the LDH-B locus. He (1983) also reported that the aconitase 1 locus was sex-linked and the male was heterogametic in *Rana clamitans*. In *Rana pipiens*, WRIGHT and RICHARDS (1983) and WRIGHT, RICHARDS, FROST, CAMOZZI and KUNZ (1983) reported that the peptidase C and SOD-1 loci were sex-linked and the male was heterogametic.

The present authors in 1983 mated a male with a female in *Rana brevipoda* collected from Konko, Okayama Prefecture, and three males with three females in *Rana nigromaculata* collected from Hiro, Hiroshima Prefecture. They (1989) assumed from a preliminary experiment that in both species the males were heterogametic and the sex-determining genes were probably linked with the MPI locus on chromosome No. 4. Thereafter, they performed experiments to examine the presence of linkages between the sex-determining genes and seven loci controlling seven enzymes located on chromosomes Nos. 3 and 4.

MATERIALS AND METHODS

The frogs used in the present study were as follows. 1. Eleven females (BB.Ko♀, Nos. 1~11) and 10 males (BB.Ko♂, Nos. 1~9 and 11) of the Konko population belonging to the Typical race of *Rana brevipoda* ITO collected from Konko-cho, Okayama Prefecture, and three females (BB.Ma♀, Nos. 1~3) and eight males (BB.Ma♂, Nos. 1~8) of the Maibara population belonging to the Nagoya race of *Rana brevipoda* collected from Maibara-cho, Shiga Prefecture (KAWAMURA, 1962), and six male hybrids (BB.MK♂, Nos. 1~6) and four male hybrids (BB.KM♂, Nos. 1~4) produced from reciprocal crossings between the Maibara and Konko populations of *Rana brevipoda*. 2. Three females (NN.Hr♀, Nos. 1~3) and three males (NN.Hr♂, Nos. 1~3) of the Hiro population of *Rana nigromaculata* HALLOWELL collected from Hiro, Kure city, Hiroshima Prefecture, seven females (NN.Km♀, Nos. 1~7) and three males (NN.Km♂, Nos. 1~3) of

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
Malic enzyme	ME	1.1.1.40	〃	T-C pH 7.0
Mannose phosphate isomerase	MPI	5.3.1.8	〃	〃
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

TABLE 2

Genotypes at seven loci of each

Kind	Sex	No.	Year used	Genotype						
				ENO	HK	LDH-B	ME-B	MPI	Pep-B	SORDH
BB.Ko	♀	1	1983	—	—	*cc*	—	*dd*	—	—
		2	1985	—	—	*cc*	—	*dd*	—	—
		3	1986	—	—	*cc*	—	*aa*	—	—
		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*	*dd*	*aa*	*bb*	*ff*
		10	1988	*aa*	—	*cc*	*dd*	*ad*	*bb*	*ff*
		11	1988	*aa*	—	*cc*	*dd*	*ad*	*ab*	*ff*
	♂	1	1983	—	—	*cc*	—	*ad*	—	—
		2	1985	*aa*	—	*cc*	*dd*	*ad*	—	—
		3	1985	*aa*	—	*cc*	*dd*	*ad*	—	—
		4	1985	*aa*	—	*cc*	*dd*	*ad*	—	—
		5	1986	*aa*	*aa*	*cc*	*dd*	*aa*	*bb*	*ff*
		6	1988	*aa*	*aa*	*cc*	*dd*	*aa*	*ab*	*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*
		11	1989	*aa*	*aa*	*cc*	—	*aa*	*bb*	*ff*
BB.Ma	♀	1	1986	*aa*	*ab*	*cd*	—	*cc*	*bb*	*ff*
		2	1988	*aa*	*ab*	*dd*	*fg*	*cd*	*bb*	*ff*
		3	1988	*aa*	*aa*	*dd*	*df*	*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*
		5	1986	—	*aa*	*dd*	—	*de*	*bb*	*ff*
		6	1986	—	*aa*	*dd*	—	*de*	*bb*	*ff*
		7	1986	—	*ac*	*dd*	—	*de*	*bb*	*ff*
		8	1986	—	*aa*	*dd*	—	*de*	*bb*	*ff*
BB.MK	♂	1	1988	*aa*	*ab*	*dc*	*fd*	*ca*	*bb*	*ff*
		2	1988	*aa*	*aa*	*dc*	*fd*	*ca*	*bb*	*ff*
		3	1988	*aa*	*aa*	*dc*	*fd*	*ca*	*bb*	*ff*
		4	1988	*aa*	*aa*	*dc*	*fd*	*ca*	*bb*	*ff*
		5	1990	*aa*	*aa*	*dc*	—	*ca*	*bb*	*ff*
		6	1990	*aa*	*aa*	*dc*	—	*ca*	*bb*	*ff*
BB.KM	♂	1	1988	*aa*	*aa*	*cd*	*df*	*ae*	*bb*	*ff*
		2	1988	*aa*	*aa*	*cd*	*df*	*ae*	*bb*	*ff*
		3	1988	*aa*	—	*cd*	*dd*	*ae*	*bb*	*ff*
		4	1988	*aa*	—	*cd*	*dd*	*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*

—, not examined.

individual used in the present study

Kind	Sex	No.	Year used	Genotype						
				ENO	HK	LDH-B	ME-B	MPI	Pep-B	SORDH
NN.Hr	♀	1	1983	—	—	*bb*	—	*dd*	—	—
		2	1983	—	—	*bb*	—	*dd*	—	—
		3	1983	—	—	*bb*	—	*dd*	—	—
	♂	1	1983	—	—	*bb*	—	*gd*	—	—
		2	1983	—	—	*bb*	—	*gd*	—	—
		3	1983	—	—	*bb*	—	*gd*	—	—
NN.Km	♀	1	1985	—	—	*bb*	—	*dd*	—	—
		2	1985	—	—	*bb*	—	*dd*	—	—
		3	1985	—	—	*bb*	—	*dd*	—	—
		4	1985	—	—	*bb*	—	*dd*	—	—
		5	1985	—	—	*bb*	—	*dd*	—	—
		6	1985	—	—	*bb*	—	*dd*	—	—
		7	1985	—	—	*bb*	—	*dd*	—	—
	♂	1	1985	*bb*	—	*bb*	—	*gd*	*bb*	*bb*
		2	1985	*bb*	—	*bb*	—	*gd*	*bb*	*bb*
		3	1985	*bb*	—	*bb*	—	*gd*	*bb*	*bb*
NN.Ao	♀	1	1988	*bb*	*cc*	*bb*	*gg*	*dd*	*cc*	*bb*
NN.Ka	♀	1	1988	*bb*	*cd*	*bb*	*gh*	*dd*	*bb*	*ab*
		2	1988	*bb*	*cc*	*bb*	*gg*	*dd*	*bb*	*bb*
		3	1988	*bb*	*dd*	*bb*	*gi*	*gd*	*bb*	*ab*
		4	1988	*bb*	*cc*	*bb*	*ii*	*dd*	*bb*	*bb*
		5	1990	*bb*	—	*bb*	*gg*	*dg*	*bb*	*bc*
		6	1990	*bb*	—	*bb*	*gh*	*dg*	*bb*	*bb*
		7	1990	*bb*	—	*bb*	*gg*	*dg*	*bb*	*bb*
		8	1990	*bb*	—	*bb*	*gg*	*dg*	*bb*	*bb*
		9	1990	*bb*	*dc*	*bb*	*gg*	*dg*	*bb*	*bb*
		10	1990	*bb*	—	*bb*	*hi*	*dg*	*bb*	*bb*
		11	1990	*bb*	—	*bb*	*ii*	*dg*	*bb*	*bb*
		12	1990	*bb*	—	*bb*	*gg*	*dg*	*bb*	*bb*
		13	1990	*bb*	—	*bb*	*gg*	*dg*	*bb*	*bb*
		14	1990	*bb*	—	*bb*	*gg*	*dg*	*bb*	*bb*
		15	1990	*bb*	*dc*	*bb*	*gg*	*dg*	*bb*	*bb*
	♂	1	1988	*bb*	*bc*	*bb*	*gg*	*gd*	*bb*	*ab*
		2	1988	*bb*	*cc*	*bb*	*gg*	*gd*	*bb*	*ab*
		3	1988	*ba*	*dc*	*bb*	*hh*	*dd*	*bb*	*ab*
		4	1988	*bb*	*cc*	*bb*	*gi*	*dd*	*bb*	*ab*
		5	1988	*bb*	*cc*	*bb*	*hg*	*gd*	*bb*	*ab*
		6	1988	*bb*	*cc*	*bb*	*hg*	*gd*	*bb*	*db*
		7	1988	*bb*	*cc*	*bb*	*gh*	*gg*	*bb*	*cb*
		8	1988	*bb*	*cc*	*bb*	*gg*	*dd*	*bb*	*cb*
		9	1988	*bb*	*cc*	*bb*	*gg*	*gg*	*bb*	*cb*
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*

the Kumano population of *Rana nigromaculata* collected from Kumano-cho, Aki-gun, Hiroshima Prefecture, 15 females (NN.Ka♀, Nos. 1~15) and nine males (NN.Ka♂, Nos. 1~9) of the Kaita population of *Rana nigromaculata* collected from Kaita-cho, Aki-gun, Hiroshima Prefecture, and one female (NN.Ao♀, No. 1) of the Aomori population of *Rana nigromaculata* collected from Hirosaki city, Aomori Prefecture. 3. Four female hybrids (CN♀, Nos. 1~4) between two female *Rana plancyi chosenica* OKADA and a male *Rana nigromaculata* of the Hiro population. Four female hybrids (NB♀, Nos. 1~4) between a female *R. nigromaculata* of the Aomori population and a male *R. brevipoda* of the Maibara population.

All the mating experiments were conducted by the artificial fertilization method. Ovulation was accelerated by injection of suspension of bullfrog pituitaries into the body cavity. Tadpoles were fed on boiled spinach or chard. Metamorphosed frogs were fed on tropical crickets, *Gryllus bimaculatus* DE GEER (NISHIOKA and MATSUURA, 1977).

The enzymes extracted from livers and skeletal muscles of the above mentioned 91 frogs and their offspring used in the mating experiments were analyzed by the method of horizontal starch-gel electrophoresis. The kinds of enzymes analyzed, their abbreviations, E. C. Nos., samples and buffer systems are shown in Table 1. The method of electrophoresis has been reported previously by NISHIOKA, OHTANI and SUMIDA (1980). The detection of each enzyme was performed by the method of BREWER (1970) and HARRIS and HOPKINSON (1976) with a slight modification. In order to judge the presence of linkage between the locus of each enzyme and the sex-determining genes, contingency χ^2 values were calculated at the significance level of $p<0.01$ (CAMPBELL, 1974; WRIGHT, RICHARDS and NACE, 1980; WRIGHT and RICHARDS, 1983).

The genotypes of the seven loci in 91 frogs 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. Rana brevipoda of the Konko population belonging to the Typical race

A. Matings with males whose genotypes of the MPI, LDH-B, Pep-B and ME-B loci were heterozygous

1. Linkage between the sex-determining genes and the MPI locus

In 1983 and 1985, as four male *R. brevipoda* (BB.Ko♂, Nos. 1~4) in which the genotype of the MPI locus on chromosome No. 4 was heterozygous *ad* were obtained, these males were mated with two female *R. brevipoda* of the Konko population (BB.Ko♀, Nos. 1 and 2) and seven female *R. nigromaculata* of the Kumano population (NN.Km♀, Nos. 1~7), which were homozygous *dd* at the MPI locus. From these nine matings, BB.Ko♀, Nos. 1 and 2 × BB.Ko♂, Nos.

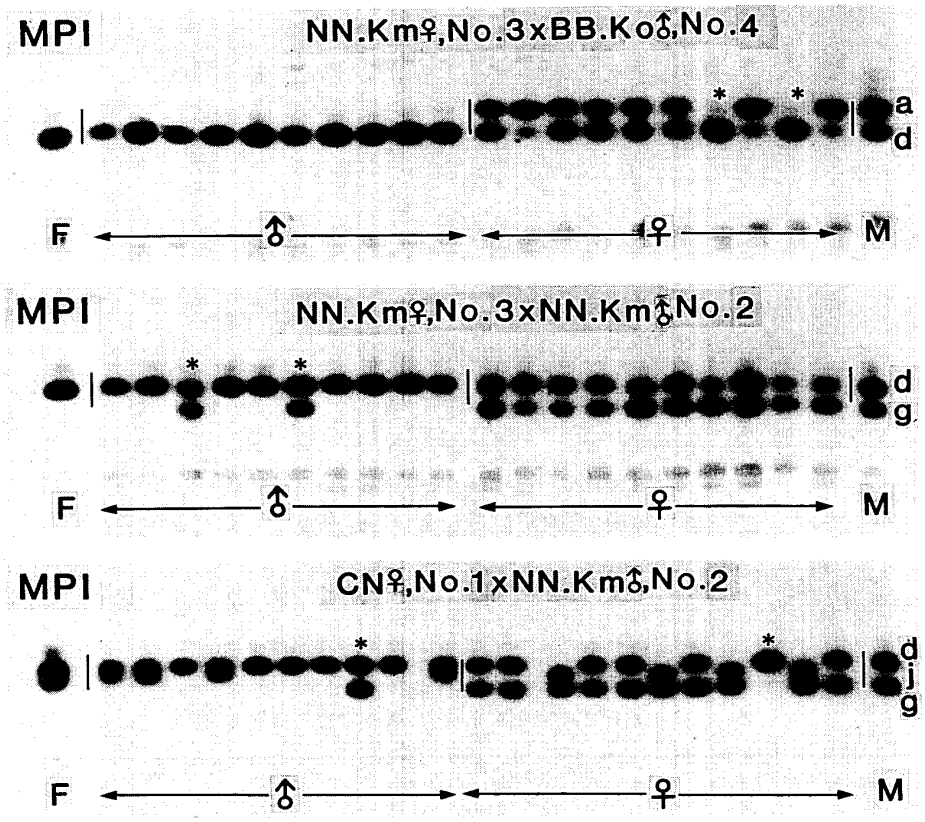

Fig. 1. Electrophoretic patterns of MPI enzyme from female and male offspring and their parents. The upper gel slice shows the MPI patterns of the offspring produced from NN.Km♀, No. 3 × BB.Ko♂, No. 4 (Table 3). The genotypes of female (F) and male (M) parents were *dd* and *ad*, respectively. All the 10 male offspring were *dd*, while eight of the 10 female offspring were *ad* and the remaining two female offspring were *dd**. The middle gel slice shows the offspring produced from NN.Km♀, No. 3 × NN.Km♂, No. 2 (Table 8). The genotypes of female and male parents were *dd* and *gd*, respectively. Eight of the 10 male offspring were *dd*, while the other two male offspring were *dg**. All the 10 female offspring were *dg*. The lower gel slice shows the offspring produced from CN♀, No.1 × NN.Km♂, No. 2 (Table 8). The genotypes of female and male parents were *jd* and *gd*, respectively. Nine of the 10 male offspring were *jd* or *dd* and the remaining one male was *dg**. While 10 of the 11 female offspring were *jg* or *dg* and the remaining one female was *dd**. *, Recombinants

1 and 2, and NN.Km♀, Nos. 1~7 × BB.Ko♂, Nos. 3 and 4, 947 offspring consisting of 473 females and 474 (50.1%) males were produced. These offspring were analyzed by the electrophoretic method to examine the linkage between the sex-determining genes and the MPI locus (Fig. 1). The results showed that 413 of the 473 females were heterozygous *da*, while the other 60 were homozygous *dd*. On the other hand, 418 of the 474 males were homozygous *dd*, while the other 56 were heterozygous *da*. The sex-determining genes agreed with the MPI genotype in constitution, as 831 (87.8%) of the offspring were males or females which were parental ($\chi^2=539.84$, $P<0.00001$). The sex-determining genes disagreed with the MPI genotype in 116 (12.2%) offspring, which were recombinants. Thus, it was assumed that the sex-determining genes in *R. brevipoda* of the Konko population linked with the MPI locus with a recombination rate of 12.2% (Table 3; Fig. 2a, b).

TABLE 3

Inheritance of the MPI, LDH-B and Pep-B loci in matings with heterozygous males of *Rana brevipoda* from the Konko population

