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Geographic Variability of Sex-Linked Loci in the Japanese Brown Frog, *Rana japonica*

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ABSTRACT

Rana japonica has been reported to be of the male heterogametic type in the sex-determining mechanism. In order to detect the sex-linked loci in *R. japonica*, the linkage relationships between the sex-determining genes and 11 loci controlling eight enzymes and one blood protein were examined in 48 crosses involving 30 males heterozygous at these loci from 10 local populations by using starch-gel electrophoresis. The Ab locus was found to be linked with the sex-determining genes in the Munakata, Yamaguchi, Ochi, Saiki, Saijo, Sahara and Mobara populations, whereas the MDH-B, MPI, Pep-A and Pep-C loci were not linked with the latter in 23 crosses involving 16 heterozygous males from the seven populations. The MPI locus was linked with the sex-determining genes in the Ichinoseki and Toyama populations, whereas the Ab locus was not linked with the latter in 11 crosses involving eight heterozygous males from two populations. In the Akita population, none of the Ab, AAT-B, ADA, α-GDH, LDH-B, ME-A, ME-B and MPI loci was linked with the sex-determining genes in 14 crosses involving six heterozygous males. Thus, it is evident that the locus linked with the sex-determining gene differs with the populations.

INTRODUCTION

In the Japanese brown frog *Rana japonica*, the male is considered to be heterogametic in sex-determining mechanism. This presumption was first proposed in 1959 by KAWAMURA and YOKOTA, who examined the sex of the progeny of sex-reversed females produced by hormone treatment. MORIWAKI (1959) also reported that *Rana japonica* seemed to be male heterogametic, based on the result of his investigation of the sex of the offspring produced from a parthenogenetic male. Thereafter, KAWAMURA and NISHIOKA (1977) ascertained male heterogamety on the basis of the sex of gynogenetically produced diploids in *Rana japonica*.

The sex-linked gene was first detected by FERRIER, JAYLET, CAYROL, GASSER and BUISAN (1980) in *Pleurodeles waltl*. Thereafter, sex-linked genes were reported in the hybrids of female *Rana clamitans* and male *Rana catesbeiana* (ELINSON, 1981), *Rana clamitans* (ELINSON, 1983), *Rana pipiens* complex (WRIGHT and RICHARDS, 1983; WRIGHT, RICHARDS, FROST, CAMOZZI and KUNZ, 1983), *Pleurodeles poireti* (DOURNON, GUILLET, BOUCHER and LACROIX, 1984), *Rana nigromaculata* and *Rana brevipoda* (NISHIOKA and SUMIDA, 1989, 1994) and *Xenopus laevis* (GRAF, 1989).

Since the report of SUMIDA (1981) that the Ichinoseki population of *Rana japonica* was reproductively isolated from the Hiroshima population, intraspecific differentiation has been examined biochemically by means of the starch-gel electrophoretic method, and electrophoretic variants of enzymes and blood proteins have been found at various loci (SUMIDA and NISHIOKA, 1991, 1994). The use of these electrophoretic variants is a powerful tool for the studies of genetic inheritance and of genetic linkage in amphibians (WRIGHT, RICHARDS and NACE, 1980).

The present study was carried out in order to ascertain which genes were sex-linked in local populations of *Rana japonica* by utilizing electrophoretic variants at various loci.

MATERIALS AND METHODS

The specimens of *Rana japonica* GÜNTHER used in the present study are listed in Table 1. These animals were screened for electrophoretic variants of several enzymes and blood proteins, using horizontal starch-gel electrophoresis. The electrophoresis was carried out according to the method of NISHIOKA, OHTANI and SUMIDA (1980). Each enzyme was detected by the agar-overlay method outlined by HARRIS and HOPKINSON (1976). The detection of blood proteins was made with the amido-black staining method. Eight kinds of enzymes and one blood protein analyzed, their abbreviations and locus names, Enzyme Commission numbers (Nomenclature Committee of Biochemistry, 1992), tissue samples used and associated buffer systems are presented in Table 2. Multiple alleles at each locus were named *a*, *b*, *c*, *etc.*, according to SUMIDA and NISHIOKA (1994). Males heterozygous at each locus were chosen and crossed with females by the artificial

TABLE 1
Specimens of *Rana japonica* used in the present study

Prefecture	Locality	No. of specimens			Population (Abbreviation)	
		Total	Female	Male		
Iwate	Ichinoseki-city, Sannoseki	7	4	3	Ichinoseki	(Ic.)
Chiba	Sahara-city	4	2	2	Sahara	(Sh.)
〃	Mobara-city	3	2	1	Mobara	(Mb.)
Akita	Akita-city, Toyoiwaishidazaka	6	3	3	Akita	(Ak.)
Niigata	Joetsu-city	1	1	0	Joetsu	(Je.)
Toyama	Toyama-city	2	0	2	Toyama	(Ty.)
Shizuoka	Shizuoka-city, Minaminumagami	1	1	0	Shizuoka	(Sz.)
Hiroshima	Hiroshima-city, Fuchu-cho	2	2	0	Hiroshima	(Hr.)
〃	Higashihiroshima-city, Saijo-cho	7	3	4	Saijo	(Sj.)
〃	Saiki-gun, Saiki-cho, Iinoyama	8	5	3	Saiki	(Sk.)
Shimane	Ochi-gun, Ochi-cho	3	1	2	Ochi	(Oc.)
Yamaguchi	Yamaguchi-city, Sayama	6	3	3	Yamaguchi	(Ym.)
Fukuoka	Munakata-city, Akama	1	0	1	Munakata	(Mn.)
Total		51	27	24		