Year	Parents		No. of metamorphosed frogs	No. of examined offspring			MPI and sex				LDH-B or Pep-B and sex				Sex-linkage of MPI, LDH-B and Pep-B				
	Female	Male		Total	♀	♂ (%)	Parents Genotype ♀ ♂	Offspring Genotype	Offspring ♀	Offspring ♂	Parents Genotype ♀ ♂	Offspring Genotype	Offspring ♀	Offspring ♂	Agree (%)	Disagree	χ^2	P	Recombination rate (%)
1983	BB.Ko, No. 1	BB.Ko, No. 1	212	136	65	71 (52.2)	*dd ad*	*da*	**64**	3	*cc cc*	*cc*	—	—	MPI 132 (97.1)	4	120.47	<0.00001	2.9
								dd	1	**68**									
1985	BB.Ko, No. 2	BB.Ko, No. 2	147	146	66	80 (54.8)	*dd ad*	*da*	**66**	0	*cc cc*	*cc*	—	—	MPI 146 (100)	0	146.0	<0.00001	0
								dd	0	**80**									
	NN.Km, Nos. 1~7	BB.Ko, Nos. 3, 4	1019	665	342	323 (48.6)	*dd ad*	*da*	**283**	53	*bb cc*	*bc*	—	—	MPI 553 (83.2)	112	292.45	<0.00001	16.8
								dd	59	**270**									
	Total		1378	947	473	474 (50.1)		*da*	**413**	56			—	—	MPI 831 (87.8)	116	539.84	<0.00001	12.2
								dd	60	**418**									
1988	BB.Ko, No. 9	BB.MK, No. 1	74	67	29	38 (56.7)	*aa ca*	*ac*	**29**	0	*cc dc*	*cd*	**29**	0	MPI → 67 (100)	0	67.0	<0.00001	0
								aa	0	**38**		*cc*	0	**38**	LDH-B				
		BB.MK, No. 2	92	68	30	38 (55.9)	*aa ca*	*ac*	**30**	1	*cc dc*	*cd*	**30**	1	MPI → 67 (98.5)	1	64.06	<0.00001	1.5
								aa	0	**37**		*cc*	0	**37**	LDH-B				
	BB.Ko, No. 10	BB.MK, No. 3	6	6	2	4 (66.7)	*ad ca*	*ac dc*	**2**	0	*cc dc*	*cd*	**2**	0	MPI → 6 (100)	0	6.0	0.02	0
								aa da	0	**4**		*cc*	0	**4**	LDH-B				
		BB.MK, No. 4	110	100	48	52 (52.0)	*ad ca*	*ac dc*	**48**	0	*cc dc*	*cd*	**48**	0	MPI →100 (100)	0	100.0	<0.00001	0
								aa da	0	**52**		*cc*	0	**52**	LDH-B				
	Total		282	241	109	132 (54.8)		*-c*	**109**	1		*cd*	**109**	1	MPI 240 (99.6)	1	237.02	<0.00001	0.4
								-a	0	131		*cc*	0	**131**	LDH-B				
1988	BB.Ma, No. 2	BB.MK, No. 1	107	77	39	38 (49.4)	*cd ca*	*cc dc*	**39**	0	*dd dc*	*dd*	**39**	0	MPI → 77 (100)	0	77.0	<0.00001	0
								ca da	0	**38**		*dc*	0	**38**	LDH-B				
		BB.MK, No. 2	108	67	32	35 (52.2)	*cd ca*	*cc dc*	**32**	0	*dd dc*	*dd*	**32**	0	MPI → 67 (100)	0	67.0	<0.00001	0
								ca da	0	**35**		*dc*	0	**35**	LDH-B				
	BB.Ma, No. 3	BB.MK, No. 3	116	105	52	53 (50.5)	*dd ca*	*dc*	**52**	1	*dd dc*	*dd*	**52**	1	MPI →104 (99.0)	1	101.04	<0.00001	1.0
								da	0	**52**		*dc*	0	**52**	LDH-B				
		BB.MK, No. 4	116	97	44	53 (54.6)	*dd ca*	*dc*	**44**	1	*dd dc*	*dd*	**44**	1	MPI → 96 (99.0)	1	93.04	<0.00001	1.0
								da	0	**52**		*dc*	0	**52**	LDH-B				
	Total		447	346	167	179 (51.7)		*-c*	**167**	2		*dd*	**167**	2	MPI 344 (99.4)	2	338.05	<0.00001	0.6
								-a	0	**177**		*dc*	0	**177**	LDH-B				
1988	NN.Ao, No. 1	BB.MK, No. 1	116	104	49	55 (52.9)	*dd ca*	*dc*	**49**	2	*bb dc*	*bd*	**49**	2	MPI →102 (98.1)	2	96.15	<0.00001	1.9
								da	0	**53**		*bc*	0	**53**	LDH-B				
		BB.MK, No. 2	95	31	17	14 (45.2)	*dd ca*	*dc*	**17**	0	*bb dc*	*bd*	**17**	0	MPI → 31 (100)	0	31.0	<0.00001	0
								da	0	**14**		*bc*	0	**14**	LDH-B				
	Total		211	135	66	69 (51.1)		*dc*	**66**	2		*bd*	**66**	2	MPI →133 (98.5)	2	127.12	<0.00001	1.5
								da	0	**67**		*bc*	0	**67**	LDH-B				
1988	NN.Ka, No. 1	BB.MK, No. 1	71	61	46	15 (24.6)	*dd ca*	*dc*	**37**	4	*bb dc*	*bd*	**37**	4	MPI → 48 (78.7)	13	20.08	<0.00001	21.3
								da	9	11		*bc*	9	**11**	LDH-B				
		BB.MK, No. 2	69	53	35	18 (34.0)	*dd ca*	*dc*	**28**	4	*bb dc*	*bd*	**28**	5	MPI 42 (79.2)	11	18.13	<0.0001	20.8
								da	7	**14**		*bc*	7	**13**	LDH-B 41 (77.4)	12	15.87	<0.0001	22.6
	Total		140	114	81	33 (28.9)		*dc*	**65**	8		*bd*	**65**	9	MPI 90 (78.9)	24	38.21	<0.00001	21.1
								da	16	**25**		*bc*	16	**24**	LDH-B 89 (78.1)	25	35.93	<0.00001	21.9

1988	BB.Ko, No. 10	BB.Ko, No. 6	102	91	38	53 (58.2)	*ad aa*	*aa*	—	—	*bb ab*	*ba*	**38**	4	Pep-B	87 (95.6)	4	75.70	<0.00001	4.4
								da	—	—		*bb*	0	**49**						
	BB.Ko, No. 11		112	95	48	47 (49.5)	*ad aa*	*aa*	—	—	*ab ab*	*aa*	**21**	0						
								da	—	—		*ab*	24	24	Pep-B	44 (93.6)	3	35.77	<0.00001	3.2
												bb	3	**23**						
	Total		214	186	86	100 (53.8)			—	—		*-a*	**59**	4	Pep-B	131 (94.9)	7	111.42	<0.00001	5.1
									—	—		*-b*	3	**72**						
1990	NN.Ka, No. 6	BB.MK, No. 5	180	134	59	75 (56.0)	*dg ca*	*dc*	**29**	5	*bb dc*	*bd*	**58**	6	MPI → LDH-B	127 (94.8)	7	107.46	<0.00001	5.2
								gc	**29**	1		*bc*	1	**69**						
								da	1	**38**										
								ga	0	**31**										
	NN.Ka, No. 7		150	103	35	68 (66.0)	*dg ca*	*dc*	**16**	4	*bb dc*	*bd*	**30**	8	MPI → LDH-B	90 (87.4)	13	57.56	<0.00001	12.6
								gc	**14**	4		*bc*	5	**60**						
								da	3	**35**										
								ga	2	**25**										
	NN.Ka, No. 8		172	75	45	30 (40.0)	*dg ca*	*dc*	**17**	4	*bb dc*	*bd*	**37**	5	MPI → LDH-B	62 (82.7)	13	32.01	<0.00001	17.3
								gc	**20**	1		*bc*	8	**25**						
								da	3	**15**										
								ga	5	**10**										
	NN.Ka, No. 9		104	59	29	30 (50.8)	*dg ca*	*dc*	**10**	1	*bb dc*	*bd*	**28**	4	MPI → LDH-B	54 (91.5)	5	40.69	<0.00001	8.5
								gc	**18**	3		*bc*	1	**26**						
								da	0	**18**										
								ga	1	**8**										
	NN.Ka, No. 10		160	105	48	57 (54.3)	*dg ca*	*dc*	**22**	9	*bb dc*	*bd*	**41**	13	MPI → LDH-B	85 (81.0)	20	40.24	<0.00001	19.0
								gc	**19**	4		*bc*	7	**44**						
								da	3	**22**										
								ga	4	**22**										
	NN.Ka, No. 11	BB.MK, No. 6	152	75	46	29 (38.7)	*dg ca*	*dc*	**20**	1	*bb dc*	*bd*	**40**	3	MPI → LDH-B	66 (88.0)	9	43.32	<0.00001	12.0
								gc	**20**	2		*bc*	6	**26**						
								da	2	**11**										
								ga	4	**15**										
	NN.Ka, No. 12		69	17	7	10 (58.8)	*dg ca*	*dc*	**2**	1	*bb dc*	*bd*	**7**	2	MPI → LDH-B	15 (88.2)	2	9.94	<0.002	11.8
								gc	**5**	1		*bc*	0	**8**						
								da	0	**2**										
								ga	0	**6**										
	NN.Ka, No. 13		338	154	51	103 (66.9)	*dg ca*	*dc*	**19**	16	*bb dc*	*bd*	**40**	25	MPI → LDH-B	117 (76.0)	37	42.88	<0.00001	24.0
								gc	**21**	10		*bc*	11	**77**						
								da	2	**36**										
								ga	9	**41**										
	NN.Ka, No. 14		318	147	84	63 (43.6)	*dg ca*	*dc*	**29**	6	*bb dc*	*bd*	**58**	15	MPI → LDH-B	106 (72.1)	41	28.74	<0.00001	27.9
								gc	**29**	9		*bc*	26	**48**						
								da	15	**21**										
								ga	11	**27**										
	NN.Ka, No. 15		99	73	41	32 (43.8)	*dg ca*	*dc*	**20**	3	*bb dc*	*bd*	**35**	5	MPI → LDH-B	62 (84.9)	11	35.63	<0.00001	15.1
								gc	**15**	2		*bc*	6	**27**						
								da	0	**18**										
								ga	6	**9**										
	Total		1742	942	445	497 (52.8)		*-c*	**374**	87		*-d*	**374**	87	MPI → LDH-B	784 (83.2)	158	417.78	<0.00001	16.8
								-a	71	**410**		*-c*	71	**410**						

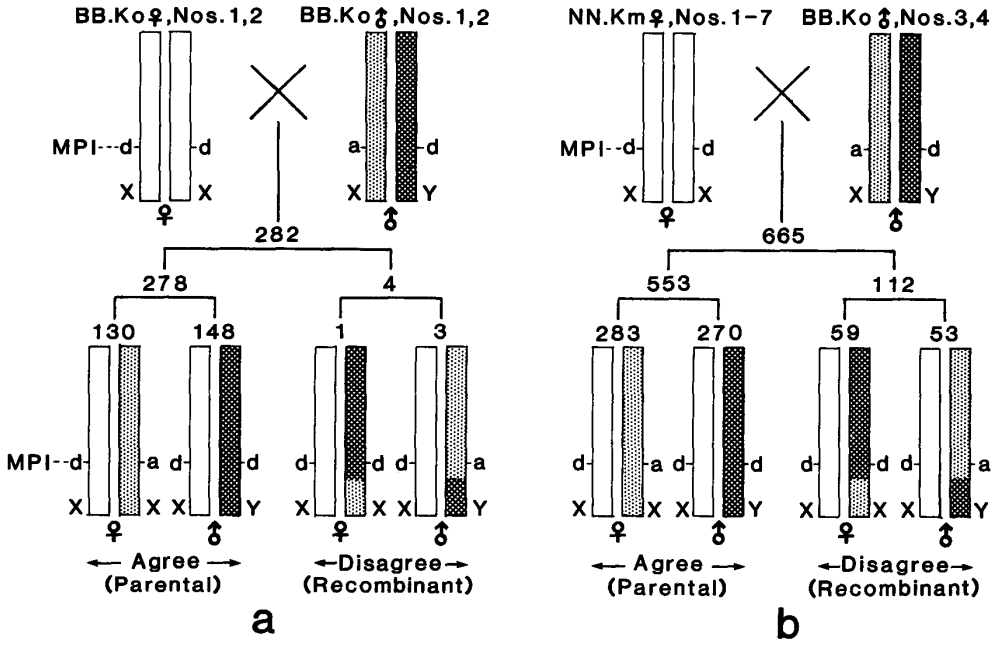

Fig. 2. Inheritance of the MPI enzyme in matings with heterozygous males and the sex-linkage of the MPI locus in *Rana brevipoda* from the Konko population. Assuming that recombinants (disagree) must have resulted from crossing-over between the sex-determining genes and the MPI locus, the linkage analyses between the sex-determining genes and the MPI locus were made.

a. Using two matings of BB.Ko♀, Nos. 1, 2 × BB.Ko♂, Nos. 1, 2 (Table 3)

b. Using seven matings of NN.Km♀, Nos. 1~7 × BB.Ko♂, Nos. 3, 4 (Table 3)

2. Linkage between the sex-determining genes and the Pep-B locus

In 1988, as a male *R. brevipoda* of the Konko population (BB.Ko♂, No. 6) in which the genotype of the Pep-B locus on chromosome No. 4 was heterozygous *ab* was obtained, this male was mated with two female *R. brevipoda* of the Konko population, one of which was homozygous *bb* (BB.Ko♀, No. 10) and the other was heterozygous *ab* (BB.Ko♀, No. 11), by two matings, BB.Ko♀, Nos. 10 and 11 × BB.Ko♂; No. 6. As there were 38 females and 53 (58.2%) males in 91 offspring obtained from a mating, BB.Ko♀, No. 10 × BB.Ko♂, No. 6, these females and males were analyzed by electrophoresis in order to examine the linkage between the sex-determining genes and the Pep-B locus. It was found that all the 38 females were heterozygous *ba*, while 49 of the 53 males were homozygous *bb* and the remaining four were heterozygous *ba*. Thus, the sex-determining genes agreed with the genotype of the Pep-B locus in constitution, that is, they were parental in 87 (95.6%) of the 91 offspring, while they disagreed with the latter, that is, they were recombinants in four offspring. Thus, it was assumed that the sex-determining genes in *R. brevipoda* of the Konko population linked with the Pep-B locus ($\chi^2=75.70$, $P<0.00001$) and the recombination rate between them was 4.4% (Table 3; Fig. 3a, b).

There were 48 females and 47 (49.5%) males in 95 offspring produced from a mating, BB.Ko♀, No. 11 × BB.Ko♂, No. 6. The results showed that all the 21

offspring which were homozygous *aa* in the genotype of the Pep–B locus were females, while of the 26 offspring which were homozygous *bb* in the genotype of the Pep–B locus, 23 were males, three were females, and all the remaining 48 were heterozygous *ab* or *ba* (*ab* and *ba* were not distinguished from each other in electrophoretic pattern). They consisted of 24 females and 24 males. The number of offspring in which the sex-determining genes agreed with the genotype of the Pep–B locus in constitution was 44 (93.6%) which were parental, while they disagreed with the latter in the other three offspring which were recombinants ($\chi^2=35.77$, $P<0.00001$). Thus, it was assumed that the sex-determining genes in *R. brevipoda* of the Konko population linked with the Pep–B locus and the recombination rate between them was 6.4% (Table 3; Fig. 3b).

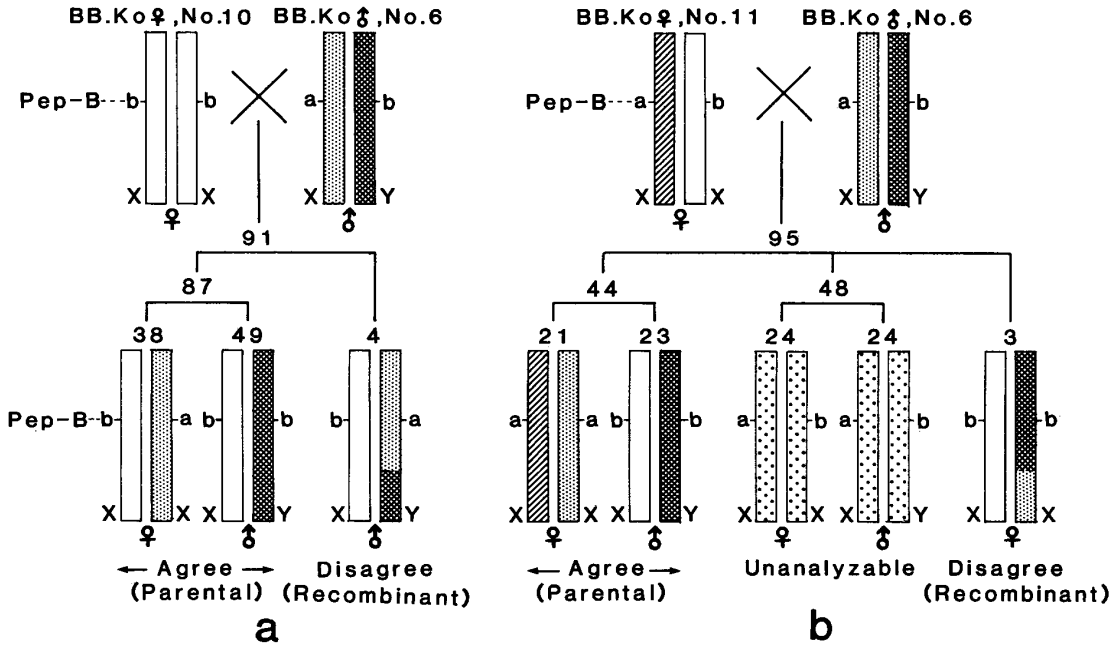

Fig. 3. Inheritance of the Pep–B enzyme in matings with a heterozygous male and the sex-linkage of the Pep–B locus in *Rana brevipoda* from the Konko population. Assuming that recombinants (disagree) must have resulted from crossing-over between the sex-determining genes and the Pep–B locus, the linkage analyses between the sex-determining genes and the Pep–B locus were made.

a. Using a mating of BB.Ko♀, No. 10 × BB.Ko♂, No. 6 (Table 3)

b. Using a mating of BB.Ko♀, No. 11 × BB.Ko♂, No. 6 (Table 3)

3. Linkage between the sex-determining genes and the MPI and LDH–B loci

a. BB.Ko♀, Nos. 9 and 10 × BB.MK♂, Nos. 1~4

In 1986, a mating, BB.Ma♀, No. 1 × BB.Ko♂, No. 5, was made between a female *R. brevipoda* of the Maibara population, Nagoya race (BB.Ma♀, No. 1), and a male *R. brevipoda* of the Konko population, Typical race (BB.Ko♂, No. 5), and hybrids (BB.MK) were obtained which were heterozygous *ca* and *dc* in the genotypes of the MPI and LDH–B loci, respectively (Table 7). In 1988, from

four matings between four male hybrids (BB.MK♂, Nos. 1~4) and two female *R. brevipoda* (BB.Ko♀, Nos. 9 and 10) of the Konko population, 241 offspring consisting of 109 females and 132 (54.8%) males were obtained. In these frogs, the presence of linkage between the sex-determining genes and the MPI and LDH-B loci was examined. It was found that the sex-determining genes agreed with the latter loci in constitution in 240 (99.6%) which were parental and disagreed in one frog which was a recombinant. Thus, it was assumed that the sex-determining genes linked with the MPI and LDH-B loci ($\chi^2=237.02$, $P<0.00001$) and the recombination rate was 0.4% (Table 3; Fig. 4a).

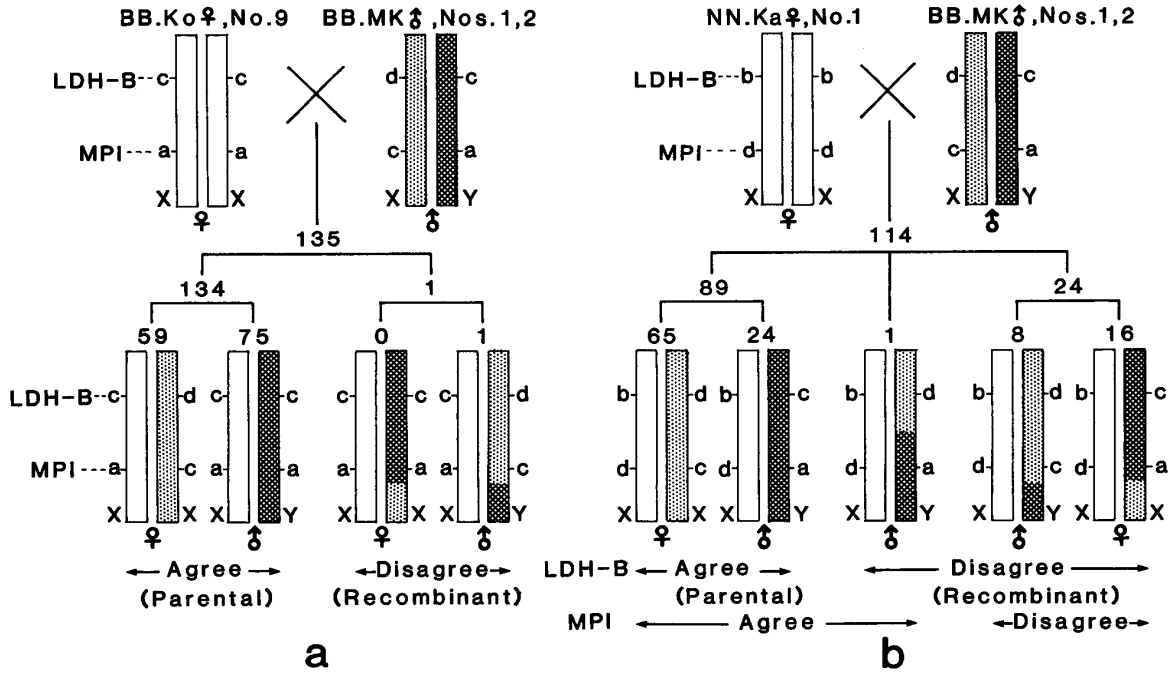

Fig. 4. Inheritance of the two enzymes, LDH-B and MPI, in matings with heterozygous males and the sex-linkage of these loci in *Rana brevipoda* from the Konko population. Assuming that recombinants (disagree) must have resulted from crossing-over between the sex-determining genes and the two loci of the LDH-B and MPI, the linkage analyses between the sex-determining genes and these two loci were made.

a. Using two matings of BB.Ko♀, No. 9 × BB.MK♂, Nos. 1, 2 (Table 3)

b. Using two matings of NN.Ka♀, No. 1 × BB.MK♂, Nos. 1, 2 (Table 3)

b. BB.Ma♀, Nos. 2 and 3 × BB.MK♂, Nos. 1~4

In 1988, from four matings between the foregoing four male hybrids of *R. brevipoda* (BB.MK♂, Nos. 1~4) and two female *R. brevipoda* of the Maibara population (BB.Ma♀, Nos. 2 and 3), 346 offspring consisting of 167 females and 179 (51.7%) males were obtained. In these frogs, the presence of linkage between the sex-determining genes and the MPI and LDH-B loci was examined (Fig. 5). It was found that the sex-determining genes agreed with the latter loci in constitution in 344 (99.4%) of the 346 frogs which were parental and disagreed in two frogs which were recombinants. Thus, it was assumed that the sex-determining genes linked with the MPI and LDH-B loci ($\chi^2=338.05$, $P<$

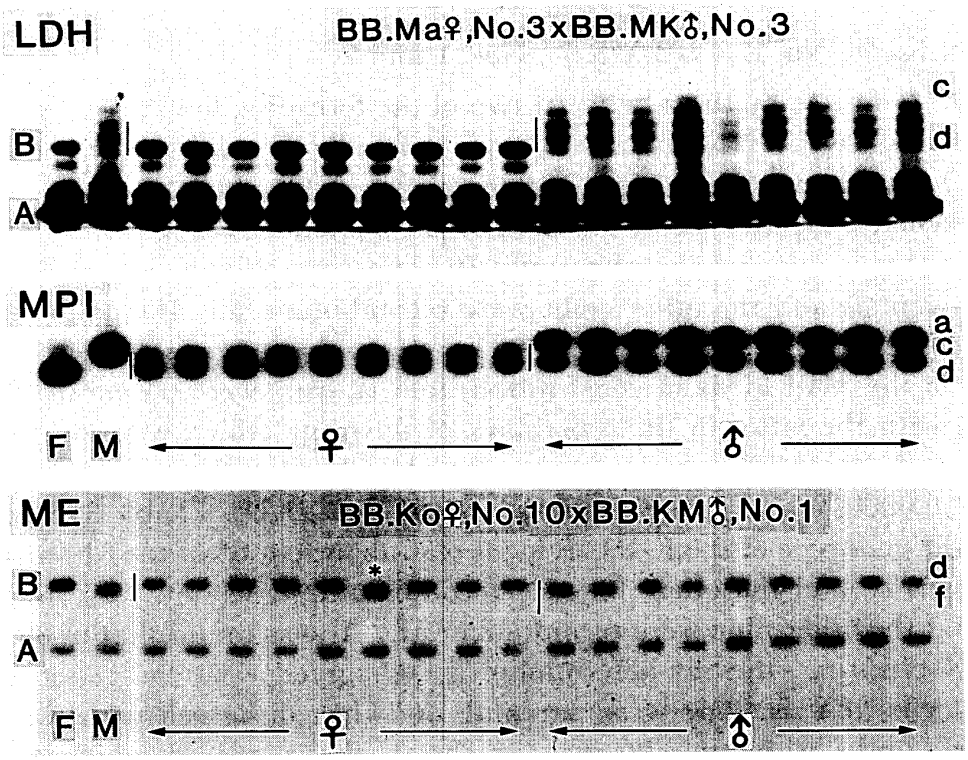

Fig. 5. Electrophoretic patterns of three enzymes from female and male offspring and their parents. The two upper gel slices show the LDH-B and MPI patterns of the offspring produced from BB.Ma♀, No. 3 × BB.MK♂, No. 3 (Table 3). At these two loci, the genotypes of male (M) parent were *dc* and *ca*, respectively, and the genotypes of female (F) parent were both *dd*. The genotypes of the LDH-B and MPI loci in the nine female offspring were *dd* and *dc*, respectively, and those in the nine male offspring were *dc* and *da*, respectively. The lower gel slice shows the ME-B pattern of the offspring produced from BB.Ko♀, No. 10 × BB.KM♂, No. 1 (Table 6). The genotypes of female and male parents were *dd* and *df*, respectively. All the nine male offspring were *df*, while eight of the nine female offspring were *dd* and the remaining one female was *df**. *, Recombinant

0.00001) and the recombination rate was 0.6% (Table 3).

c. NN.Ao♀, No. 1 × BB.MK♂, Nos. 1 and 2

In 1988, from two matings between two of the foregoing four male hybrids of *R. brevipoda* (BB.MK♂, Nos. 1 and 2) and a female *R. nigromaculata* (NN.Ao♀, No. 1), 135 offspring consisting of 66 females and 69 (51.1%) males were produced. When the genotypes of the MPI and LDH-B loci were analyzed, the 66 females were all heterozygous *dc* and the recombinant *da* was zero, while 67 of the 69 males were *da* and the other two were recombinant *dc*. In 133 (98.5%) of the 135 offspring, the sex-determining genes were found to agree with the genotypes of the MPI and LDH-B loci in constitution, that is, they were parental, while they disagreed with the latter in the remaining two offspring which were recombinants. Thus, the sex-determining genes appeared to be linked with the MPI and LDH-B loci ($\chi^2=127.12$, $P<0.00001$), and the recombination rate was 1.5% (Table 3).

d. NN.Ka♀, No. 1 × BB.MK⇕, Nos. 1 and 2

In 1988, from two matings between two of the foregoing four male hybrids of *R. brevipoda* (BB.MK⇕, Nos. 1 and 2) and a female *R. nigromaculata* (NN.Ka♀, No. 1), 114 offspring consisting of 81 females and 33 (28.9%) males were produced. When the genotype of the MPI locus was analyzed, 65 of the 81 females were heterozygous *dc* and the remaining 16 were recombinant *da*, while 25 of the 33 males were *da* and the other eight were recombinant *dc*. It was found that in 90 (78.9%) of the 114 offspring, the sex-determining genes agreed with the genotype of the MPI locus in constitution, that is, they were parental, while they disagreed with the latter in the remaining 24 offspring which were recombinants. Thus, the sex-determining genes appeared to be linked with the MPI locus ($\chi^2=$ 38.21, $P<0.00001$), and the recombination rate was 21.1% (Table 3; Fig. 4b).

When the genotype of the LDH-B locus was analyzed, 65 of the 81 females were heterozygous *bd* and the other 16 were recombinant *bc*, while 24 of the 33 males were *bc* and the other nine were recombinant *bd*. It was found that in 89 (78.1%) of the 114 offspring, the sex-determining genes agreed with the genotype of the LDH-B locus, that is, they were parental, and they disagreed with the latter in constitution in the remaining 25 offspring, that is, they were recombinants. Thus, the sex-determining genes appeared to be linked with the LDH-B locus ($\chi^2=$ 35.93, $P<0.00001$), and the recombination rate was assumed to be 21.9% (Table 3; Fig. 4b).

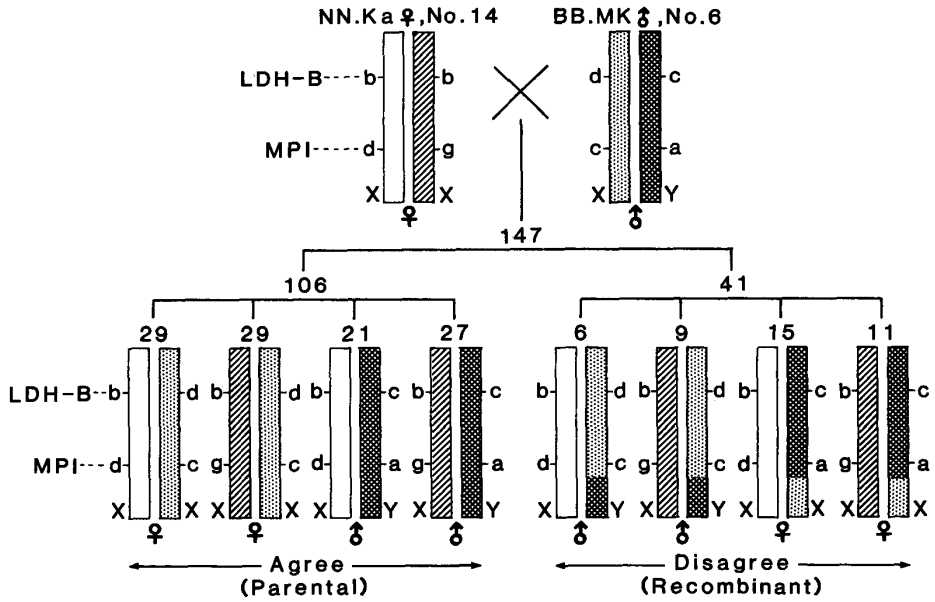

Fig. 6. Inheritance of the two enzymes, LDH-B and MPI, in a mating with a heterozygous male, NN.Ka♀, No. 14 × BB.MK⇕, No. 6 (Table 3), and the sex-linkage of these loci in *Rana brevipoda* from the Konko population. Assuming that recombinants (disagree) must have resulted from crossing-over between the sex-determining genes and the two loci of the LDH-B and MPI, the linkage analyses between the sex-determining genes and these two loci were made.

e. NN.Ka♀, Nos. 6~15 × BB.MK⇕, Nos. 5 and 6

In 1990, two males (BB.MK⇕, Nos. 5 and 6) which were produced from a mating, *R. brevipoda* from Maibara ♀ × *R. brevipoda* from Konko ⇕, and had both MPI and LDH-B whose genotypes were heterozygous *ca* and *dc*, respectively, were mated with 10 female *R. nigromaculata* of the Kaita population (NN.Ka♀, Nos. 6~15) (Fig. 6). It was found that in the 942 offspring obtained from these 10 matings, 445 were females and 497 (52.7%) were males. When the genotypes of the MPI and LDH-B loci were examined in these offspring, 374 of the 445 females showed heterozygous *dc* or *gc* at the MPI locus and *bd* at the LDH-B locus, while the remaining 71 females showed *da* or *ga* at the MPI locus and *bc* at the LDH-B locus. Of the 497 males, 410 showed *da* or *ga* at the MPI locus and *bc* at the LDH-B locus, while the remaining 87 males showed *dc* or *gc* at the MPI locus and *bd* at the LDH-B locus. The sex-determining genes agreed with the genotypes of the MPI and LDH-B loci in constitution in 784 (83.2%) of the 942 offspring, that is, they were parental, while they disagreed with the latter in the remaining 158, that is, they were recombinants. Thus, the MPI and LDH-B loci seemed to be linked with the sex-determining genes ($\chi^2=417.78$, $P<0.00001$), and the recombination rate was assumed to be 16.8% (Table 3).

4. Linkage between the sex-determining genes and the ME-B locus

In the Konko population of *R. brevipoda*, the sex-determining genes linked with the MPI and LDH-B loci on chromosome No. 4, as shown in Table 3, while it was assumed that the sex-determining genes did not link with the ME-B locus on chromosome No. 3 in the Konko population of *R. brevipoda*. As shown in Table 6, of the 241 offspring produced from four matings, BB.Ko♀, Nos. 9 and 10 × BB.MK⇕, Nos. 1~4, the sex-determining genes agreed with the genotype of the ME-B locus in 119 (49.4%) offspring which were parental, while they disagreed with the latter in 122 offspring which were recombinants ($\chi^2=0.04$, $P>0.84$). Of the 228 offspring produced from four matings, BB.Ma♀, Nos. 2 and 3 × BB.MK⇕, Nos. 1~4, the sex-determining genes agreed with the genotype of the ME-B locus in constitution in 120 (52.6%) offspring, while they disagreed with the latter in 108 offspring ($\chi^2=0.63$, $P>0.42$). Thus, it was assumed that the sex-determining genes did not link with the ME-B locus in the Konko population of *R. brevipoda* (Table 6).

5. Summary of linkage analyses between the sex-determining genes and four enzyme loci

a. Linkage between the sex-determining genes and the MPI locus

A total of 31 matings was made between 10 male *R. brevipoda* of the Konko population or MK hybrids between the Maibara and Konko populations in which the genotype of the MPI locus on chromosome No. 4 was heterozygous *ad* or *ca* and 25 female *R. brevipoda* or *R. nigromaculata*. In 2421 (88.9%) of the 2724

offspring obtained from these matings, the sex-determining genes agreed with the genotype of the MPI locus in constitution, that is, they were parental, while they disagreed with the latter in the other 303 offspring, that is, they were recombinants ($\chi^2=1646.81$, $P<0.00001$). Thus, it was assumed that the sex-determining genes linked with the MPI locus on chromosome No. 4 and the recombination rate was 11.1% (Tables 3 and 11).

b. Linkage between the sex-determining genes and the LDH-B locus

A total of 22 matings was made between six male MK hybrids produced from male *R. brevipoda* of the Konko population in which the genotype of the LDH-B locus on chromosome No. 4 was heterozygous *dc* and 16 female *R. brevipoda* and *R. nigromaculata*. In 1591 (89.5%) of the 1777 offspring obtained from these matings, the sex-determining genes agreed with the genotype of the LDH-B locus in constitution, that is, they were parental, while in the remaining 186 offspring, they disagreed, that is, they were recombinants ($\chi^2=1110.87$, $P<0.00001$) and the recombination rate between them was 10.5% (Tables 3 and 11).

c. Linkage between the sex-determining genes and the Pep-B locus

Two matings were made between a male *R. brevipoda* of the Konko population in which the genotype of the Pep-B locus was heterozygous *ab* and two female *R. brevipoda* of the Konko population. It was found that in 131 (94.9%) of the 138 offspring obtained from these matings, the sex-determining genes agreed with the genotype of the Pep-B locus in constitution, that is, they were parental, while they disagreed in the remaining seven offspring, that is, they were recombinants ($\chi^2=111.42$, $P<0.00001$). Thus, it was assumed that the sex-determining genes linked with the Pep-B locus on chromosome No. 4 and the recombination rate between them was 5.1% (Tables 3 and 11).

d. Linkage between the sex-determining genes and the ME-B locus

Eight matings were made between four male MK hybrids produced from male *R. brevipoda* of the Konko population in which the genotype of the ME-B locus on chromosome No. 3 was heterozygous *fd* and four female *R. brevipoda* of the Konko or Maibara population. When 469 of the 587 offspring obtained from these matings were analyzed, the sex-determining genes were found to agree with the genotype of the ME-B locus in constitution in 239 (51.0%) offspring, that is, they were parental, while they disagreed in 230 offspring, that is, they were recombinants ($\chi^2=0.17$, $P>0.67$). Thus, it was evident that they did not link with the latter (Tables 6 and 11).

The foregoing results showed that the sex-determining genes linked with the MPI, LDH-B and Pep-B loci on chromosome No. 4 in the Konko population of *R. brevipoda*, while they did not link with the ME-B locus on chromosome No. 3.

B. Matings with females whose genotype of the MPI locus was heterozygous

In 1988, two matings were made between two female *R. brevipoda* of the Konko population (BB.Ko♀, Nos. 10 and 11) whose genotype of the MPI locus was heterozygous *ad* and a male *R. brevipoda* of the Konko population (BB.Ko♂, No. 6) whose genotype of the MPI locus was homozygous *aa*, and six matings between one of the foregoing females (BB.Ko♀, No. 10) and six males of reciprocal hybrids (BB.KM♂, Nos. 1~4 and BB.MK♂, Nos. 3 and 4) produced from matings between the Konko and Maibara populations of *R. brevipoda*. From these matings, a total of 569 offspring consisting of 266 females and 303 (53.3%) males was produced. When the sex-determining genes were compared with the genotype of the MPI locus in constitution in the male and female offspring, they agreed in 285 (50.1%), while they disagreed in the other 284. Thus, it was evident that the sex-determining genes did not link with the MPI locus in the females ($\chi^2=0.002$, $P>0.96$) (Table 4).