TABLE 2
Enzymes and blood protein analyzed in the present study

Enzyme or blood protein	Locus	E.C.No.	Sample	Buffer system
Aspartate aminotransferase	AAT-B	2.6.1.1	Skeletal muscle	T-C pH 7.0
Adenosine deaminase	ADA	3.5.4.4	〃	〃
α-Glycerophosphate dehydrogenase	α-GDH	1.1.1.8	〃	T-C pH 6.0
Lactate dehydrogenase	LDH-B	1.1.1.27	〃	〃
Malate dehydrogenase	MDH-B	1.1.1.37	〃	〃
Malic enzyme	ME-A	1.1.1.40	〃	T-C pH 7.0
〃	ME-B	〃	〃	〃
Mannose phosphate isomerase	MPI	5.3.1.8	〃	〃
Peptidase	Pep-A	3.4.3.1	〃	T-B-E pH 8.0
〃	Pep-C	〃	〃	〃
Serum albumin	Ab	—	Blood serum	〃

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

fertilization method. The tadpoles were fed on boiled spinach, and metamorphosed froglets were fed on crickets (NISHIOKA and MATSUURA, 1977). The sex of each frog could be determined three months after metamorphosis by macroscopic examination of the gonads. The frogs were used for analysis of 11 loci by means of starch-gel electrophoresis. Linkage relationships were tested according to WRIGHT, RICHARDS and NACE (1980) and WRIGHT, RICHARDS, FROST, CAMOZZI and KUNZ (1983). Data were tabulated separately for males and females in each cross, and contingency χ^2 values were calculated to test the association of sex with genotype at each locus. The designations "parental" or "recombinant" were used according to these authors. The authors set high confidence limits ($P<0.01$) for linkage χ^2 values.

OBSERVATION

I. Inheritance and sex-linkage of 11 enzyme and blood protein loci in 10 populations of Rana japonica

1. Saiki population

The results of four crosses involving three males heterozygous at five loci, the Ab, MDH-B, MPI, Pep-A and Pep-C loci, are shown in Table 3 and the electrophoretic patterns of the offspring produced from these crosses are presented in Figs. 1 and 2. Each of Ab, MPI and Pep-C has a monomer structure so that the heterozygotes have two-banded electrophoretic patterns consisting of two primary bands. Each primary band of MPI has a weak satellite band with slower mobility. Both MDH and Pep-A have dimer structures, so that the heterozygotes show triple band patterns that consist of three regularly spaced bands, a middle primary band and two weaker bands.

TABLE 3

Inheritance of the Ab, MDH-B, MPI, Pep-A and Pep-C loci

Year	Parents		No. of metamorphosed frogs	No. of offspring examined			Ab and sex				MDH-B and sex				
							Parents	Offspring			Parents	Offspring			
	Female	Male		Total	♀	♂ (%)	Genotype ♀	Genotype	♀	♂	Genotype ♀	Genotype	♀	♂	
1990	Sk.1	Sk.1	53	39	13	26 (66.7)	*bb*	*ba*	2	**19**	—	—	—	—	—
								bb	**10**	5					
	Sk.2		160	75	23	52 (69.3)	—	—	—	—	—	—	—	—	—
		Sk.2	132	108	50	58 (53.7)	*bb*	*ba*	0	**36**	*cc*	*bc*	*cb*	27	32
								bb	**34**	6			*cc*	23	26
	Sk.3	Sk.3	122	51	21	30 (58.8)	*aa*	*aa*	0	**11**	—	—	—	—	—
								ab	**7**	0					
	Total		467	273	107	166 (60.8)			2	**66**			*cb*	27	32
									51	11			*cc*	23	26
	Parental (%)						**117 (90.0)**				55 (50.9)				
	Recombinant						**13**				53				
	χ^2						**83.20**				0.04				
	P						**<0.00001**				0.84				
	Recombination rate (%)						**10.0**				49.1				

Three crosses involving three males, Sk.♂Nos. 1~3, *ab* heterozygous at the Ab locus and three females, Sk.♀Nos. 1~3, *aa* or *bb* homozygous at this locus were analyzed (Table 3). In the cross, Sk.♀No. 1 × Sk.♂No. 1, involving a *bb* homozygous female and an *ab* heterozygous male, 19 of the male offspring were *ba* heterozygous and 10 of the female offspring were *bb* homozygous. These 29 were parental types. Only five males were *bb* homozygous, and only two females were *ba* heterozygous. These seven were recombinant types ($\chi^2=13.44$, $P<0.001$). In the cross, Sk.♀No. 2 × Sk.♂No. 2, involving a *bb* homozygous female and an *ab* heterozygous male, most of the male offspring (36) were *ba* heterozygous, whereas all female offspring examined (34) were *bb* homozygous with six *bb* homozygous males. Seventy were parental types and six were recombinant types ($\chi^2=53.89$, $P<0.00001$). In the cross, Sk.♀No. 3 × Sk.♂No. 3, involving an *aa* homozygous female and an *ab* heterozygous male, all males examined (11) were *aa* homozygous and all females examined (7) were *ab* heterozygous. All 18 offspring examined were parental types ($\chi^2=18.00$, $P<0.0001$). In these crosses, male offspring tended to inherit one paternal allele, *a*, whereas female offspring tended to inherit the other paternal allele, *b*. In total, 117 consisting of 66 males and 51