TABLE 4

Inheritance of the MPI locus in matings with heterozygous females of *Rana brevipoda* from the Konko population

Year	Parents		No. of metamorphosed frogs	No. of examined offspring			MPI and sex				Sex-linkage of MPI				
							Parents	Offspring							
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂	Agree (%)	Disagree	χ^2	P	Recombination rate (%)
1988	BB.Ko, No. 10	BB.KM, No. 1	108	89	45	44 (49.4)	*ad ae*	*aa ae*	19	27	53 (59.6)	36	3.25	0.07	40.4
								da de	26	17					
		BB.KM, No. 2	112	88	40	48 (54.5)	*ad ae*	*aa ae*	16	26	50 (56.8)	38	1.64	0.20	43.2
								da de	24	22					
		BB.KM, No. 3	101	87	46	41 (47.1)	*ad ae*	*aa ae*	24	18	40 (46.0)	47	0.56	0.45	54.0
								da de	22	23					
		BB.KM, No. 4	87	80	34	46 (57.5)	*ad ae*	*aa ae*	16	17	35 (43.8)	45	1.25	0.26	56.2
								da de	18	29					
		BB.MK, No. 3	6	6	2	4 (66.7)	*ad ca*	*ac aa*	1	3	4 (66.7)	2	0.67	0.41	33.3
								dc da	1	1					
		BB.MK, No. 4	110	100	48	52 (52.0)	*ad ca*	*ac aa*	32	29	45 (45.0)	55	1.00	0.31	55.0
								dc da	16	23					
		BB.Ko, No. 6	102	64	25	39 (60.9)	*ad aa*	*aa*	12	16	29 (45.3)	35	0.56	0.45	54.7
								da	13	23					
	BB.Ko, No. 11	BB.Ko, No. 6	112	55	26	29 (52.7)	*ad aa*	*aa*	10	13	29 (52.7)	26	0.16	0.68	47.2
								da	16	16					
	Total		738	569	266	303 (53.3)		*a-*	130	149	285 (50.1)	284	0.002	0.96	49.9
								d-	136	154					

II. Rana brevipoda of the Maibara population belonging to the Nagoya race

A. Matings with males whose genotypes of the MPI, LDH-B and ME-B loci were heterozygous

1. Linkage between the sex-determining genes and the MPI and LDH-B loci

As four (BB.Ma♂, Nos. 1~4) of 43 male *R. brevipoda* of the Nagoya race

TABLE 5

Inheritance of the MPI and LDH-B loci in matings with heterozygous males of *Rana breripoda* from the Maibara population

Year	Parents		No. of metamorphosed frogs	No. of examined offspring			MPI and sex				LDH-B and sex				Sex-linkage of MPI and LDH-B					
	Female	Male		Total	♀	♂ (%)	Parents	Offspring			Parents	Offspring			Agree (%)		Disagree	χ^2	P	Recombination rate (%)
							Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂						
1986	BB.Ko, Nos. 6~8	BB.Ma, No.1	70	69	34	35 (50.7)	*aa da*	*ad*	14	21	*cc cd*	*cc*	13	21	MPI	28 (40.6)	41	2.50	0.11	59.4
								aa	20	14		*cd*	21	14	LDH-B	27 (39.1)	42	3.26	0.07	60.9
	BB.Ko, Nos. 4~8	BB.Ma, No.2	165	153	79	74 (48.4)	*aa ae*	*aa*	54	49	*cc cd*	*cc*	50	51	MPI	79 (51.6)	74	0.16	0.68	48.4
								ae	25	25		*cd*	29	23	LDH-B	73 (47.7)	80	0.32	0.57	52.3
	BB.Ko, Nos. 6~8	BB.Ma, No.3	44	42	21	21 (50.0)	*aa de*	*ad*	12	10	*cc cd*	*cc*	12	11	MPI	23 (54.8)	19	0.38	0.53	45.2
								ae	9	11		*cd*	9	10	LDH-B	22 (52.4)	20	0.10	0.75	47.6
	BB.Ko, Nos. 7, 8	BB.Ma, No.4	41	37	22	15 (40.5)	*aa de*	*ad*	12	6	*cc cd*	*cc*	13	6	MPI	21 (56.8)	16	0.68	0.41	43.3
								ae	10	9		*cd*	9	9	LDH-B	22 (59.5)	15	1.32	0.24	40.5
	BB.Ko, Nos. 3~8	BB.Ma, Nos. 5~8	256	211	106	105 (49.8)	*aa de*	*ad*	62	49	—	—	—	—	MPI	118 (55.9)	93	2.96	0.08	44.1
								ae	44	56	—	—	—	—						
	Total		576	512	262	250 (48.8)			154	135			88	89	MPI	269 (52.5)	243	1.32	0.25	47.5
									108	115			68	56	LDH-B	144 (47.8)	157	0.56	0.45	52.2
1988	BB.Ko, No.10	BB.KM, No.1	108	89	45	44 (49.4)	*ad ae*	*aa da*	23	20	*cc cd*	*cc*	23	20	MPI → LDH-B	47 (52.8)	42	0.28	0.59	47.2
								ae de	22	24		*cd*	22	24						
		BB.KM, No.2	112	88	40	48 (54.5)	*ad ae*	*aa da*	20	23	*cc cd*	*cc*	19	23	MPI	45 (51.1)	43	0.05	0.83	48.9
								ae de	20	25		*cd*	21	25	LDH-B	44 (50.0)	44	0	1.00	50.0
		BB.KM, No.3	101	87	46	41 (47.1)	*ad ae*	*aa da*	20	17	*cc cd*	*cc*	20	17	MPI → LDH-B	44 (50.6)	43	0.01	0.91	49.4
								ae de	26	24		*cd*	26	24						
		BB.KM, No.4	87	80	34	46 (57.5)	*ad ae*	*aa da*	18	23	*cc cd*	*cc*	18	23	MPI → LDH-B	41 (51.3)	39	0.05	0.82	48.8
								ae de	16	23		*cd*	16	23						
	BB.Ma, No.3	BB.KM, No.1	116	93	35	58 (62.4)	*dd ae*	*da*	18	24	*dd cd*	*dc*	18	24	MPI → LDH-B	52 (55.9)	41	1.30	0.25	44.1
								de	17	34		*dd*	17	34						
		BB.KM, No.2	109	87	41	46 (52.9)	*dd ae*	*da*	17	28	*dd cd*	*dc*	17	28	MPI → LDH-B	35 (40.2)	52	3.32	0.06	59.8
								de	24	18		*dd*	24	18						
		BB.KM, No.3	64	63	28	35 (55.6)	*dd ae*	*da*	10	20	*dd cd*	*dc*	10	20	MPI → LDH-B	25 (39.7)	38	2.68	0.10	60.3
								de	18	15		*dd*	18	15						
		BB.KM, No.4	102	87	37	50 (57.5)	*dd ae*	*da*	19	21	*dd cd*	*dc*	19	21	MPI → LDH-B	48 (55.2)	39	0.93	0.33	44.8
								de	18	29		*dd*	18	29						
	Total		799	674	306	368 (54.6)			145	176			144	176	MPI	337 (50.0)	337	0	1.00	50.0
									161	192			162	192	LDH-B	336 (49.9)	338	0.01	0.93	50.1

collected from Maibara in 1986 were heterozygous in the genotypes of the MPI and LDH-B loci and four other males (BB.Ma♂, Nos. 5~8) were heterozygous in the genotype of the MPI locus, these eight males were mated with six female *R. brevipoda* (BB.Ko♀, Nos. 3~8) of the Konko population (Typical race). It was found that 262 of the 512 offspring were females and 250 (48.8%) were males. When the presence of linkage between the sex-determining genes and the MPI locus was examined, they agreed in 269 (52.5%), while they disagreed in 243 ($\chi^2=1.32$, $P>0.25$). In 301 offspring obtained from 13 matings, BB.Ko♀, Nos. 4~8 × BB.Ma♂, Nos. 1~4, the sex-determining genes agreed with the genotype of the LDH-B locus in 144 (47.8%), while they disagreed in 157 ($\chi^2=0.56$, $P>0.45$). Thus, it was evident that the sex-determining genes did not link with the LDH-B nor MPI locus (Table 5).

In 1988, four males consisting of two males (BB.KM♂, Nos. 1 and 2) produced from a mating in 1986, BB.Ko♀, No. 8 × BB.Ma♂, No. 5, one male (BB.KM♂, No. 3) produced from a mating in 1986, BB.Ko♀, No. 8 × BB.Ma♂, No. 2, and one male (BB.KM♂, No. 4) produced from a mating in 1986, BB.Ko♀, No. 8 × BB.Ma♂, No. 3, were mated with a female *R. brevipoda* (BB.Ko♀, No. 10) obtained from the Konko population and a female *R. brevipoda* (BB.Ma♀, No. 3) obtained from the Maibara population. These four males were all heterozygous in the genotypes of the MPI and LDH-B loci and produced 674 offspring from eight matings with the foregoing two females. Of the 674 offspring, 306 were females and 368 (54.6%) were males. The sex-determining genes agreed with the genotype of the MPI locus in constitution in 337 (50.0%), while they disagreed with the latter in the other 337 ($\chi^2=0$, $P=1.0$). The sex-determining genes agreed with the genotype of the LDH-B locus in constitution in 336 (49.9%), while they disagreed in the other 338 ($\chi^2=0.01$, $P>0.93$). Thus, it was clear that the sex-determining genes did not link with the MPI and LDH-B loci in the Maibara population of *R. brevipoda* (Table 5).

2. Linkage between the sex-determining genes and the ME-B locus

a. BB.Ko♀, No. 10 and BB.Ma♀, No. 3 × BB.KM♂, Nos. 1 and 2

In 1988, four matings were made between two male *R. brevipoda* (BB.KM♂, Nos. 1 and 2) which were obtained from a mating in 1986, BB.Ko♀, No. 8 × BB.Ma♂, No. 5, and in which the genotype of the ME-B locus on chromosome No. 3 was heterozygous *df* and the foregoing two female *R. brevipoda* (BB.Ko♀, No. 10 and BB.Ma♀, No. 3) of the Konko and Maibara populations which were homozygous *dd* and heterozygous *df*, respectively. From two matings of BB.Ko♀, No. 10 × BB.KM♂, Nos. 1 and 2, 177 offspring composed of 85 females and 92 (52.0%) males were produced. When the sex-determining genes were compared with the genotype of the ME-B locus, it was found that they agreed with the latter in constitution in 173 (97.7%) offspring including 84 females and 89 males, that is, they were parental, while they disagreed in four offspring including one female and three males, that is, they were recombinants. Thus, it

TABLE 6

Inheritance of the ME-B locus in matings with heterozygous males of *Rana brevipoda*

Year	Parents		No. of metamorphosed frogs	No. of examined offspring			ME-B and sex				Sex-linkage of ME-B				
							Parents	Offspring							
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂	Agree (%)	Disagree	χ^2	P	Recombination rate (%)
1988	BB.Ko, No. 9	BB.MK, No. 1	74	67	29	38 (56.7)	*dd fd*	*df*	13	16	35 (52.2)	32	0.13	0.71	47.8
								dd	16	22					
		BB.MK, No. 2	92	68	30	38 (55.9)	*dd fd*	*df*	13	20	31 (45.6)	37	0.53	0.46	54.4
								dd	17	18					
	BB.Ko, No. 10	BB.MK, No. 3	6	6	2	4 (66.7)	*dd fd*	*df*	1	1	4 (66.7)	2	0.67	0.41	33.3
								dd	1	3					
		BB.MK, No. 4	110	100	48	52 (52.0)	*dd fd*	*df*	26	29	49 (49.0)	51	0.04	0.84	51.0
								dd	22	23					
		Total	282	241	109	132 (54.8)			53	66	119 (49.4)	122	0.04	0.84	50.6
									56	66					
1988	BB.Ma, No. 2	BB.MK, No. 1	107	77	39	38 (49.4)	*fg fd*	*ff gf*	14	15	37 (48.1)	40	0.12	0.73	51.9
								fd gd	25	23					
		BB.MK, No. 2	108	67	32	35 (52.2)	*fg fd*	*ff gf*	14	12	37 (55.2)	30	0.73	0.39	44.8
								fd gd	18	23					
	BB.Ma, No. 3	BB.MK, No. 3	116	105	52	53 (50.5)	*df fd*	*ff*	11	15					
								df	32	27	22 (47.8)	24	0.09	0.76	52.2
								dd	9	11					
		BB.MK, No. 4	116	97	44	53 (54.6)	*df fd*	*ff*	7	7					
								df	30	29	24 (63.2)	14	2.63	0.10	36.8
								dd	7	17					
		Total	447	346	167	179 (51.7)			46	49	120 (52.6)	108	0.63	0.42	47.4
									59	74					
1988	BB.Ko, No. 10	BB.KM, No. 1	108	89	45	44 (49.4)	*dd df*	*dd*	**44**	2	**86 (96.6)**	**3**	**77.40**	**<0.00001**	**3.4**
								df	1	**42**					
		BB.KM, No. 2	112	88	40	48 (54.5)	*dd df*	*dd*	**40**	1	**87 (98.9)**	**1**	**84.05**	**<0.00001**	**1.1**
								df	0	**47**					
		Total	220	177	85	92 (52.0)		*dd*	**84**	3	**173 (97.7)**	**4**	**161.36**	**<0.00001**	**2.3**
								df	1	**89**					
	BB.Ma, No. 3	BB.KM, No. 1	116	93	35	58 (62.4)	*df df*	*dd*	**22**	0					
								df	12	31	**49 (98.0)**	**1**	**40.08**	**<0.00001**	**2.0**
								ff	1	**27**					
		BB.KM, No. 2	109	87	41	46 (52.9)	*df df*	*dd*	**24**	1					
								df	17	19	**50 (98.0)**	**1**	**47.08**	**<0.00001**	**2.0**
								ff	0	**26**					
		Total	225	180	76	104 (57.8)		*dd*	**46**	1					
								df	29	50	**99 (98.0)**	**2**	**93.16**	**<0.00001**	**2.0**
								ff	1	**53**					

was assumed that the sex-determining genes linked with the ME-B locus in the Maibara population of *R. brevipoda* ($\chi^2=161.36$, $P<0.00001$), and the recombination rate was 2.3% (Table 6; Fig. 7a).

There were 76 females and 104 (57.8%) males in the 180 offspring produced from two matings, BB.Ma♀, No. 3 × BB.KM♂, Nos. 1 and 2. The results

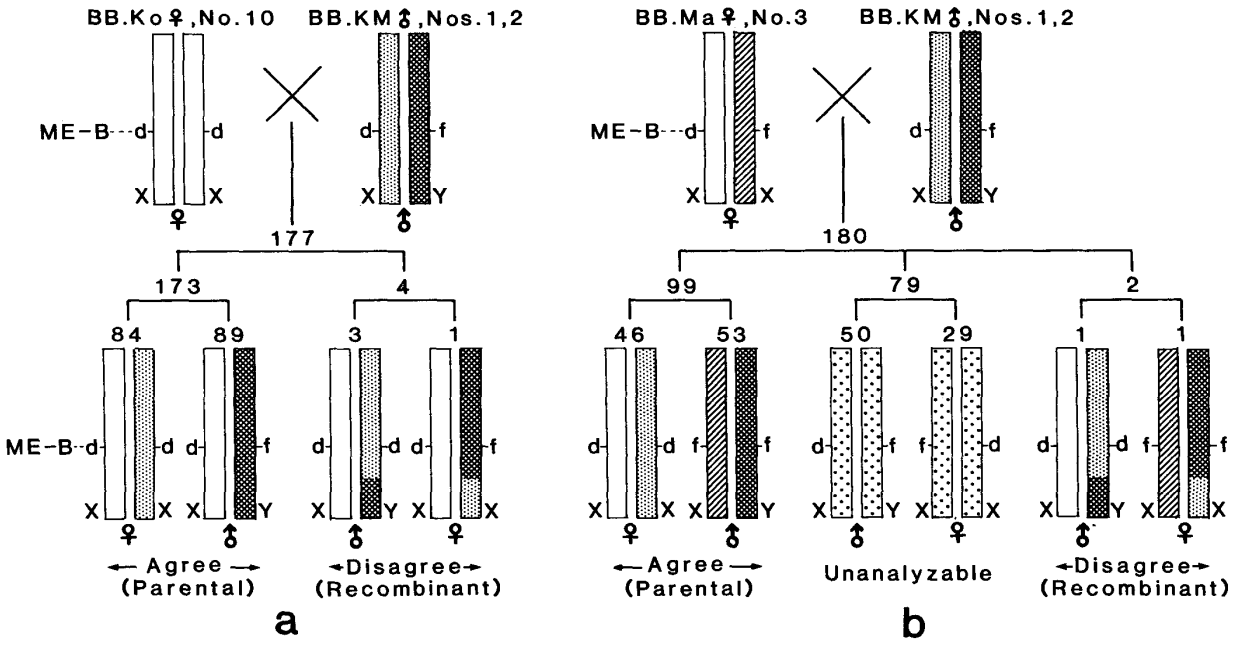

Fig. 7. Inheritance of the ME-B enzyme in matings with heterozygous males and the sex-linkage of the ME-B locus in *Rana brevipoda* from the Maibara population. Assuming that recombinants (disagree) must have resulted from crossing-over between the sex-determining genes and the ME-B locus, the linkage analyses between the sex-determining genes and the ME-B locus were made.

a. Using two matings of BB.Ko♀, No. 10 × BB.KM♂, Nos. 1, 2 (Table 6)

b. Using two matings of BB.Ma♀, No. 3 × BB.KM♂, Nos. 1, 2 (Table 6)

showed that of the 47 offspring which were homozygous *dd* in the genotype of the ME-B locus, 46 were females and one was a male, while of the 54 offspring which were homozygous *ff* in the genotype of the ME-B locus, 53 were males and one was a female. All the remaining 79 offspring including 29 females and 50 males were heterozygous *df* which were unanalyzable. The sex-determining genes agreed with the genotype of the ME-B locus in constitution in 99 (98.0%) offspring, that is, they were parental, while they disagreed with the latter in the other two offspring, that is, they were recombinants. Thus, it was assumed that the sex-determining genes in *R. brevipoda* of the Maibara population linked with the ME-B locus on chromosome No. 3 and the recombination rate between them was 2.0% ($\chi^2=93.16$, $P<0.00001$) (Table 6; Fig. 7b).

3. Summary of linkage analyses between the sex-determining genes and three enzyme loci

a. Linkage between the sex-determining genes and the MPI locus

Sixteen matings were made between 12 male *R. brevipoda* of the Maibara population or KM hybrids produced from male *R. brevipoda* of the Maibara population in which the genotype of the MPI locus on chromosome No. 4 was heterozygous *da*, *de* or *ae* and eight female *R. brevipoda* of the Konko or Maibara population. The sex-determining genes agreed with the genotype of the MPI

locus in constitution in 606 (51.1%) of the 1186 offspring obtained from these matings, while they disagreed in the remaining 580 ($\chi^2=0.57$, $P>0.45$). Thus, it was evident that the sex-determining genes did not link with the MPI locus (Tables 5 and 11).

b. Linkage between the sex-determining genes and the LDH-B locus

Twelve matings were made between eight male *R. brevipoda* of the Maibara population in which the genotype of the LDH-B locus on chromosome No. 4 was heterozygous *cd* and seven female *R. brevipoda* of the Konko or Maibara population in which the genotype of the LDH-B locus was homozygous *cc* or *dd*. The sex-determining genes agreed with the genotype of the LDH-B locus in constitution in 480 (49.2%) of the 975 offspring obtained from these matings, while they disagreed in the other 495 ($\chi^2=0.23$, $P>0.63$). Thus, it was evident that the sex-determining genes did not link with the LDH-B locus (Tables 5 and 11).

c. Linkage between the sex-determining genes and the ME-B locus

Four matings were made between two male KM hybrids produced from male *R. brevipoda* of the Maibara population in which the genotype of the ME-B locus on chromosome No. 3 was heterozygous *df* and two female *R. brevipoda* of the Konko or Maibara population. In 272 (97.8%) of the 278 offspring obtained from these matings, the sex-determining genes agreed with the genotype of the ME-B locus in constitution, that is, they were parental, while they disagreed in only six offspring, that is, they were recombinants ($\chi^2=254.52$, $P<0.00001$). Thus, it was assumed that the sex-determining genes linked with ME-B locus on chromosome No. 3 in the Maibara population of *R. brevipoda* and the recombination rate was 2.2% (Tables 6 and 11).

The foregoing results showed that the Maibara population of *R. brevipoda* differed from the Konko population of this species in that the sex-determining genes linked with the ME-B locus on chromosome No. 3.

B. Matings with females whose genotypes of the LDH-B, MPI, HK and ME-B loci were heterozygous

1. Linkage analyses between the sex-determining genes and the LDH-B locus

In 1986, a mating was made between a female *R. brevipoda* of the Maibara population (BB.Ma♀, No. 1) whose genotype of the LDH-B locus was heterozygous *cd* and a male *R. brevipoda* of the Konko population (BB.Ko♂, No. 5) whose genotype of the LDH-B locus was homozygous *cc*. From this mating, 30 offspring consisting of 16 females and 14 (46.7%) males were produced. When the sex-determining genes were compared with the genotype of the LDH-B locus in these males and females, 17 (56.7%) offspring agreed in constitution, while 13 disagreed ($\chi^2=0.53$, $P>0.46$). Thus, it was evident that the sex-determining genes did not link with the LDH-B locus (Table 7).