in crosses with heterozygous males of the Saiki population

MPI and sex				Pep-A and sex				Pep-C and sex			
Parents	Offspring			Parents	Offspring			Parents	Offspring		
Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂
ab *bc*	*ab*	1	10	— —	—	—	—	*ab* *be*	*ab*	4	8
	bb	1	3						*bb*	4	7
	ac	6	3						*ae*	4	4
	bc	5	10						*be*	1	7
ab *bc*	*ab*	4	10	— —	—	—	—	*ab* *be*	*ab*	4	10
	bb	5	16						*bb*	8	15
	ac	3	12						*ae*	4	14
	bc	11	14						*be*	7	13
ab *bc*	*ab*	15	12	— —	—	—	—	— —	—	—	—
	bb	7	10								
	ac	15	18								
	bc	13	18								
bc *ba*	*bb*	2	6	*bb* *ab*	*ba*	7	12	— —	—	—	—
	cb	2	8		*bb*	14	18				
	ba	9	13								
	ca	8	3								
		37	75		*ba*	7	12			20	40
		70	91		*bb*	14	18			16	38
145 (53.1)				26 (51.0)				58 (50.9)			
128				25				56			
1.06				0.02				0.04			
0.30				0.89				0.85			
46.9				49.0				49.1			

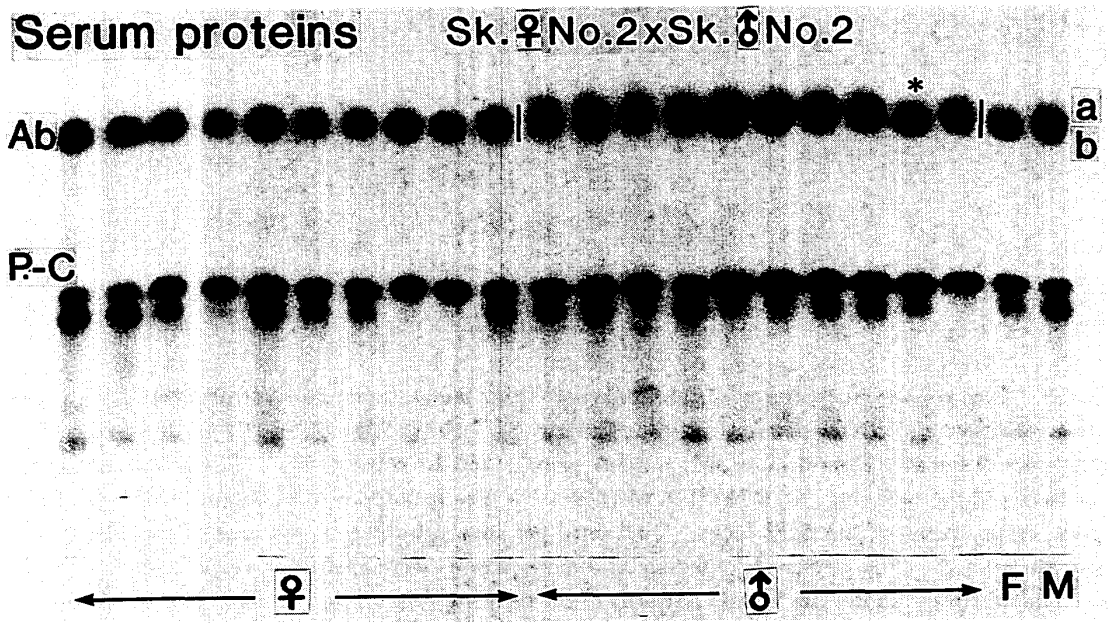

Fig. 1. Electrophoretic patterns of serum proteins from female and male offspring, Sk.♀No. 2 × Sk.♂ No. 2, and their parents. At the Ab locus, the genotypes of female (F) and male (M) parents were *bb* and *ab*, respectively. All the female offspring were *bb* homozygous and male offspring were predominantly *ab* heterozygous at this locus. A recombinant male was marked with an asterisk.