2. Linkage analyses between the sex-determining genes and the MPI locus

In 1988, five matings were made between a female *R. brevipoda* of the Maibara population (BB.Ma♀, No. 2) whose genotype of the MPI locus was heterozygous *cd* and five males consisting of two male *R. brevipoda* of the foregoing MK hybrids (BB.MK♂, Nos. 1 and 2) whose genotype of the MPI locus was heterozygous *ca*, two male *R. nigromaculata* of the Kaita population (NN.Ka♂, Nos. 1 and 2) whose genotype of the MPI locus was heterozygous *gd* and a male *R. nigromaculata* of the Kaita population (NN.Ka♂, No. 4) whose genotype of the MPI locus was homozygous *dd*. From these matings, 363 offspring consisting of 155 females and 208 (57.3%) males were produced. When the sex-determining genes of these offspring were compared with the genotype of the MPI locus, they agreed in constitution in 193 (53.2%) offspring, while they disagreed in 170 offspring ($\chi^2=1.46$, $P>0.22$). Thus, it was evident that the sex-determining genes did not link with the MPI locus (Table 7).

3. Linkage analyses between the sex-determining genes and the HK locus

In 1988, three matings were made between a female *R. brevipoda* of the Maibara population (BB.Ma♀, No. 2) whose genotype of the HK locus was heterozygous *ab* and a male *R. nigromaculata* of the Kaita population (NN.Ka♂, No. 1) whose genotype of the HK locus was heterozygous *bc* and two male *R. nigromaculata* of the Kaita population (NN.Ka♂, Nos. 2 and 4) whose genotype of the HK locus was homozygous *cc*. From these matings, 171 offspring consisting of 79 females and 92 (53.8%) males were produced. When the sex-determining genes of these offspring were compared with the genotype of the HK locus, the former agreed with the latter in 96 (56.1%) offspring , while they disagreed in the other 75 ($\chi^2=2.58$, $P>0.10$). Thus, it was evident that the sex-determining genes did not link with the HK locus (Table 7).

4. Linkage analyses between the sex-determining genes and the ME–B locus

In 1988, four matings between two female *R. brevipoda* of the Maibara population (BB.Ma♀, Nos. 2 and 3) whose genotypes of the ME–B locus were *fg* and *df* and four male hybrids obtained from matings between the Maibara and Konko populations of *R. brevipoda* (BB.MK♂, Nos. 1 and 2, and BB.KM♂, Nos. 3 and 4) whose genotypes of the ME–B locus were heterozygous *fd* and homozygous *dd*. From these matings, 248 offspring consisting of 118 females and 130 (52.4%) males were obtained. When the sex-determining genes were compared with the genotype of the ME–B locus, they agreed with the latter in 123 (49.6%) offspring, while they disagreed in the other 125 ($\chi^2=0.02$, $P>0.89$). Thus, it was evident that the sex-determining genes did not link with the ME–B locus (Table 7).

TABLE 7

Inheritance of the MPI, LDH-B, HK and ME-B loci in matings with

Year	Parents		No. of metamorphosed frogs	No. of examined offspring			MPI and sex				LDH-B and sex			
							Parents	Offspring			Parents	Offspring		
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂
1986	BB.Ma, No. 1	BB.Ko, No. 5	33	30	16	14 (46.7)	*cc aa*	*ca*	16	14	*cd cc*	*cc*	10	7
												dc	6	7
1988	BB.Ma, No. 2	BB.MK, No. 1	107	77	39	38 (49.4)	*cd ca*	*cc ca*	18	19	— —	—	—	—
								dc da	21	19				
		BB.MK, No. 2	108	67	32	35 (52.2)	*cd ca*	*cc ca*	18	18	— —	—	—	—
								dc da	14	17				
		NN.Ka, No. 1	109	95	50	45 (47.4)	*cd gd*	*cg cd*	23	31	— —	—	—	—
								dg dd	27	14				
		NN.Ka, No. 2	91	62	7	55 (88.7)	*cd gd*	*cg cd*	3	28	— —	—	—	—
								dg dd	4	27				
		NN.Ka, No. 4	94	62	27	35 (56.5)	*cd dd*	*cd*	13	17	— —	—	—	—
								dd	14	18				
	Total		509	363	155	208 (57.3)			75	113	— —	—	—	—
									80	95				
1988	BB.Ma, No. 3	BB.KM, No. 3	64	51	20	31 (60.8)	— —	—	—	—	— —	—	—	—
		BB.KM, No. 4	102	53	27	26 (49.1)	— —	—	—	—	— —	—	—	—
	Total		166	104	47	57 (54.8)	— —	—	—	—	— —	—	—	—

III. Rana nigromaculata of the Hiro population

A. Matings with males whose genotype of the MPI locus was heterozygous

In 1983, three matings were made between three female *R. nigromaculata* of the Hiro population (NN.Hr♀, Nos. 1~3) which were homozygous *dd* in the genotype of the MPI locus and three male *R. nigromaculata* of the Hiro population (NN.Hr♂, Nos. 1~3) which were heterozygous *gd*. When the genotypes of the MPI locus in 235 offspring including 114 females and 121 (51.5%) males produced from these matings were examined in 1985, 106 of the 114 females showed heterozygous *dg* and the other eight showed homozygous *dd*. Of the 121 males, 108 showed homozygous *dd* and the other 13 showed heterozygous *dg*. In 214 (91.1%) of the 235 offspring, the sex-determining genes agreed with the genotype of the MPI locus, that is, they were parental, while in 21 offspring, they disagreed with the latter, that is, they were recombinants ($\chi^2=158.51$, $P<0.00001$). It was assumed that the sex-determining genes linked with the MPI locus on chromosome No. 4 in the Hiro population of *R. nigromaculata* and the recombination rate was 8.9% (Tables 8 and 11).

heterozygous females of *Rana brevipoda* from the Maibara population

HK and sex				ME-B and sex				Sex-linkage of MPI, LDH-B, HK and ME-B					
Parents	Offspring			Parents	Offspring			Agree (%)		Dis-agree	χ^2	P	Recombination rate (%)
Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂						
— —	—	—	—	— —	—	—	—	LDH-B	17 (56.7)	13	0.53	0.46	43.3
— —	—	—	—	*fg fd*	*ff fd*	22	18	MPI	40 (51.9)	37	0.12	0.73	48.1
					gf gd	17	20	ME-B	35 (45.5)	42	0.64	0.42	54.5
— —	—	—	—	*fg fd*	*ff fd*	14	14	MPI	32 (47.8)	35	0.13	0.71	52.2
					gf gd	18	21	ME-B	32 (47.8)	35	0.13	0.71	52.2
ab bc	*ab ac*	23	28	— —	—	—	—	MPI	58 (61.1)	37	4.64	0.04	38.9
	bb bc	27	17					HK	55 (57.9)	40	2.37	0.12	42.1
ab cc	*ac*	1	8	— —	—	—	—	MPI	32 (51.6)	30	0.06	0.79	48.4
	bc	1	4					HK	9 (64.3)	5	1.14	0.28	35.7
ab cc	*ac*	13	18	— —	—	—	—	MPI	31 (50.0)	31	0	1.00	50.0
	bc	14	17					HK	32 (51.6)	30	0.06	0.79	48.4
		37	54			36	32	MPI	193 (53.2)	170	1.46	0.22	46.8
		42	38			35	41	HK	96 (56.1)	75	2.58	0.10	43.9
								ME-B	67 (46.5)	77	0.69	0.40	53.5
— —	—	—	—	*df dd*	*dd*	10	13	ME-B	23 (45.1)	28	0.49	0.48	54.9
					fd	10	18						
— —	—	—	—	*df dd*	*dd*	10	16	ME-B	33 (62.3)	20	3.19	0.07	37.7
					fd	17	10						
— —	—	—	—			20	29	ME-B	56 (53.8)	48	0.62	0.43	46.2
— —	—	—	—			27	28						

B. Matings with females whose genotypes of the MPI, SORDH and LDH-B loci were heterozygous

In 1985, seven matings were made between four female hybrids (CN♀, Nos. 1~4) which were obtained from two female *R. plancyi chosenica* and a male *R. nigromaculata* of the Hiro-population and were all heterozygous in the genotypes of the MPI, SORDH and LDH-B loci and three male *R. nigromaculata* of the Kumano population. In a total of 235 offspring obtained from these matings, the sex-determining genes were compared with the genotypes of the foregoing three loci. The results showed that the sex-determining genes agreed with the genotype of the MPI locus in constitution in 134 (57.0%) of the 235 offspring, while they disagreed in the other 101 offspring ($\chi^2=4.63$, $P>0.03$). The sex-determining genes agreed with the genotype of the SORDH locus in constitution in 131 (55.7%), while they disagreed in the other 104 offspring ($\chi^2=3.10$, $P>0.07$). The sex-determining genes agreed also with the genotype of the LDH-B locus in constitution in 137 (58.3%), while they disagreed in the other 98 offspring ($\chi^2=6.47$, $P>0.01$). Thus, it was assumed that the sex-determining genes did not link with the MPI, SORDH and LDH-B loci on chromosome No. 4 (Table 13).

TABLE 8

Inheritance of the MPI locus in matings with heterozygous males of *Rana nigromaculata*

Year	Parents		No. of metamorphosed frogs	No. of examined offspring			MPI and sex				Sex-linkage of MPI				
							Parents	Offspring							
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂	Agree (%)	Disagree	χ^2	P	Recombination rate (%)
1983	NN.Hr, No. 1	NN.Hr, No. 1	153	74	35	39 (52.7)	*dd gd*	*dg*	**33**	3	69 (93.2)	5	55.35	<0.00001	6.8
								dd	2	**36**					
	NN.Hr, No. 2	NN.Hr, No. 2	162	90	44	46 (51.1)	*dd gd*	*dg*	**41**	8	79 (87.8)	11	51.38	<0.00001	12.2
								dd	3	**38**					
	NN.Hr, No. 3	NN.Hr, No. 3	149	71	35	36 (50.7)	*dd gd*	*dg*	**32**	2	66 (93.0)	5	52.41	<0.00001	7.0
								dd	3	**34**					
	Total		464	235	114	121 (51.5)		*dg*	**106**	13	214 (91.1)	21	158.51	<0.00001	8.9
								dd	8	**108**					
1985	NN.Km, No. 1	NN.Km, No. 1	132	36	20	16 (44.4)	*dd gd*	*dg*	**17**	0	33 (91.7)	3	25.0	<0.00001	8.3
								dd	3	**16**					
	NN.Km, No. 2	NN.Km, No. 1	105	40	17	23 (57.5)	*dd gd*	*dg*	**16**	0	39 (97.5)	1	36.1	<0.00001	2.5
								dd	1	**23**					
	NN.Km, No. 3	NN.Km, No. 2	124	89	33	56 (62.9)	*dd gd*	*dg*	**32**	11	77 (86.5)	12	47.47	<0.00001	13.5
								dd	1	**45**					
	NN.Km, No. 4	NN.Km, No. 2	137	85	29	56 (65.9)	*dd gd*	*dg*	**25**	7	74 (87.1)	11	46.69	<0.00001	12.9
								dd	4	**49**					
	NN.Km, No. 5	NN.Km, No. 3	85	10	1	9 (90.0)	*dd gd*	*dg*	**1**	0	10 (100)	0	10.0	<0.002	0
								dd	0	**9**					
	NN.Km, No. 6	NN.Km, No. 3	94	63	28	35 (55.6)	*dd gd*	*dg*	**24**	12	47 (74.6)	16	15.25	<0.00001	25.4
								dd	4	**23**					
	Total		677	323	128	195 (60.4)		*dg*	**115**	30	280 (86.7)	43	173.90	<0.00001	13.3
								dd	13	**165**					
1985	BB.Ko, No. 2	NN.Km, No. 1	137	73	32	41 (56.2)	*dd gd*	*dg*	**32**	0	73 (100)	0	73.0	<0.00001	0
								dd	0	**41**					
		NN.Km, No. 2	127	109	50	59 (54.1)	*dd gd*	*dg*	**49**	5	103 (94.5)	6	86.32	<0.00001	5.5
								dd	1	**54**					
		NN.Km, No. 3	136	95	47	48 (50.5)	*dd gd*	*dg*	**47**	2	93 (97.9)	2	87.17	<0.00001	2.1
								dd	0	**46**					
	Total		400	277	129	148 (53.4)		*dg*	**128**	7	269 (97.1)	8	245.92	<0.00001	2.9
								dd	1	**141**					
1985	CN, No. 1	NN.Km, No. 1	103	20	8	12 (60.0)	*jd gd*	*jg dg*	**8**	0	20 (100)	0	20.0	<0.00001	0
								jd dd	0	**12**					
	CN, No. 2	NN.Km, No. 1	118	19	7	12 (63.2)	*nd gd*	*ng dg*	**7**	0	19 (100)	0	19.0	<0.00001	0
								nd dd	0	**12**					
	CN, No. 3	NN.Km, No. 1	118	23	4	19 (82.6)	*jd gd*	*jg dg*	**4**	7	16 (69.6)	7	3.52	0.06	30.4
								jd dd	0	**12**					
	CN, No. 1	NN.Km, No. 2	123	79	33	46 (58.2)	*jd gd*	*jg dg*	**30**	4	72 (91.1)	7	53.48	<0.00001	8.9
								jd dd	3	**42**					
	CN, No. 4	NN.Km, No. 2	90	48	18	30 (62.5)	*nd gd*	*ng dg*	**18**	4	44 (91.7)	4	33.33	<0.00001	8.3
								nd dd	0	**26**					
	CN, No. 1	NN.Km, No. 3	91	32	9	23 (71.9)	*jd gd*	*jg dg*	**8**	1	30 (93.8)	2	24.5	<0.00001	6.2
								jd dd	1	**22**					
	CN, No. 2	NN.Km, No. 3	90	14	6	8 (57.1)	*nd gd*	*ng dg*	**6**	1	13 (92.9)	1	10.29	<0.002	7.1
								nd dd	0	**7**					
	Total		733	235	85	150 (63.8)		*-g*	**81**	17	214 (91.1)	21	158.51	<0.00001	8.9
								-d	4	**133**					

IV. Rana nigromaculata of the Kumano population

A. Matings with males whose genotype of the MPI locus was heterozygous

a. NN.Km♀, Nos. 1~6 × NN.Km♂, Nos. 1~3

In 1985, six matings were made between three male *R. nigromaculata* of the Kumano population (NN.Km♂, Nos. 1~3) which were heterozygous *gd* in the genotype of the MPI locus and six female *R. nigromaculata* of the Kumano population (NN.Km♀, Nos. 1~6) which were homozygous *dd* in the same locus, and 323 offspring consisting of 128 females and 195 (60.4%) males were obtained. When the relationship between the sex-determining genes and the genotype of the MPI locus was examined in these offspring (Fig. 1), it was found that 115 of the 128 females were heterozygous *dg*, while the remaining 13 were homozygous *dd*, and that 165 of the 195 males were homozygous *dd*, while the remaining 30 were heterozygous *dg*. In 280 (86.7%) of the 323 offspring, the sex-determining genes agreed with the genotype of the MPI locus, that is, they were parental, while in 43, they disagreed with the latter, that is, they were recombinants ($\chi^2=173.90$, $P<0.00001$). Thus, it was assumed that the sex-determining genes linked with the MPI locus and the recombination rate was 13.3% (Table 8).

b. BB.Ko♀, No. 2 × NN.Km♂, Nos. 1~3

In 1985, three matings were made between a female *R. brevipoda* of the Konko population (BB.Ko♀, No. 2) which was *dd* in the genotype of the MPI locus and the foregoing three male *R. nigromaculata* which were heterozygous *gd* in the genotype of the MPI locus. These matings produced 277 offspring consisting of 129 females and 148 (53.4%) males. When the genotype of the MPI locus was examined in these offspring, it was found that 128 of the 129 females were heterozygous *dg*, while the remainder was homozygous *dd*, and that 141 of the 148 males were homozygous *dd*, while the other seven were heterozygous *dg*. In 269 (97.1%) of the 277 offspring, the sex-determining genes agreed with the genotype of the MPI locus, that is, they were parental, while in eight, they disagreed with the latter, that is, they were recombinants ($\chi^2=245.92$, $P<0.00001$). Thus, it was assumed that the sex-determining genes linked with the MPI locus and the recombination rate was 2.9% (Table 8).

c. CN♀, Nos. 1~4 × NN.Km♂, Nos. 1~3

In 1983, two matings were made between two female *R. plancyi chosenica* from Korea, which was heterozygous *jn* in the genotype of the MPI locus and a male *R. nigromaculata* of the Hiro population, which was homozygous *dd* in the genotype of the MPI locus, in order to produce the hybrids (CN) having heterozygous *jd* or *nd* in the genotype of the MPI locus. In 1985, seven matings were made between four females of these hybrids (CN♀, Nos. 1~4) and the foregoing three male *R. nigromaculata* of the Kumano population. As there were 85 females and 150 (63.8%) males among the 235 offspring, the genotype of the MPI locus was

examined in these offspring (Fig. 1). It was found that 81 of 85 females were heterozygous *jg*, *ng* or *dg*, while the other four females were heterozygous *jd* or homozygous *dd*, and that 133 of the 150 males were *jd*, *nd* or *dd*, while the other 17 males were *jg*, *ng* or *dg*. In 214 (91.1%) of the 235 offspring, the sex-determining genes agreed with the genotype of the MPI locus, that is, they were parental, while in 21, they disagreed with the latter, that is, they were recombinants ($\chi^2=158.51$, $P<0.00001$). Thus, it was assumed that the sex-determining genes linked with the MPI locus and the recombination rate was 8.9% (Table 8).

d. Summary of linkage analyses between the sex-determining genes and the MPI locus on chromosome No. 4 of male *Rana nigromaculata* of the Kumano population

Sixteen matings were made between three male *R. nigromaculata* of the Kumano population in which the genotype of the MPI locus was heterozygous *gd* and 11 females including six female *R. nigromaculata* of the Kumano population in which the genotype of the MPI locus was homozygous *dd*, a female *R. brevipoda* of the Konko population, and four female hybrids between *R. plancyi chosenica* and *R. nigromaculata* in which the genotype of the MPI locus was heterozygous. It was found that the sex-determining genes agreed with the genotype of the MPI locus in constitution in 763 (91.4%) of the 835 offspring obtained from these matings, that is, they were parental, while they disagreed in the other 72 offspring, that is, they were recombinants ($\chi^2=571.83$, $P<0.00001$). Thus, it was assumed that the sex-determining genes linked with the MPI locus and the recombination rate was 8.6% (Tables 8 and 11).