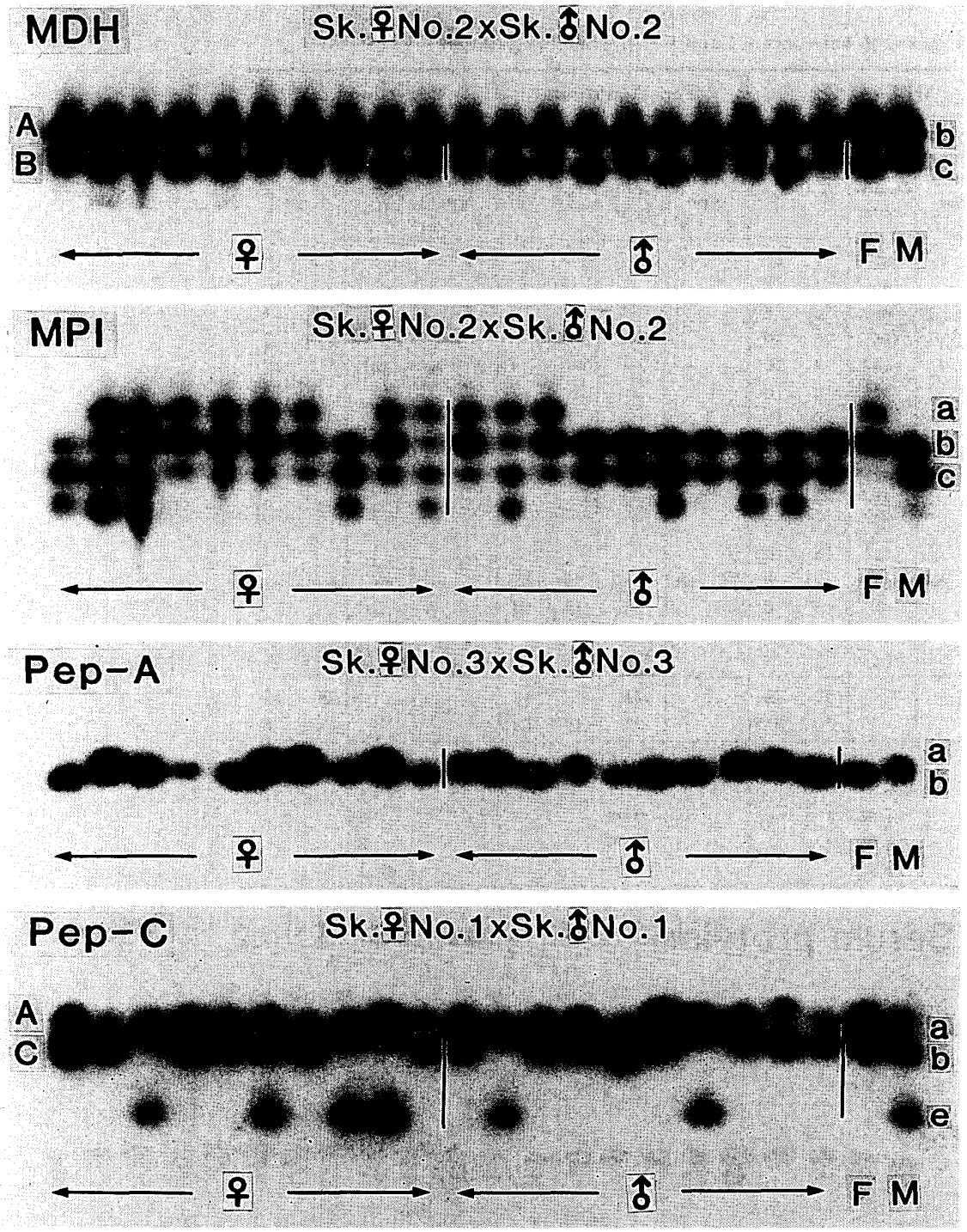

Fig. 2. Electrophoretic patterns of four enzymes from female and male offspring and their parents. The first gel shows the MDH patterns of the offspring, Sk.♀No. 2 × Sk.♂No. 2. At the MDH-B locus, the genotypes of female (F) and male (M) parents were *cc* and *bc*, respectively. The second gel shows the MPI patterns of the offspring, Sk.♀No. 2 × Sk.♂No. 2. The genotypes of male and female parents were *ab* and *bc*, respectively, at the MPI locus. The third gel shows the Pep-A patterns of the offspring, Sk.♀No. 3 × Sk.♂No. 3. The genotypes of female and male parents were *bb* and *ab*, respectively, at the Pep-A locus. The fourth gel shows the Pep-C patterns of the offspring, Sk.♀No. 1 × Sk.♂No. 1. The substrate L-leucyl-alanine also detected the Pep-A on this gel. The genotypes of female and male parents were *ab* and *be*, respectively, at the Pep-C locus. These electrophoretic patterns showed that none of these four loci was linked with the sex-determining genes.

females were parental types and 13 consisting of 11 males and two females were recombinant types and the recombination rate was 10.0% ($\chi^2=83.20$, $P<0.00001$). These values show that the Ab locus was closely linked with the sex-determining genes.

In one cross involving a male heterozygous at the MDH-B locus, 55 offspring were parental types, 53 were recombinant types and the recombination rate was 49.1% ($\chi^2=0.04$, $P>0.8$). In four crosses involving three males heterozygous at the MPI locus, a total of 145 was parental types, a total of 128 was recombinant types and the recombination rate was 46.9% ($\chi^2=1.06$, $P>0.3$). In one cross involving a male heterozygous at the Pep-A locus, 26 were parental types, 25 were recombinant types and the recombination rate was 49.0% ($\chi^2=0.02$, $P>0.8$). In two crosses involving a male heterozygous at the Pep-C locus, a total of 58 was parental types, a total of 56 was recombinant types and the recombination rate was 49.1% ($\chi^2=0.04$, $P>0.8$). These values show that the MDH-B, MPI, Pep-A and Pep-C loci were not linked with the sex-determining genes.

2. Munakata population

One cross involving a male, Mn. ♂ No. 1, *bc* heterozygous at the Ab locus, and a female, Sk. ♀ No. 4, *ab* heterozygous at this locus was analyzed (Table 4). In this cross, most of the male offspring (10) inherited one paternal allele, *b*, whereas all female offspring (11) inherited the other paternal allele, *c*. These 21 were parental types. Only two male offspring inherited paternal allele *c*. These two were recombinant types. The recombination rate was 8.7% ($\chi^2=15.70$, $P<0.0001$). These values show that the Ab locus was closely linked with the sex-determining genes.

3. Yamaguchi population

Three crosses involving three males, Ym. ♂ Nos. 1~3, *ab* heterozygous at the Ab locus and two females, Ym. ♀ No. 1 and Sj. ♀ No. 1, *aa* homozygous or *ab* heterozygous at this locus were analyzed (Table 4). In the cross, Ym. ♀ No. 1 × Ym. ♂ No. 1, involving an *aa* homozygous female and an *ab* heterozygous male, most of the male offspring (60) were *ab* heterozygous, whereas all female offspring (49) were *aa* homozygous, with two *aa* homozygous males. In two crosses, Sj. ♀ No. 1 × Ym. ♂ Nos. 1 and 2, of which both parents were *ab* heterozygous, there were approximately equal numbers of male (27) and female (30) *ab* heterozygous offspring, whereas all *aa* homozygous individuals (30) were females and all *bb* homozygous individuals (18) were males. In total, 157 consisting of 79 females and 78 males were parental types, two males were recombinant types and the recombination rate was 1.3% ($\chi^2=151.10$, $P<0.00001$). These values show that the Ab locus was closely linked with the sex-determining genes.

4. Ochi population

Three crosses involving two males, Oc. ♂ Nos. 1 and 2, *ba* or *ab* heterozygous at the Ab locus and three females, Oc. ♀ No. 1 and Sj. ♀ Nos. 1 and 2, *bb* homozygous

TABLE 4

Inheritance of the Ab locus in crosses with heterozygous males of the Munakata, Yamaguchi and Ochi populations

Year	Parents		No. of metamorphosed frogs	No. of offspring examined			Ab and sex				Sex-linkage of Ab				
							Parents	Offspring			Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂					
1991	Sk.4	Mn.1	24	23	11	12 (52.2)	*ab* *bc*	*ab*	0	**4**	**21 (91.3)**	**2**	**15.70**	**<0.0001**	**8.7**
								bb	0	**6**					
								ac	**4**	1					
								bc	**7**	1					
1992	Ym.1	Ym.1	287	111	49	62 (55.9)	*aa* *ab*	*aa*	**49**	2	**109 (98.2)**	**2**	**103.14**	**<0.00001**	**1.8**
								ab	0	**60**					
1993	Sj.1	Ym.2	78	52	33	19 (36.5)	*ab* *ab*	*aa*	**17**	0	**27 (100)**	**0**	**27.00**	**<0.00001**	**0**
								ab	16	9					
								bb	0	**10**					
		Ym.3	76	53	27	26 (49.1)	*ab* *ab*	*aa*	**13**	0	**21 (100)**	**0**	**21.00**	**<0.00001**	**0**
								ab	14	18					
								bb	0	**8**					
Total			441	216	109	107 (49.5)		*aa*	**79**	2	**157 (98.7)**	**2**	**151.10**	**<0.00001**	**1.3**
								ab	30	27					
								bb	0	**78**					
1992	Oc.1	Oc.1	15	8	1	7 (87.5)	*bb* *ba*	*bb*	0	**7**	**8 (100)**	**0**	**8.00**	**<0.01**	**0**
								ba	**1**	0					
1993	Sj.1	Oc.2	82	57	31	26 (45.6)	*ab* *ab*	*aa*	0	**12**	**28 (96.6)**	**1**	**25.14**	**<0.00001**	**3.4**
								ab	15	13					
								bb	**16**	1					
	Sj.2		85	57	28	29 (50.9)	*ab* *ab*	*aa*	0	**6**	**26 (81.3)**	**6**	**12.50**	**<0.001**	**18.8**
								ab	8	17					
								bb	**20**	6					
Total			182	122	60	62 (50.8)			0	**25**	**62 (89.9)**	**7**	**43.84**	**<0.00001**	**10.1**
									23	30					
									37	7					

or *ab* heterozygous at this locus were examined (Table 4). In the cross, Oc.♀No. 1 × Oc.♂No. 1, involving a *bb* homozygous female and a *ba* heterozygous male, all male offspring (7) were *bb* homozygous and one female was *ba* heterozygous. In two crosses, Sj.♀Nos. 1 and 2 × Oc.♂No. 2, of which both parents were *ab* heterozygous, 23 female and 30 male offspring were *ab* heterozygous, whereas all *aa* homozygous offspring (18) were males and most of *bb* homozygous offspring (36) were females, with seven *bb* homozygous males. In total, 62 consisting of 37 females and 25 males were parental types, seven males were recombinant types and the recombination rate was 10.1% ($\chi^2=43.84$, $P<0.00001$). These values indicate the close sex-linkage of the Ab locus.