V. Rana nigromaculata of the Kaita population

A. Matings with males whose genotypes of the MPI, ENO, SORDH, HK and ME-B loci were heterozygous

In 1988, 19 matings were made between nine male *R. nigromaculata* of the Kaita population (NN.Ka♂, Nos. 1~9) which were heterozygous in the genotype of the MPI, ENO, SORDH, HK or ME-B locus and six females including a female *R. nigromaculata* of the Aomori population (NN.Ao♀, No. 1), three female *R. nigromaculata* of the Kaita population (NN.Ka♀, Nos. 2~4), a female *R. brevipoda* of the Maibara population (BB.Ma♀, No. 2) and a female *R. brevipoda* of the Konko population (BB.Ko♀, No. 9), in order to examine the relationship between the sex-determining genes and the genotypes of the foregoing enzymes in the offspring produced from these matings. It was found that in five (NN.Ka♂, Nos. 1, 3, 4, 7 and 8) of the nine males, the sex-determining genes agreed with the genotype of the MPI, ENO, SORDH or HK locus situated on chromosome No. 4, while in the other four males (NN.Ka♂, Nos. 2, 5, 6 and 9), the sex-determining genes did not link with the loci of four kinds of enzymes situated on chromosome No. 4 nor with the ME-B locus situated on chromosome No. 3 (Tables 9, 10 and 11).

1. Five males, NN.Ka♂, Nos. 1, 3, 4, 7 and 8

a. Linkage between the sex-determining genes and the MPI locus

In 1988, three matings were made between a male *R. nigromaculata* of the Kaita population (NN.Ka♂, No. 1) in which the genotype of the MPI locus was heterozygous *gd* and three females including a female *R. nigromaculata* of the Aomori population (NN.Ao♀, No. 1) in which the genotype of the MPI locus was homozygous *dd*, a female *R. brevipoda* of the Konko population (BB.Ko♀, No. 9) in which the genotype of the MPI locus was homozygous *aa* and a female *R. brevipoda* of the Maibara population (BB.Ma♀, No. 2) in which the genotype of the MPI locus was heterozygous *cd*. A total of 216 offspring consisting of 125 females and 91 (42.1%) males was produced. When the relationship of the sex-determining genes and the genotype of the MPI locus was examined, it was found that they agreed in 199 (92.1%) in constitution, that is, they were parental, while they disagreed, that is, they were recombinants in 17 ($\chi^2=153.35$, $P<0.00001$). Thus, it was assumed that the sex-determining genes in this male linked with the MPI locus and the recombination rate was 7.9% (Tables 9 and 11; Fig. 8a, b).

b. Linkage between the sex-determining genes and the SORDH locus

In 1988, nine matings were made between five male *R. nigromaculata* of the Kaita population (NN.Ka♂, Nos. 1, 3, 4, 7 and 8) in which the genotype of the SORDH locus was heterozygous *ab* or *cb* 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 genotype of the SORDH locus was homozygous *bb* and two female *R. brevipoda* of the Maibara and Konko populations (BB.Ma♀, No. 2 and BB.Ko♀, No. 9) in which the genotype of the SORDH locus was homozygous *ff*. From these matings, 568 offspring consisting of 288 females and 280 (49.3%) males were produced. When the relationship between the sex-determining genes and the genotype of the SORDH locus was examined, they agreed, that is, they were parental in 533 (93.8%), and disagreed, that is, they were recombinants in 35 offspring ($\chi^2=436.63$, $P<0.00001$). Thus, it was assumed that the sex-determining genes in the five males linked with the SORDH locus and the recombination rate was 6.2% (Tables 9, 10 and 11; Fig. 8a, b).

c. Linkage between the sex-determining genes and the HK locus

In 1988, four matings were made between two male *R. nigromaculata* of the Kaita population (NN.Ka♂, Nos. 1 and 3) in which the genotype of the HK locus was heterozygous *bc* or *dc* 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 genotype of the HK locus was homozygous *cc*, a female *R. brevipoda* of the Maibara population (BB.Ma♀, No. 2) whose genotype of the HK locus was heterozygous *ab* and a female *R. brevipoda* of the Konko population (BB.Ko♀, No. 9) whose genotype of the HK locus was homozygous *aa*. From these matings,

TABLE 9

Inheritance of the MPI, ENO, SORDH and HK loci in matings with

Year	Parents		No. of metamorphosed frogs	No. of examined offspring			MPI or ENO and sex			
							Parents	Offspring		
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂
1988	NN.Ao, No. 1	**NN.Ka, No. 1**	111	70	49	21 (30.0)	*dd gd*	*dg*	**33**	0
								dd	16	**21**
	BB.Ma, No. 2		109	95	50	45 (47.4)	*cd gd*	*cg*	**23**	1
								dg	**27**	0
								cd	0	**30**
								dd	0	**14**
	BB.Ko, No. 9		65	51	26	25 (49.0)	*aa gd*	*ag*	**26**	0
								ad	0	**25**
	Total		285	216	125	91 (42.1)			**109**	1
									16	**90**
1988	NN.Ao, No. 1	NN.Ka, No. 2	113	25	12	13 (52.0)	*dd gd*	*dg*	5	6
								dd	7	7
	BB.Ma, No. 2		91	62	7	55 (88.7)	*cd gd*	*cg*	2	9
								dg	3	14
								cd	1	19
								dd	1	13
	BB.Ko, No. 9		94	61	18	43 (70.5)	*aa gd*	*ag*	9	22
								ad	9	21
	Total		298	148	37	111 (75.0)			19	51
									18	60
1988	NN.Ka, No. 2	**NN.Ka, No. 3**	122	70	24	46 (65.7)	*bb ba*	*bb*	**24**	8
								ba	0	**38**
1988	NN.Ao, No. 1	**NN.Ka, No. 4**	107	56	37	19 (33.9)	*dd dd*	*dd*	—	—
	BB.Ma, No. 2		94	62	27	35 (56.5)	*cd dd*	*cd*	—	—
								dd	—	—
	BB.Ko, No. 9		58	24	11	13 (54.2)	*aa dd*	*ad*	—	—
	Total		259	142	75	67 (47.2)			—	—
									—	—

286 offspring consisting of 149 females and 137 (47.9%) males were produced. When the relationship between the sex-determining genes and the genotype of the HK locus was examined in these offspring, it was found that they agreed in constitution, that is, they were parental in 261 (91.3%) offspring, while they disagreed, that is, they were recombinants in 25 ($\chi^2=194.74$, $P<0.00001$). Thus, it was assumed that the sex-determining genes in the two males linked with

heterozygous males of *Rana nigromaculata* from the Kaita population

SORDH and sex				HK and sex				Sex-linkage of MPI, ENO, SORDH and HK				
Parents		Offspring		Parents		Offspring		Agree (%)	Dis-agree	χ^2	P	Recombination rate (%)
Genotype ♀ ♂		Genotype	♀ ♂	Genotype ♀ ♂		Genotype	♀ ♂					
bb *ab*		*ba*	**33** 0	*cc* *bc*		*cb*	**33** 0	MPI				
		bb	16 **21**			*cc*	16 **21**	SORDH **54 (77.1)**	**16**	**20.62**	**<0.00001**	**22.9**
								HK				
ff *ab*		*fa*	**50** 1	*ab* *bc*		*ab*	**23** 1	MPI				
		fb	0 **44**			*bb*	**27** 0	SORDH **94 (98.9)**	**1**	**91.04**	**<0.00001**	**1.1**
						ac	0 **27**	HK				
						bc	0 **17**					
ff *ab*		*fa*	**26** 0	*aa* *bc*		*ab*	**26** 0	MPI				
		fb	0 **25**			*ac*	0 **25**	SORDH **51 (100)**	**0**	**51.0**	**<0.00001**	**0**
								HK				
			109 1				**109** 1	MPI				
			16 **90**				16 **90**	SORDH **199 (92.1)**	**17**	**153.35**	**<0.00001**	**7.9**
								HK				
bb *ab*		*ba*	5 6	*cc* *cc*		*cc*	— —	MPI 12 (48.0)	13	0.04	0.84	52.0
		bb	7 7					SORDH				
ff *ab*		*fa*	5 23	*ab* *cc*		*ac*	— —	MPI 37 (59.7)	25	2.32	0.12	40.3
		fb	2 32			*bc*	— —	SORDH				
ff *ab*		*fa*	9 22	*aa* *cc*		*ac*	— —	MPI 30 (49.2)	31	0.02	0.89	50.8
		fb	9 21					SORDH				
			19 51				— —	MPI 79 (53.4)	69	0.68	0.41	46.6
			18 60				— —	SORDH				
bb *ab*		*ba*	**24** 8	*cc* *dc*		*cd*	**24** 8	ENO				
		bb	0 **38**			*cc*	0 **38**	SORDH **62 (88.6)**	**8**	**41.66**	**<0.00001**	**11.4**
								HK				
bb *ab*		*ba*	**32** 0	*cc* *cc*		*cc*	— —	SORDH **51 (91.1)**	**5**	**37.79**	**<0.00001**	**8.9**
		bb	5 **19**									
ff *ab*		*fa*	**26** 1	*ab* *cc*		*ac*	— —	SORDH **60 (96.8)**	**2**	**54.26**	**<0.00001**	**3.2**
		fb	1 **34**			*bc*	— —					
ff *ab*		*fa*	**11** 0	*aa* *cc*		*ac*	— —	SORDH **24 (100)**	**0**	**24.0**	**<0.00001**	**0**
		fb	0 **13**									
			69 1				— —	SORDH **135 (95.1)**	**7**	**115.38**	**<0.00001**	**4.9**
			6 **66**				— —					

the HK locus and the recombination rate was 8.7% (Tables 9 and 11; Fig. 8a, b).

d. Linkage between the sex-determining genes and the ENO locus

In 1988, a mating was made between a male *R. nigromaculata* of the Kaita population (NN.Ka♂, No. 3) in which the genotype of the ENO locus was heterozygous *ba* and a female *R. nigromaculata* of the Kaita population (NN.Ka♀,

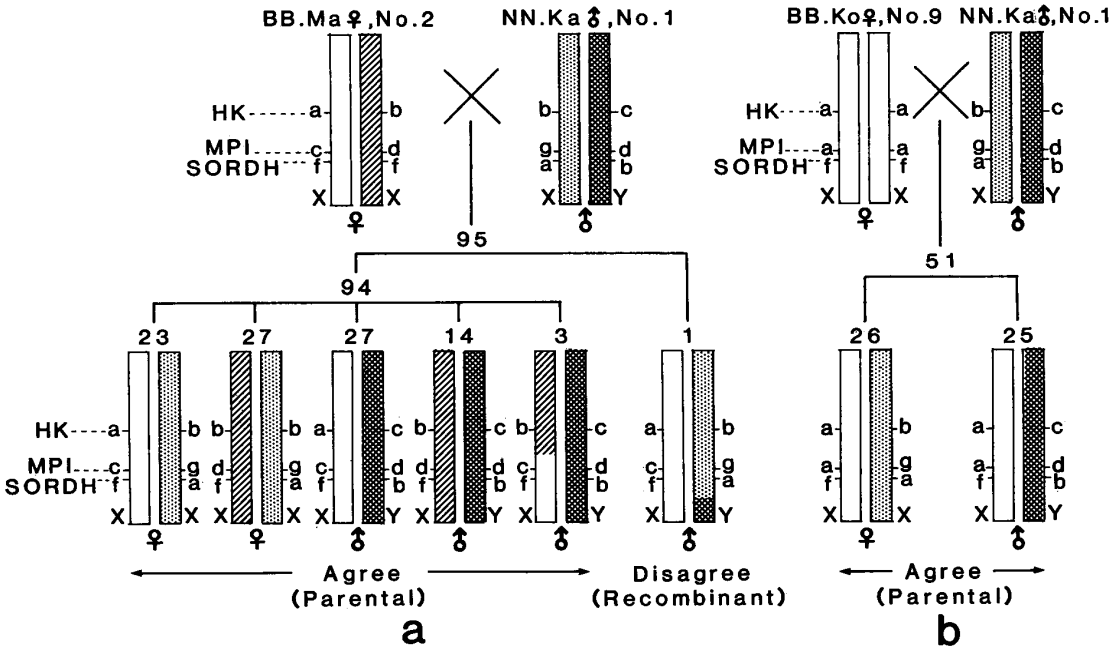

Fig. 8. Inheritance of the three enzymes, HK, MPI and SORDH, in matings with a heterozygous male and the sex-linkage of these three loci in *Rana nigromaculata* from the Kaita population. Assuming that a recombinant (disagree) must have resulted from crossing-over between these three loci of the HK, MPI and SORDH and the sex-determining genes, the linkage analyses between the sex-determining genes and these three loci were made.

a. Using a mating of BB.Ma♀, No. 2 × NN.Ka♂, No. 1 (Table 9)
b. Using a mating of BB.Ko♀, No. 9 × NN.Ka♂, No. 1 (Table 9)

No. 2). From this mating, 70 offspring consisting of 24 females and 46 (65.7%) males were produced. When the relationship between the sex-determining genes and the genotype of the ENO locus was examined in these offspring, they agreed in constitution, that is, they were parental in 62 (88.6%) offspring, while they disagreed, that is, they were recombinants in the other eight ($\chi^2=41.66$, $P<0.00001$). Thus, it was assumed that the sex-determining genes in this male linked with the ENO locus and the recombination rate was 11.4% (Tables 9 and 11).

2. Four males, NN.Ka♂, Nos. 2, 5, 6 and 9, in which enzymes were not sex-linked

a. Linkage between the sex-determining genes and the MPI locus

In 1988, nine matings were made between three male *R. nigromaculata* of the Kaita population (NN.Ka♂, Nos. 2, 5 and 6) in which the genotype of the MPI locus was heterozygous *gd* and six females including a female *R. nigromaculata* of the Aomori population (NN.Ao♀, No. 1) whose genotype of the MPI locus was homozygous *dd*, two female *R. nigromaculata* of the Kaita population (NN.Ka♀, Nos. 2 and 4) whose genotype of the MPI locus was homozygous *dd*, a female *R. nigromaculata* of the Kaita population (NN.Ka♀, No. 3) whose genotype of the

MPI locus was heterozygous *gd*, a female *R. brevipoda* of the Konko population (BB.Ko♀, No. 9) whose genotype of the MPI locus was homozygous *aa*, and a female *R. brevipoda* of the Maibara population (BB.Ma♀, No. 2) whose genotype of the MPI locus was heterozygous *cd*. From these matings, 667 offspring consisting of 319 females and 348 (52.2%) males were produced. When the genotype of the MPI locus was examined in 297 females and 335 males of the foregoing offspring, it could not be analyzed in 30 females and 77 males which were produced from NN.Ka♀, No. 3 (*gd* in genotype) × NN.Ka♂, Nos. 5 and 6 (*gd* in genotype), as in these females and males, the genotype was *gd* or *dg* which could not be distinguished from each other by electrophoretic patterns. When the sex-determining genes and the genotype of the MPI locus were compared in 525 offspring consisting of 267 females and 258 males other than the foregoing 30 females and 77 males, they agreed in constitution in 290 (55.2%) offspring and disagreed in the other 235 ($\chi^2=5.76$, $0.01<P<0.02$). Thus, it was evident that the sex-determining genes did not link with the MPI locus in the three males (NN.Ka♂, Nos. 2, 5 and 6) which were used to produce offspring (Tables 9, 10 and 11).

b. Linkage between the sex-determining genes and the SORDH locus

In 1988, of three male *R. nigromaculata* of the Kaita population which were used in the foregoing matings and whose genotype of the MPI locus was heterozygous *gd*, two (NN.Ka♂, Nos. 2 and 5) were heterozygous *ab* in the genotype of the SORDH locus and the other (NN.Ka♂, No. 6) was heterozygous *db*. Another male *R. nigromaculata* of the Kaita population (NN.Ka♂, No. 9) was heterozygous *cb* in the genotype of the SORDH locus. Of the four female *R. nigromaculata* used in the foregoing matings, a female *R. nigromaculata* of the Aomori population (NN.Ao♀, No. 1) and two female *R. nigromaculata* of the Kaita population (NN.Ka♀, Nos. 2 and 4) were homozygous *bb* in the genotype of the SORDH locus, and the remaining female *R. nigromaculata* of the Kaita population (NN.Ka♀, No. 3) was heterozygous *ab*. Two female *R. brevipoda* of the Maibara and Konko populations (BB.Ma♀, No. 2 and BB.Ko♀, No. 9) were homozygous *ff* in the genotype of the SORDH locus. From 10 matings between these four males and six females, a total of 734 offspring consisting of 383 females and 351 (47.8%) males was produced. Of these offspring, 395 consisting of 207 females and 188 males were examined on the relationship between the sex-determining genes and the genotype of the SORDH locus. It was found that they agreed in constitution in 219 (55.4%) offspring, while disagreed in 176 ($\chi^2=4.68$, $P>0.03$). Thus, in the four male *R. nigromaculata* (NN.Ka♂, Nos. 2, 5, 6 and 9), it seemed evident that the sex-determining genes were not linked with the SORDH locus (Tables, 9, 10 and 11).