5. Saijo population

Seven crosses involving four males, Sj.♂Nos. 1~4, *ab* heterozygous at the Ab

TABLE 5

Inheritance of the Ab locus in crosses with heterozygous males of the Saijo population

Year	Parents		No. of metamorphosed frogs	No. of offspring examined			Ab and sex				Sex-linkage of Ab				
							Parents	Offspring							
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂	Parental (%)	Recombinant	χ^2	P	Recombination rate (%)
1991	Sk.4	Sj.1	35	18	8	10 (55.6)	*ab* *ab*	*aa*	**2**	0	**4 (100)**	**0**	**4.00**	**0.05**	**0**
								ab	6	8					
								bb	0	**2**					
	Sk.5		54	25	7	18 (72.0)	*ab* *ab*	*aa*	**5**	3	**12 (80.0)**	**3**	**5.40**	**0.02**	**20.0**
								ab	2	8					
								bb	0	**7**					
1992	Je.1	Sj.2	83	67	28	39 (58.2)	*bc* *ab*	*ba*	**15**	8	**53 (79.1)**	**14**	**22.70**	**<0.00001**	**20.9**
								ca	**10**	3					
								bb	1	**12**					
								cb	2	**16**					
1993	Sj.1	Sj.3	82	42	18	24 (57.1)	*ab* *ab*	*aa*	**9**	0	**20 (100)**	**0**	**20.00**	**<0.00001**	**0**
								ab	9	13					
								bb	0	**11**					
	Sj.2		29	29	10	19 (65.5)	*ab* *ab*	*aa*	**5**	2	**11 (84.6)**	**2**	**6.23**	**0.01**	**15.4**
								ab	5	11					
								bb	0	**6**					
	Sj.3		29	29	6	23 (79.3)	*ab* *ab*	*aa*	**4**	2	**15 (88.2)**	**2**	**9.94**	**<0.001**	**11.8**
								ab	2	10					
								bb	0	**11**					
		Sj.4	53	53	12	41 (77.4)	*ab* *ba*	*bb*	**8**	7	**21 (75.0)**	**7**	**7.00**	**<0.01**	**25.0**
								ba	4	21					
								aa	0	**13**					
Total			365	263	89	174 (66.2)			**58**	25	**136 (82.9)**	**28**	**71.12**	**<0.00001**	**17.1**
									28	71					
									3	**78**					

locus, and six females, Sk.♀Nos. 4 and 5, Je.♀No.1 and Sj.♀ Nos. 1~3, *ab* or *bc* heterozygous at this locus were analyzed (Table 5). In the cross, Je.♀No. 1 × Sj.♂No. 2, involving a *bc* heterozygous female and an *ab* heterozygous male, most of the female offspring (25) inherited one paternal allele, *a*, whereas most of the male offspring (28) inherited the other paternal allele, *b*. Only three females inherited paternal allele *b*, and 11 males inherited paternal allele *a*. In the other six crosses involving five females, Sk.♀Nos. 4 and 5 and Sj.♀Nos. 1~3, *ab* heterozygous at the Ab locus, and three males, Sj.♂Nos. 1, 3 and 4, *ab* heterozygous at this locus, 28 females and 71 males were *ab* heterozygous, whereas all *bb* homozygous offspring (50) were males and most of the *aa* homozygous offspring (33) were females, with 14 *aa* homozygous males. In total, 136 consisting of 58 females and 78 males were parental types, 28 consisting of three females and 25 males were recombinant types and the recombination rate was 17.1% ($\chi^2=71.12$, $P<0.00001$). These values show that the Ab locus was linked with the sex-determining genes. Of the 28 recombinants, males were far more numerous than females. It is probable that some of the male recombinants are considered to be

TABLE 6
Inheritance of the Ab and MPI loci in crosses with

Year	Parents		No. of metamorphosed frogs	No. of offspring examined			Ab and sex			
							Parents	Offspring		
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂
1991	Sh.1	Sh.1	59	33	12	21 (63.6)	— —	—	—	—
	Sh.2		83	20	9	11 (55.0)	— —	—	—	—
1992	Je.1	Sh.2	110	87	47	40 (46.0)	*bc* *bc*	*bb*	**21**	0
								bc	24	25
								cc	2	**15**
Total			252	140	68	72 (51.4)			**21**	0
									24	25
									2	**15**
1993	Mb.1	Mb.1	149	55	27	28 (50.9)	*bc* *bc*	*bb*	**11**	0
								bc	16	8
								cc	0	**20**
	Mb.2		105	95	52	43 (45.3)	*cc* *bc*	*cb*	**44**	0
								cc	0	**31**
Total			254	150	79	71 (47.3)			**55**	0
									16	8
									0	**51**

genetic females and sex-reversed males, because the sex-reversal from female to male sometimes occurs in the artificial crossings.

6. Sahara population

Three crosses involving two males heterozygous at two loci, the Ab and MPI loci, were analyzed (Table 6). In the cross, Je.♀No. 1 × Sh.♂No. 2, of which both parents were *bc* heterozygous at the Ab locus, almost equal numbers of male (25) and female (24) offspring were *bc* heterozygous, whereas all *bb* homozygous individuals (21) were females and most of the *cc* homozygous individuals (15) were males, with two *cc* homozygous females. In this cross, 36 consisting of 21 females and 15 males were parental types, two females were recombinant types and the recombination rate was 5.3% ($\chi^2=30.42$, $P<0.00001$). In two crosses involving a male heterozygous at the MPI locus, a total of 27 was parental types, a total of 26 was recombinant types and the recombination rate was 49.1% ($\chi^2=0.02$, $P>0.8$). These results show that the Ab locus was linked with the sex-determining genes, whereas the MPI locus was not linked with the latter.

7. Mobara population

Two crosses involving a male heterozygous at two loci, the Ab and MPI loci,

heterozygous males of the Sahara and Mobara populations

MPI and sex				Sex-linkage of Ab and MPI					
Parents	Offspring			Parental (%)		Recombinant	χ^2	P	Recombination rate (%)
Genotype ♀ ♂	Genotype	♀	♂						
cd ce	*cc*	3	3	MPI	17 (51.5)	16	0.03	0.86	48.5
	dc	2	7						
	ce	3	6						
	de	4	5						
dd ce	*dc*	6	7	MPI	10 (50.0)	10	0	1.00	50.0
	de	3	4						
— —	—	—	—	**Ab**	**36 (94.7)**	**2**	**30.42**	**<0.00001**	**5.3**
		11	17	**Ab**	**36 (94.7)**	**2**	**30.42**	**<0.00001**	**5.3**
		10	15	MPI	27 (50.9)	26	0.02	0.89	49.1
dd cd	*dc*	16	16	**Ab**	**31 (100)**	**0**	**31.00**	**<0.00001**	**0**
	dd	11	12	MPI	28 (50.9)	27	0.02	0.89	49.1
cc cd	*cc*	25	16	**Ab**	**75 (100)**	**0**	**75.00**	**<0.00001**	**0**
	cd	27	27	MPI	52 (54.7)	43	0.85	0.36	45.3
		41	32	**Ab**	**106 (100)**	**0**	**106.00**	**<0.00001**	**0**
		38	39	MPI	80 (53.3)	70	0.67	0.41	46.7

were analyzed (Table 6). In the cross, Mb. ♀No. 1 × Mb. ♂No. 1, of which both parents were *bc* heterozygous at the Ab locus, 16 female and eight male offspring were *bc* heterozygous, whereas all *bb* homozygous individuals (11) were females and all *cc* homozygous individuals (20) were males. In the other cross, Mb. ♀ No. 2 × Mb. ♂No. 1, involving a female, *cc* homozygous at the Ab locus and a male, *bc* heterozygous at this locus, all females (44) were *cb* heterozygous and all males (31) were *cc* homozygous. In these two crosses, male offspring tended to inherit one paternal allele, *c*, whereas female offspring tended to inherit the other paternal allele, *b*. A total of 106 offspring, consisting of 55 females and 51 males, was parental types and there were no recombinant types ($\chi^2=106.00$, $P<0.00001$). In the same two crosses involving a male heterozygous at the MPI locus, a total of 80 offspring was parental types, a total of 70 offspring was recombinant types and the recombination rate was 46.7% ($\chi^2=0.67$, $P>0.4$). These results show that the Ab locus was linked with the sex-determining genes, but the MPI locus was not linked with the latter.

8. Ichinoseki population

Four crosses involving three males heterozygous at two loci, the Ab and MPI loci, were analyzed (Table 7). In two crosses, Ic. ♀Nos. 1 and 2 × Ic. ♂No. 1,

TABLE 7

Inheritance of the Ab and MPI loci in crosses with

Year	Parents		No. of metamorphosed frogs	No. of offspring examined			Ab and sex			
							Parents	Offspring		
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂
1993	Ic.1	Ic.1	53	31	19	12 (38.7)	*bc* *bc*	*bb*	4	1
								bc	7	7
								cc	8	4
	Ic.2		60	31	16	15 (48.4)	*bc* *bc*	*bb*	4	1
								bc	8	9
								cc	4	5
	Ic.3	Ic.2	107	101	57	44 (43.6)	— —	—	—	—
	Ic.4	Ic.3	68	35	11	24 (68.6)	*bb* *bc*	*bb*	5	11
								bc	6	13
Total			288	198	103	95 (48.0)			13	13
									15	16
									18	22
1988	Hr.1	Ty.1	139	94	34	60 (63.8)	— —	—	—	—
	Hr.2		77	50	19	31 (62.0)	— —	—	—	—
	Sz.1		105	76	38	38 (50.0)	— —	—	—	—
1992	Oc.1	88(Hr.1×Ty.1)1	75	40	15	25 (62.5)	*bb* *ab*	*ba*	5	11
								bb	10	14
		88(Hr.1×Ty.1)2	63	63	24	39 (61.9)	— —	—	—	—
	Ym.2	88(Hr.2×Ty.1)1	102	44	19	25 (56.8)	*bb* *ab*	*ba*	11	10
								bb	8	15
	Ym.3	88(Sz.1×Ty.2)1	97	49	23	26 (53.1)	*bb* *ac*	*ba*	12	17
								bc	11	9
Total			658	416	172	244 (58.7)			28	38
									29	38