c. Linkage between the sex-determining genes and the ME–B locus

In 1988, six matings were made between two male *R. nigromaculata* of the Kaita

TABLE 10

Inheritance of the MPI, SORDH and ME-B loci in matings with

Year	Parents		No. of metamorphosed frogs	No. of examined offspring			MPI and sex			
							Parents	Offspring		
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂
1988	NN.Ka, No. 2	NN.Ka, No. 5	102	62	55	7 (11.3)	*dd gd*	*dg*	28	2
								dd	27	5
	NN.Ka, No. 3		121	111	39	72 (64.9)	*gd gd*	*gg*	13	14
								dg	16	37
								dd	10	21
	NN.Ka, No. 4		111	17	10	7 (41.2)	*dd gd*	*dg*	7	1
								dd	3	6
	Total		334	190	104	86 (45.3)			48	17
									16	37
									40	32
1988	NN.Ka, No. 2	NN.Ka, No. 6	202	163	117	46 (28.2)	*dd gd*	*dg*	58	17
								dd	44	22
	NN.Ka, No. 3		119	94	26	68 (72.3)	*gd gd*	*gg*	6	15
								dg	14	40
								dd	6	13
	NN.Ka, No. 4		83	72	35	37 (51.4)	*dd gd*	*dg*	15	14
								dd	13	17
	Total		404	329	178	151 (45.9)			79	46
									14	40
									63	52
1988	NN.Ka, No. 2	**NN.Ka, No. 7**	97	78	38	40 (51.3)	*dd gg*	*dg*	—	—
		NN.Ka, No. 8	119	62	26	36 (58.1)	*dd dd*	*dd*	—	—
		NN.Ka, No. 9	104	67	64	3 (4.5)	*dd gg*	*dg*	—	—

population (NN.Ka♂, Nos. 5 and 6) in which the genotype of the ME-B locus was heterozygous *hg* and three female *R. nigromaculata* of the Kaita population (NN.Ka♀, Nos. 2~4) in which the genotype of the ME-B locus was *gg*, *gi* or *ii*. From these matings, 519 offspring consisting of 282 females and 237 (45.7%) males were produced. When the relationship between the sex-determining genes and the genotype of the ME-B locus was examined, it was found that they agreed with the latter in constitution in 257 (49.5%), while they disagreed in 262 ($\chi^2=$

heterozygous males of *Rana higromaculata* from the Kaita population

SORDH and sex				ME-B and sex				Sex-linkage of MPI, SORDH and ME-B						
Parents	Offspring			Parents	Offspring									
Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂	Agree (%)		Disagree	χ^2	P	Recombination rate (%)
bb ab	*ba*	9	0	*gg hg*	*gh*	24	2	MPI	33 (53.2)	29	0.26	0.61	46.8
	bb	7	3		*gg*	31	5	SORDH	12 (63.2)	7	1.32	0.25	36.8
								ME-B	29 (46.8)	33	0.26	0.61	53.2
ab ab	*aa*	2	4	*gi hg*	*gh*	10	22	MPI	34 (58.6)	24	1.72	0.18	41.4
	ab	6	5		*ih*	9	19	SORDH	2 (25.0)	6	2.00	0.15	75.0
	bb	2	0		*gg*	8	12	ME-B	50 (45.0)	61	1.09	0.29	55.0
					ig	12	19						
bb ab	*ba*	5	1	*ii hg*	*ih*	6	3	MPI	13 (76.5)	4	4.76	0.03	23.5
	bb	1	2		*ig*	4	4	SORDH	7 (77.8)	2	2.78	0.09	22.2
								ME-B	10 (58.8)	7	0.53	0.46	41.2
		16	5			49	46	MPI	80 (58.4)	57	3.86	0.05	41.6
		6	5			—	—	SORDH	21 (58.3)	15	1.00	0.31	41.7
		10	5			55	40	ME-B	89 (46.8)	101	0.76	0.38	53.2
bb db	*bd*	31	6	*gg hg*	*gh*	51	22	MPI	80 (56.7)	61	2.56	0.10	43.3
	bb	21	12		*gg*	66	24	SORDH	43 (61.4)	27	3.66	0.05	38.6
								ME-B	75 (46.0)	88	1.04	0.30	54.0
ab db	*ad*	1	5	*gi hg*	*gh*	7	18	MPI	19 (47.5)	21	0.10	0.75	52.5
	bd	0	3		*ih*	7	17	SORDH	11 (52.4)	10	0.05	0.82	47.6
	ab	0	8		*gg*	7	19	ME-B	47 (50.0)	47	0	1.00	50.0
	bb	2	2		*ig*	5	14						
bb db	*bd*	14	13	*ii hg*	*ih*	20	11	MPI	32 (54.2)	27	0.42	0.51	45.8
	bb	11	15		*ig*	15	26	SORDH	29 (54.7)	24	0.47	0.49	45.3
								ME-B	46 (63.9)	26	5.56	0.02	36.1
		46	27			85	68	MPI	131 (54.6)	109	2.02	0.15	45.4
		—	—			—	—	SORDH	83 (57.6)	61	3.36	0.06	42.4
		34	37			93	83	ME-B	168 (51.1)	161	0.15	0.69	48.9
bb cb	*bc*	**37**	1	*gg gh*	*gg*	—	—	SORDH	**76 (97.4)**	**2**	**70.21**	**<0.00001**	**2.6**
	bb	1	**39**		*gh*	—	—						
bb cb	*bc*	**26**	1	*gg gg*	*gg*	—	—	SORDH	**61 (98.4)**	**1**	**58.06**	**<0.00001**	**1.6**
	bb	0	**35**										
bb cb	*bc*	34	1	*gg gg*	*gg*	—	—	SORDH	36 (53.7)	31	0.37	0.54	46.3
	bb	30	2										

0.05, $P>0.82$). Thus, it was assumed that the sex-determining genes did not link with the ME-B locus on chromosome No. 3. (Tables 10 and 11).

3. Offspring of *R. nigromaculata* of the Kaita population mated in 1988

a. Offspring of a male, NN.Ka♂, No. 6, in which the sex-determining genes did not link with the MPI locus

In 1990, 11 matings were made between 11 female *R. nigromaculata* (NN.Ka♀,

TABLE 11

Linkage between seven enzymes and sex determining genes on chromosomes No. 3 and No. 4 in two populations of *Rana brevipoda* and three populations of *R. nigromaculata*

Species			*Rana brevipoda*		*Rana nigromaculata*			
Population			Konko	Maibara	Hiro	Kumano	Kaita	
No. of analyzed males			6	8	3	3	5	4
Chromosome No. 4	MPI—Sex	No. of frogs	**2724**	1186	**235**	**835**	**216**	525
		Agree (%)	**2421 (88.9)**	606 (51.1)	**214 (91.1)**	**763 (91.4)**	**199 (92.1)**	290 (55.2)
		Disagree	**303**	580	**21**	**72**	**17**	235
		χ^2	**1646.81**	0.57	**158.51**	**571.83**	**153.35**	5.76
		P	**<0.00001**	0.45	**<0.00001**	**<0.00001**	**<0.00001**	0.02
		Rec. rate (%)	**11.1**	48.9	**8.9**	**8.6**	**7.9**	44.8
	LDH-B—Sex	No. of frogs	**1777**	975	—	—	—	—
		Agree (%)	**1591 (89.5)**	480 (49.2)	—	—	—	—
		Disagree	**186**	495	—	—	—	—
		χ^2	**1110.87**	0.23	—	—	—	—
		P	**<0.00001**	0.63	—	—	—	—
		Rec. rate (%)	**10.5**	50.8	—	—	—	—
	Pep-B—Sex	No. of frogs	**138**	—	—	—	—	—
		Agree (%)	**131 (94.9)**	—	—	—	—	—
		Disagree	**7**	—	—	—	—	—
		χ^2	**111.42**	—	—	—	—	—
		P	**<0.00001**	—	—	—	—	—
		Rec. rate (%)	**5.1**	—	—	—	—	—
	SORDH—Sex	No. of frogs	—	—	—	—	**568**	395
		Agree (%)	—	—	—	—	**533 (93.8)**	219 (55.4)
		Disagree	—	—	—	—	**35**	176
		χ^2	—	—	—	—	**436.63**	4.68
		P	—	—	—	—	**<0.00001**	0.04
		Rec. rate (%)	—	—	—	—	**6.2**	44.6
	ENO—Sex	No. of frogs	—	—	—	—	**70**	—
		Agree (%)	—	—	—	—	**62 (88.6)**	—
		Disagree	—	—	—	—	**8**	—
		χ^2	—	—	—	—	**41.66**	—
		P	—	—	—	—	**<0.00001**	—
		Rec. rate (%)	—	—	—	—	**11.4**	—
	HK—Sex	No. of frogs	—	—	—	—	**286**	—
		Agree (%)	—	—	—	—	**261 (91.3)**	—
		Disagree	—	—	—	—	**25**	—
		χ^2	—	—	—	—	**194.74**	—
		P	—	—	—	—	**<0.00001**	—
		Rec. rate (%)	—	—	—	—	**8.7**	—
Chromosome No. 3	ME-B—Sex	No. of frogs	469	**278**	—	—	—	519
		Agree (%)	239 (51.0)	**272 (97.8)**	—	—	—	257 (49.5)
		Disagree	230	**6**	—	—	—	262
		χ^2	0.17	**254.52**	—	—	—	0.05
		P	0.67	**<0.00001**	—	—	—	0.82
		Rec. rate (%)	49.0	**2.2**	—	—	—	50.5

TABLE 12

Inheritance of the MPI locus in matings with heterozygous males of *Rana nigromaculata*

Year	Parents		No. of metamorphosed frogs	No. of examined offspring			MPI and sex				Sex-linkage of MPI				
							Parents	Offspring							
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂	Agree (%)	Disagree	χ^2	*P*	Recombination rate (%)
1990	NN.Ka, No. 5	88NN.Ka^{2}Ka^6, No. 1	21	19	15	4 (21.1)	*dg dg*	*dd*	4	1					
								dg	10	2	5 (71.4)	2	1.29	0.25	28.6
								gg	1	1					
	NN.Ka, No. 6		144	110	97	13 (11.8)	*dg dg*	*dd*	22	6					
								dg	56	7	22 (46.8)	25	0.19	0.66	53.2
								gg	19	0					
	NN.Ka, No. 7		137	102	90	12 (11.8)	*dg dg*	*dd*	24	2					
								dg	46	9	25 (53.2)	22	0.19	0.66	46.8
								gg	20	1					
	NN.Ka, No. 8		123	104	92	12 (11.5)	*dg dg*	*dd*	15	1					
								dg	48	9	17 (36.2)	30	3.60	0.05	63.8
								gg	29	2					
	NN.Ka, No. 9		85	72	50	22 (30.6)	*dg dg*	*dd*	15	5					
								dg	29	13	19 (63.3)	11	2.13	0.14	36.7
								gg	6	4					
	NN.Ka, No. 10		106	78	59	19 (24.4)	*dg dg*	*dd*	13	4					
								dg	38	12	16 (57.1)	12	0.57	0.44	42.9
								gg	8	3					
	NN.Ka, No. 11	88NN.Ka^{2}Ka^6, No. 2	14	14	12	2 (14.3)	*dg dg*	*dd*	3	0					
								dg	6	1	4 (57.1)	3	0.14	0.70	42.9
								gg	3	1					
	NN.Ka, No. 12		92	73	55	18 (24.7)	*dg dg*	*dd*	13	6					
								dg	31	6	19 (52.8)	17	0.11	0.73	47.2
								gg	11	6					
	NN.Ka, No. 13		108	83	56	27 (32.5)	*dg dg*	*dd*	15	4					
								dg	30	17	21 (58.3)	15	1.0	0.31	41.7
								gg	11	6					
	NN.Ka, No. 14		29	20	10	10 (50.0)	*dg dg*	*dd*	2	3					
								dg	6	6	3 (57.5)	5	0.5	0.47	62.5
								gg	2	1					
	NN.Ka, No. 15	88NN.Ka^{2}Ka^6, No. 3	45	36	34	2 (5.6)	*dg dg*	*dd*	8	1					
								dg	15	0	9 (42.9)	12	0.43	0.51	57.1
								gg	11	1					
	Total		904	711	570	141 (19.8)		*dd*	134	33					
								dg	315	82	160 (51.0)	154	0.11	0.73	49.0
								gg	121	26					
1990	NN.Ka, No. 5	88NN.Ka^{2}Ka^7, No. 1	21	15	8	7 (46.7)	*dg dg*	*dd*	**3**	1					
								dg	5	5	4 (80.0)	1	1.8	0.17	20.0
								gg	0	**1**					
	NN.Ka, No. 6		121	90	35	55 (61.1)	*dg dg*	*dd*	**16**	2					
								dg	19	30	39 (95.1)	2	33.39	<0.00001	4.9
								gg	0	**23**					
	NN.Ka, No. 7		101	94	45	49 (52.1)	*dg dg*	*dd*	**22**	3					
								dg	23	22	46 (93.9)	3	37.73	<0.00001	6.1
								gg	0	**24**					
	NN.Ka, No. 8		143	128	67	61 (47.7)	*dg dg*	*dd*	**30**	3					
								dg	37	36	52 (94.5)	3	43.65	<0.00001	5.5
								gg	0	**22**					
	NN.Ka, No. 9		36	33	16	17 (51.5)	*dg dg*	*dd*	**5**	2					
								dg	11	4	16 (88.9)	2	10.89	<0.001	11.1
								gg	0	**11**					
	NN.Ka, No. 10		41	11	3	8 (72.7)	*dg dg*	*dd*	**1**	1					
								dg	2	4	4 (80.0)	1	1.8	0.17	20.0
								gg	0	**3**					
	NN.Ka, No. 11	88NN.Ka^{2}Ka^7, No. 2	87	48	29	19 (39.6)	*dg dg*	*dd*	**9**	0					
								dg	16	14	14 (77.8)	4	5.56	0.02	22.2
								gg	4	**5**					
	NN.Ka, No. 12		19	16	4	12 (75.0)	*dg dg*	*dd*	**3**	0					
								dg	1	5	10 (100)	0	10.0	<0.002	0
								gg	0	**7**					
	NN.Ka, No. 13		69	53	26	27 (50.9)	*dg dg*	*dd*	**15**	4					
								dg	11	17	21 (84.0)	4	11.56	<0.0007	16.0
								gg	0	**6**					
	NN.Ka, No. 14		31	25	12	13 (52.0)	*dg dg*	*dd*	**8**	1					
								dg	4	3	17 (94.4)	1	14.22	<0.0002	5.6
								gg	0	**9**					
	Total		669	513	245	268 (52.2)		*dd*	**112**	17					
								dg	129	140	223 (91.4)	21	167.23	<0.00001	8.6
								gg	4	**111**					

TABLE 13
Inheritance of the MPI, SORDH LDH-B and ME-B loci

Year	Parents		No. of metamorphosed frogs	No. of examined offspring			MPI and sex			
							Parents	Offspring		
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂
1985	CN, No. 1	NN.Km, No. 1	103	20	8	12 (60.0)	*jd gd*	*jg jd*	5	5
								dg dd	3	7
		NN.Km, No. 2	123	79	33	46 (58.2)	*jd gd*	*jg jd*	15	18
								dg dd	18	28
		NN.Km, No. 3	91	32	9	23 (71.9)	*jd gd*	*jg jd*	8	13
								dg dd	1	10
	CN, No. 2	NN.Km, No. 1	118	19	7	12 (63.2)	*nd gd*	*ng nd*	6	8
								dg dd	1	4
		NN.Km, No. 3	90	14	6	8 (57.1)	*nd gd*	*ng nd*	5	2
								dg dd	1	6
	CN, No. 3	NN.Km, No. 1	118	23	4	19 (82.6)	*jd gd*	*jg jd*	2	10
								dg dd	2	9
	CN, No. 4	NN.Km, No. 2	90	48	18	30 (62.5)	*nd gd*	*ng nd*	8	9
								dg dd	10	21
	Total		733	235	85	150 (63.8)			49	65
									36	85
1988	NN.Ka, No. 3	NN.Ka, No. 6	119	94	26	68 (72.3)	— —	—	—	—
1990	NN.Ka, No. 6	BB.MK, No. 5	180	134	59	75 (56.0)	*dg ca*	*dc da*	30	43
								gc ga	29	32
	NN.Ka, No. 7		150	103	35	68 (66.0)	*dg ca*	*dc da*	19	39
								gc ga	16	29
	NN.Ka, No. 8		172	75	45	30 (40.0)	*dg ca*	*dc da*	20	19
								gc ga	25	11
	NN.Ka, No. 9		104	59	29	30 (50.8)	*dg ca*	*dc da*	10	19
								gc ga	19	11
	NN.Ka, No. 10		160	105	48	57 (54.3)	*dg ca*	*dc da*	25	31
								gc ga	23	26
	NN.Ka, No. 11	BB.MK, No. 6	152	75	46	29 (38.7)	*dg ca*	*dc da*	22	12
								gc ga	24	17
	NN.Ka, No. 12		69	17	7	10 (58.8)	*dg ca*	*dc da*	2	3
								gc ga	5	7
	NN.Ka, No. 13		338	154	51	103 (66.9)	*dg ca*	*dc da*	21	52
								gc ga	30	51
	NN.Ka, No. 14		318	147	84	63 (42.9)	*dg ca*	*dc da*	44	27
								gc ga	40	36
	NN.Ka, No. 15		99	73	41	32 (43.8)	*dg ca*	*dc da*	20	21
								gc ga	21	11
	Total		1742	942	445	497 (52.8)			213	266
									232	231

in matings with heterozygous females

SORDH and sex				LDH-B or ME-B and sex				Sex-linkage of MPI, SORDH, LDH-B and ME-B					
Parents	Offspring			Parents	Offspring								
Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂	Agree (%)		Disagree	χ^2	P	Recombination rate (%)
eb bb	*eb*	3	5	*ab bb*	*ab*	6	2	MPI	12 (60.0)	8	0.08	0.37	40.0
	bb	5	7		*bb*	2	10	SORDH	10 (50.0)	10	0	1.00	50.0
								LDH-B	16 (80.0)	4	7.20	<0.008	20.0
eb bb	*eb*	17	21	*ab bb*	*ab*	13	23	MPI	43 (54.4)	36	0.62	0.43	45.6
	bb	16	25		*bb*	20	23	SORDH	42 (53.2)	37	0.32	0.57	46.8
								LDH-B	36 (45.6)	43	0.62	0.43	54.4
eb bb	*eb*	8	14	*ab bb*	*ab*	6	14	MPI	18 (56.3)	14	0.50	0.47	43.8
	bb	1	9		*bb*	3	9	SORDH	17 (53.1)	15	0.13	0.72	46.9
								LDH-B	15 (46.9)	17	0.13	0.72	53.1
eb bb	*eb*	6	6	*ab bb*	*ab*	5	7	MPI	10 (52.6)	9	0.05	0.81	47.4
	bb	1	6		*bb*	2	5	SORDH	12 (63.2)	7	1.32	0.25	36.8
								LDH-B	10 (52.6)	9	0.05	0.81	47.4
eb bb	*eb*	5	2	*ab bb*	*ab*	5	2	MPI	11 (78.6)	3	4.57	0.04	21.4
	bb	1	6		*bb*	1	6	SORDH	11 (78.6)	3	4.57	0.04	21.4
								LDH-B	11 (78.6)	3	4.57	0.04	21.4
eb bb	*eb*	2	11	*ab bb*	*ab*	4	8	MPI	11 (47.8)	12	0.04	0.83	52.2
	bb	2	8		*bb*	0	11	SORDH	10 (43.5)	13	0.39	0.53	56.5
								LDH-B	15 (65.2)	8	2.13	0.14	34.8
eb bb	*eb*	8	9	*ab bb*	*ab*	11	7	MPI	29 (60.4)	19	2.08	0.14	39.6
	bb	10	21		*bb*	7	23	SORDH	29 (60.4)	19	2.08	0.14	39.6
								LDH-B	34 (70.8)	14	8.33	<0.004	29.2
		49	68			50	63	MPI	134 (57.0)	101	4.63	0.03	43.0
		36	82			35	87	SORDH	131 (55.7)	104	3.10	0.07	44.3
								LDH-B	137 (58.3)	98	6.47	0.02	41.7
ab db	*ad ab*	1	13	*gi hg*	*gh gg*	14	37	SORDH	15 (71.4)	6	3.86	0.04	28.6
	bd bb	2	5		*ih ig*	12	31	ME-B	49 (52.1)	45	0.17	0.67	47.9
—	—	—	—	—	—	—	—						
								MPI	72 (53.7)	62	0.75	0.38	46.3
—	—	—	—	—	—	—	—						
								MPI	55 (53.4)	48	0.48	0.49	46.6
—	—	—	—	—	—	—	—						
								MPI	44 (58.7)	31	2.25	0.13	41.3
—	—	—	—	—	—	—	—						
								MPI	38 (64.4)	21	4.90	0.03	35.6
—	—	—	—	—	—	—	—						
								MPI	54 (51.4)	51	0.09	0.76	49.0
—	—	—	—	—	—	—	—						
								MPI	36 (48.0)	39	0.12	0.72	52.0
—	—	—	—	—	—	—	—						
								MPI	8 (47.1)	9	0.06	0.80	52.9
—	—	—	—	—	—	—	—						
								MPI	82 (53.2)	72	0.65	0.42	46.8
—	—	—	—	—	—	—	—						
								MPI	67 (45.6)	80	1.15	0.28	54.4
—	—	—	—	—	—	—	—						
								MPI	42 (57.5)	31	1.66	0.19	42.5
—	—	—	—	—	—	—	—						
								MPI	498 (52.9)	444	3.10	0.07	47.1

TABLE 14
Inheritance of the SORDH, MPI, ENO, HK, Pep-B and LDH-B loci

Year	Parents		No. of metamorphosed frogs	No. of examined offspring			SORDH and sex				MPI and sex			
							Parents	Offspring			Parents	Offspring		
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂
1989	NB, No. 1	BB.Ko, No. 7	14	13	3	10 (76.9)	*bf* *ff*	*bf*	0	4	*dc* *aa*	*da*	0	4
								ff	3	6		*ca*	3	6
	NB, No. 2	BB.Ko, No. 9	2	2	0	2 (100)	*bf* *ff*	*bf*	0	2	*dc* *aa*	*da*	0	2
								ff	0	0		*ca*	0	0
	NB, No. 3	BB.Ko, No. 7	26	24	13	11 (45.8)	*bf* *ff*	*bf*	8	5	*dc* *aa*	*da*	6	5
								ff	5	6		*ca*	7	6
	NB, No. 3	BB.Ko, No. 8	56	51	33	18 (35.3)	*bf* *ff*	*bf*	16	10	*dc* *dd*	*dd*	17	10
								ff	17	8		*cd*	16	8
	NB, No. 4	BB.Ko, No. 9	22	22	10	12 (54.5)	*bf* *ff*	*bf*	6	5	*dc* *aa*	*da*	5	5
								ff	4	7		*ca*	5	7
	NB, No. 4	BB.Ko, No. 11	35	29	12	17 (58.6)	*bf* *ff*	*bf*	6	10	*dc* *aa*	*da*	6	10
								ff	6	7		*ca*	6	7
Total			155	141	71	70 (49.6)		*bf*	36	36		*d-*	34	36
								ff	35	34		*c-*	37	34
Agree (%)							70 (49.6)				68 (48.2)			
Disagree							71				73			
χ^2							0.01				0.18			
P							0.93				0.67			
Recombination rate (%)							50.4				51.8			

Nos. 5~15) collected from Kaita which were heterozygous *dg* in the genotype of the MPI locus and three male *R. nigromaculata* (88NN.Ka^2 Ka^6 ♂, Nos. 1~3) which were produced from a mating, NN.Ka♀, No. 2 × NN.Ka♂, No. 6, and were heterozygous *dg* in the genotype of the MPI locus. From these matings, 711 offspring consisting of 570 females and 141 (19.8%) males were produced. When the relationship between the sex-determining genes and the genotype of the MPI locus was examined, it was found that 134 of the 570 females were *dd* and 121 were *gg*, while 315 were *dg*. Of the 141 males, 33 were *dd* and 26 were *gg*, while 82 were *dg*. It was found that in 160 (51.0%) of 314 offspring other than the 397 having *dg* (unanalyzable), the sex-determining genes agreed in constitution with the genotype of the MPI locus, that is, they were parental, while they disagreed with the latter in the other 154, that is, they were recombinants (χ^2=0.11, $P>$ 0.73). Thus, it was assumed that the sex-determining genes did not link with the MPI locus in the next generation of the offspring (Table 12).

b. Offspring of a male, NN.Ka♂, No. 7, in which the sex-determining genes were linked with the MPI locus

In 1990, 10 matings were made between 10 (NN.Ka♀, Nos. 5~14) of the 11 females used in the foregoing matings and two males (88 NN.Ka^2 Ka^7 ♂, Nos. 1 and 2) which were produced from a mating, NN.Ka♀, No. 2 × NN.Ka♂, No. 7.

in matings with heterozygous hybrid females

ENO and sex				HK and sex				Pep-B and sex				LDH-B and sex			
Parents	Offspring			Parents	Offspring			Parents	Offspring			Parents	Offspring		
Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂
ba aa	*ba*	0	3	*ca aa*	*ca*	1	4	*cb bb*	*cb*	1	5	*bd cc*	*bc*	0	7
	aa	3	7		*aa*	2	6		*bb*	2	5		*dc*	3	3
ba aa	*ba*	0	2	*ca aa*	*ca*	0	1	*cb bb*	*cb*	0	1	*bd cc*	*bc*	0	1
	aa	0	0		*aa*	0	1		*bb*	0	1		*dc*	0	1
ba aa	*ba*	6	5	*ca aa*	*ca*	6	2	*cb bb*	*cb*	6	2	*bd cc*	*bc*	6	3
	aa	7	6		*aa*	7	9		*bb*	7	9		*dc*	7	8
ba aa	*ba*	16	10	*ca aa*	*ca*	16	12	*cb bb*	*cb*	17	12	*bd cc*	*bc*	18	13
	aa	17	8		*aa*	17	6		*bb*	16	6		*dc*	15	5
ba aa	*ba*	5	6	*ca aa*	*ca*	4	2	*cb bb*	*cb*	4	3	*bd cc*	*bc*	3	5
	aa	5	6		*aa*	6	10		*bb*	6	9		*dc*	7	7
ba aa	*ba*	6	9	*ca aa*	*ca*	6	6	*cb bb*	*cb*	6	5	*bd cc*	*bc*	7	6
	aa	6	8		*aa*	6	11		*bb*	6	12		*dc*	5	11
	ba	33	35		*ca*	33	27		*cb*	34	28		*bc*	34	35
	aa	38	35		*aa*	38	43		*bb*	37	42		*dc*	37	35
68 (48.2)				76 (53.9)				76 (53.9)				69 (48.9)			
73				65				65				72			
0.18				0.86				0.86				0.06			
0.67				0.35				0.35				0.80			
51.8				46.1				46.1				51.1			

The genotype of the MPI locus was heterozygous *dg*. From these matings, 513 offspring consisting of 245 females and 268 (52.2%) males were produced. When the genotype of the MPI locus was examined, 112 of the 245 females were *dd*, four were *gg* and the remaining 129 were *dg*, while 111 of the 268 males were *gg*, 17 were *dd* and the remaining 140 were *dg*. When the 129 females and 140 males of the heterozygous *dg*, which were unanalyzable, were eliminated, the sex-determining genes in 223 (91.4%) of 244 offspring agreed with the genotype of the MPI locus in constitution, that is, they were parental, while they disagreed with the latter, that is, they were recombinants in the other 21 ($\chi^2=167.23$, $P<0.00001$). Thus, it was assumed that the sex-determining genes linked with the MPI locus and the recombination rate was 8.6% (Table 12).

B. Matings with females whose genotypes of the MPI, SORDH and ME-B loci were heterozygous

a. NN.Ka♀, No. 3 × NN.Ka♂, No. 6

In 1988, a mating was made between a female *R. nigromaculata* (NN.Ka♀, No. 3) of the Kaita population whose genotypes of the SORDH and ME-B loci were each heterozygous and a male *R. nigromaculata* (NN.Ka♂, No. 6) of the Kaita population. When the sex-determining genes were compared with the genotype

of the SORDH locus in 21 of the 94 offspring obtained from the foregoing mating, it was found that they agreed with the latter in 15 (71.4%) offspring, while they disagreed in six ($\chi^2=3.86$, $P>0.04$). When the sex-determining genes were compared with the genotype of the ME-B locus in constitution in the 94 offspring, they agreed in 49 (52.1%) offspring, while they disagreed in 45 ($\chi^2=0.17$, $P>0.67$). Thus, it was assumed that the sex-determining genes did not link with the SORDH nor ME-B locus (Table 13).

b. NN.Ka♀, Nos. 6~15 × BB.MK♂, Nos. 5 and 6

In 1990, 10 matings were made between 10 female *R. nigromaculata* (NN.Ka♀, Nos. 6~15) of the Kaita population whose genotype of the MPI locus was heterozygous and two male *R. brevipoda* (BB.MK♂, Nos. 5 and 6). In 498 (52.9%) of the 942 offspring obtained from these matings, it was found that the sex-determining genes agreed with the genotype of the MPI locus in constitution, while they disagreed in the other 444 offspring ($\chi^2=3.10$, $P>0.07$). Thus, it was assumed that the sex-determining genes did not link with the MPI locus (Table 13).

c. NB♀, Nos. 1~4 × BB.Ko♂, Nos. 7~9 and 11

In 1989, six matings were made between four female hybrids (NB♀, Nos. 1~4) which were obtained from a mating between a female *R. nigromaculata* of the Aomori population and a male *R. brevipoda* of the Maibara population and were heterozygous in the genotypes of the SORDH, MPI, ENO, HK, Pep-B and LDH-B loci and four male *R. brevipoda* of the Konko population. In the 141 offspring obtained from these matings, 71 were females and 70 (49.6%) were males. The sex-determining genes were compared with the genotypes of the foregoing six loci. The results showed that the sex-determining genes agreed with the genotype of the SORDH locus in constitution in 70 (49.6%) of the 141 offspring, while they disagreed in the other 71 offspring ($\chi^2=0.01$, $P>0.9$). The sex-determining genes agreed with the genotypes of the MPI and ENO loci in constitution in 68 (48.2%), while they disagreed in the other 73 offspring ($\chi^2=0.18$, $P>0.6$). The sex-determining genes agreed with the genotypes of HK and Pep-B loci in constitution in 76 (53.9%), while they disagreed in the other 65 offspring ($\chi^2=0.86$, $P>0.3$). The sex-determining genes agreed also with the genotype of the LDH-B locus in constitution in 69 (48.9%), while they disagreed in the other 72 offspring ($\chi^2=0.06$, $P>0.7$). Thus, it was assumed that the sex-determining genes did not link with the SORDH, MPI, ENO, HK, Pep-B and LDH-B loci on chromosome No. 4 (Table 14).

DISCUSSION

KAWAMURA and YOKOTA (1959) were first to report that the male is heterogametic (XY) in *Rana japonica* by clarifying that the offspring of males which were sex-reversed from females by injection of testosterone propionate were almost

females. By the same kind of experiments, it was confirmed that *R. nigromaculata*, *R. brevipoda*, *R. tsushimensis* and *R. rugosa* collected from the neighboring districts of Hiroshima city were of the XX–XY type, and in addition, *Bombina orientalis* from Korea was also of the XX–XY type (KAWAMURA and NISHIOKA, 1977). Recently, the sex-determining mechanisms were clarified by cytological studies in a good number of species of anurans and urodeles (SCHMID, NANDA, STEINLEIN, KAUSCH, EPPLEN and HAAF, 1991). While most of the species were of the XX–XY type, there were a small number of species which were of the ZZ–ZW type. In contrast to these studies, it was found that the sex-determining mechanism was not always the same in one and the same species. According to NISHIOKA, KODAMA, SUMIDA and RYUZAKI (1993) and NISHIOKA, HANADA, MIURA and RYUZAKI (1994), there were many differences in sex-determining mechanism in *R. rugosa* distributed widely in Japan. They drew a dendrogram of 40 populations of this species by UPGMA method on the basis of genetic distances among them. This dendrogram showed that this species was first differentiated into the eastern and western groups. The eastern group was then divided into the northern, southern and intermediate subgroups. The northern subgroup was distributed in Hokkaido, Tohoku and Hokuriku regions and a part of Chubu region and included frogs which were of the ZZ–ZW type in sex-determining mechanism. The southern subgroup was distributed in parts of Chubu and Kinki regions and included frogs of the XX–XY type. In these frogs, the X and Y chromosomes could be clearly identified. In the frogs of the intermediate subgroup distributed in Kanto and a part of Chubu region and those of the western group, the sex chromosome could not be identified, although it was evident that they were of the XX–XY type in sex-determining mechanism.

The existence of sex-linked locus on sex chromosomes was first found in *Pleurodeles*. When FERRIER, JAYLET, CAYROL, GASSER and BUISAN (1980) developed the eggs of female *Pleurodeles waltl* in which the genotype of peptidase 1 was *ab* by gynogenesis, the offspring were diploids and consisted of 34 *aa* males, two *ab* females, and 22 *bb* females which were ZZ, ZW and WW in sex chromosomes, respectively. When they crossed *ab* females (ZW) with *aa* males (ZZ), 34 *aa* (ZZ) were all males and 28 *ab* (ZW) were all females. As all the 14 offspring produced from crossings between *bb* females (WW) and *aa* males (ZZ) obtained by gynogenesis were *ab* (ZW) females, these authors considered that the peptidase 1 locus was sex-linked. DOURNON, GUILLET, BOUCHER and LACROIX (1984) also reported that the peptidase 1 locus was sex-linked in *P. poireti*. The female heterogamety was previously reported by LACROIX (1968, 1970) in *P. waltl* and *P. poireti*, as he observed a heteromorphic region in bivalent (lampbrush) chromosome No. 4.

GRAF (1989a) reported that the sex-determining gene locus (or region) was linked with mitochondrial malic enzyme (mME) locus in *Xenopus laevis* which was of the ZZ–ZW type in sex-determining mechanism, and the recombination rate between them was 6.1%. Moreover, he (1989b) reported that the recombination rate between the mME and GPD–1 loci was 23% and that between the GPD–1

and sSOD-1 loci was also 23%.

ELINSON (1981) obtained backcross hybrids between male hybrids, *Rana clamitans* ♀ × *R. catesbeiana* ♂, in which the genotype of the LDH-B locus was heterozygous, and female *R. catesbeiana* whose genotype of the LDH-B locus was homozygous, and clarified that the sex-determining genes of *R. catesbeiana* were linked with the LDH-B locus, and that *R. catesbeiana* was of male heterogamety. ELINSON (1983) further reported that in *R. clamitans*, the aconitase 1 locus was linked with the sex-determining genes and that the male was heterogametic. These results contrasted with MENGDEN's report (1981) which clarified the female heterogamety in *R. clamitans* from the study of the chromosomal banding patterns.

WRIGHT and RICHARDS (1983) reported that in *R. pipiens* the male was of heterogamety and that the sex-determining genes were linked with the Pep-C and SOD-1 loci, by analyzing the offspring between a male whose genotypes of the Pep-C and SOD-1 loci were heterozygous and a female whose genotypes of the same loci were homo- or heterozygous. It was found that the recombination rates between the sex-determining genes and the Pep-C and SOD-1 loci were 12.1% and 8.6%, respectively, and that between the Pep-C and SOD-1 loci was 6.9%. WRIGHT, RICHARDS, FROST, CAMOZZI and KUNZ (1983) also clarified that the sex-determining genes were linked with the SOD-1 locus, and that the recombination rate between them was 8.6% and that between the SOD-1 and Pep-C loci was 7.1%, by analyzing the backcross progeny between the male hybrids, *R. palustris* ♀ × *R. pipiens* ♂, and female *R. pipiens*. They also reported that the sex-determining genes in *R. blairi* were linked with the SOD-1 locus by analyzing the backcross progeny produced between female *R. sphenocephala* and male hybrids, *R. sphenocephala* ♀ × *R. blairi* ♂. In *R. berlandieri*, they reported that the sex-determining genes were not linked with the Pep-C and SOD-1 loci, while they were linked with the ADH-2 locus and also the sex-determining genes were linked with the Ab, PGM 1, F16DP and β-GLU loci by analyzing the backcross progeny between female *R. sphenocephala* and male hybrids, *R. sphenocephala* ♀ × *R. berlandieri* ♂. They further assumed that in *R. sphenocephala* the sex-determining genes were linked with the aconitase 1 locus by analyzing 21 offspring consisting of 14 *ab* males, six *aa* females and one *aa* male which were obtained from a mating between a female which was homozygous *aa* and a male which was heterozygous *ab* in the genotype of the aconitase 1 locus.

SUMIDA and NISHIOKA (1994) examined the linkages between the sex-determining genes and 11 gene loci controlling eight enzymes and one blood protein in 10 populations of *R. japonica* distributed in Japan, and clarified that the Ab locus was linked with the sex-determining genes in the Munakata, Yamaguchi, Ochi, Saiki, Saijo, Sawara and Mobara populations, while the MDH-B, MPI, Pep-A and Pep-C loci were not linked with the latter. In the Ichinoseki and Toyama populations, the MPI locus was linked with the sex-determining genes, while the latter were not linked with the Ab locus. It was also clarified that each of the Ab, MPI, AAT-B, ADA, α-GDH, LDH-B, ME-A and ME-B loci was not linked with the sex-determining genes in the Akita population.

The linkages between the sex-determining genes and the loci controlling various enzymes in the *R. nigromaculata* group consisting of two species, one subspecies and many local populations were clarified by the present study. They found that the sex-determining genes were linked with three loci, the MPI, LDH-B and Pep-B loci, on chromosome No. 4 in the Konko population of *R. brevipoda*, and the recombination rates were assumed to be 11.1%, 10.5% and 5.1%, respectively. In the Hiro and Kumano populations of *R. nigromaculata*, the MPI locus was linked with the sex-determining genes and the recombination rates were assumed to be 8.9% and 8.6%, respectively. In five of the nine males of the Kaita population of *R. nigromaculata*, the four loci, the MPI, SORDH, ENO and HK loci, were linked with the sex-determining genes, and the recombination rates were assumed to be 7.9%, 6.2%, 11.4% and 8.7%, respectively. In the remaining four males of the Kaita population, the sex-determining genes were not linked with the MPI nor SORDH locus on chromosome No. 4, as well as the ME-B locus on chromosome No. 3. In the Maibara population of *R. brevipoda*, the ME-B locus on chromosome No. 3 was linked with the sex-determining genes and the recombination rate was assumed to be 2.2%, while the loci of the enzymes located on chromosome No. 4 were not linked with the sex-determining genes. Thus, in the Konko population of *R. brevipoda* and the Hiro and Kumano populations of *R. nigromaculata*, it was considered that the sex-determining genes were located together with six loci, the SORDH, MPI, ENO, HK, Pep-B and LDH-B loci on chromosome No. 4. Furthermore, in the Maibara population of *R. brevipoda*, it was assumed that the sex-determining genes were located together with the ME-B locus on chromosome No. 3.

It seems to be apparent that the Kaita population of *R. nigromaculata* consisted of two groups, the first of which had the sex-determining genes situated on chromosome No. 4 and was of male heterogamety (XX-XY type), while the second group probably had them situated on some chromosomes other than Nos. 3 and 4 and was of male or female heterogamety. The female heterogamety of the second group may be speculated by the fact that the sex ratios in the offspring of males of the second group were very irregular. The presence of two kinds of populations, XX-XY and ZZ-ZW types in sex-determining mechanisms, in one and the same species and the fact that the offspring between the populations of XX-XY and ZZ-ZW types were irregular in sex ratio were reported in *R. rugosa* by NISHIOKA, MIURA and SAITOH (1993) and NISHIOKA and HANADA (1994).

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