involving a male, *dc* heterozygous at the MPI locus and two females, *cc* homozygous at this locus, all female offspring (35) were *cd* heterozygous, whereas most of the male offspring (25) were *cc* homozygous, with only two *cd* heterozygous males. In the cross, Ic.♀No. 3 × Ic.♂No. 2, involving a male, *ce* heterozygous at the MPI locus and a female, *cc* homozygous at this locus, all female offspring (57) were *cc* homozygous, whereas all male offspring (44) were *ce* heterozygous. In these crosses, male offspring tended to inherit one paternal allele and female offspring the other. In total, 161 offspring, consisting of 92 females and 69 males, were

heterozygous males of the Ichinoseki and Toyama populations

MPI and sex				Sex-linkage of Ab and MPI					
Parents	Offspring			Parental (%)		Recombinant	χ^2	P	Recombination rate (%)
Genotype ♀ ♂	Genotype	♀	♂						
cc dc	*cd*	**19**	1	Ab	8 (47.1)	9	0.06	0.81	52.9
	cc	0	**11**	**MPI**	**30 (96.8)**	**1**	**27.13**	**<0.00001**	**3.2**
cc dc	*cd*	**16**	1	Ab	9 (64.3)	5	1.14	0.29	35.7
	cc	0	**14**	**MPI**	**30 (96.8)**	**1**	**27.13**	**<0.00001**	**3.2**
cc ce	*cc*	**57**	0	**MPI**	**101 (100)**	**0**	**101.00**	**<0.00001**	**0**
	ce	0	**44**						
— —	—	—	—	Ab	18 (51.4)	17	0.03	0.87	48.6
		92	2	Ab	35 (53.0)	31	0.24	0.62	47.0
		0	**69**	**MPI**	**161 (98.8)**	**2**	**155.10**	**<0.00001**	**1.2**
ac cd	*ac*	**21**	6	**MPI**	**86 (91.5)**	**8**	**64.72**	**<0.00001**	**8.5**
	cc	**13**	2						
	ad	0	**30**						
	cd	0	**22**						
cc cd	*cc*	**19**	9	**MPI**	**41 (82.0)**	**9**	**20.48**	**<0.00001**	**18.0**
	cd	0	**22**						
ab cd	*ac*	**13**	0	**MPI**	**71 (93.4)**	**5**	**57.32**	**<0.00001**	**6.6**
	bc	**25**	5						
	ad	0	**19**						
	bd	0	**14**						
cc bd	*cb*	**15**	1	Ab	21 (52.5)	19	0.10	0.75	47.5
	cd	0	**24**	**MPI**	**39 (97.5)**	**1**	**36.10**	**<0.00001**	**2.5**
cc cd	*cc*	**24**	9	**MPI**	**54 (85.7)**	**9**	**32.14**	**<0.00001**	**14.3**
	cd	0	**30**						
cc cd	*cc*	**19**	5	Ab	18 (40.9)	26	1.45	0.23	59.1
	cd	0	**20**	**MPI**	**39 (88.6)**	**5**	**26.27**	**<0.00001**	**11.4**
bc bc	*bb*	**12**	1	Ab	28 (57.1)	21	1.00	0.32	42.9
	bc	11	13	**MPI**	**24 (96.0)**	**1**	**21.16**	**<0.00001**	**4.0**
	cc	0	**12**						
		161	38	Ab	67 (50.4)	66	0.01	0.93	49.6
		11	13	**MPI**	**354 (90.3)**	**38**	**254.74**	**<0.00001**	**9.7**
		0	**193**						

parental types, only two male offspring were recombinant types and the recombination rate was 1.2% ($\chi^2=155.10$, $P<0.00001$). In three crosses involving two males heterozygous at the Ab locus, a total of 35 was parental types, a total of 31 was recombinant types and the recombination rate was 47.0% ($\chi^2=0.24$, $P>0.6$). These results show that the MPI locus was closely linked with the sex-determining genes, whereas the Ab locus was not linked with the latter.

9. Toyama population

Seven crosses involving five males heterozygous at two loci, the Ab and MPI loci, were analyzed (Table 7 and Fig. 3). In the cross, Ym.♀No. 3 × 88 (Sz.1 × Ty.2)♂No. 1, of which both parents were *bc* heterozygous at the MPI locus, there were almost equal numbers of male (13) and female (11) *bc* heterozygous offspring, whereas all *cc* homozygous individuals (12) were males and most of the *bb* homozygous individuals (12) were females, with only one *bb* homozygous male. In the cross, Oc.♀No. 1 × 88(Hr.1 × Ty.1)♂No. 1, involving a male, *bd* heterozygous at the MPI locus and a female, *cc* homozygous at this locus, all female offspring (15) were *cb* heterozygous, whereas most of the male offspring (24) were *cd* heterozygous, with only one *cb* heterozygous male. In the other five crosses involving three males, Ty.♂No. 1, 88 (Hr.1 × Ty.1)♂No. 2 and 88 (Hr.2 × Ty.1)♂No. 1, *cd* heterozygous at the MPI locus, and five females, Hr.♀Nos. 1 and 2, Sz.♀No. 1, Oc.♀No. 1 and Ym.♀No. 2, *ac* or *ab* heterozygous or *cc* homozygous at this locus, all 134 female offspring inherited one paternal allele, *c*,

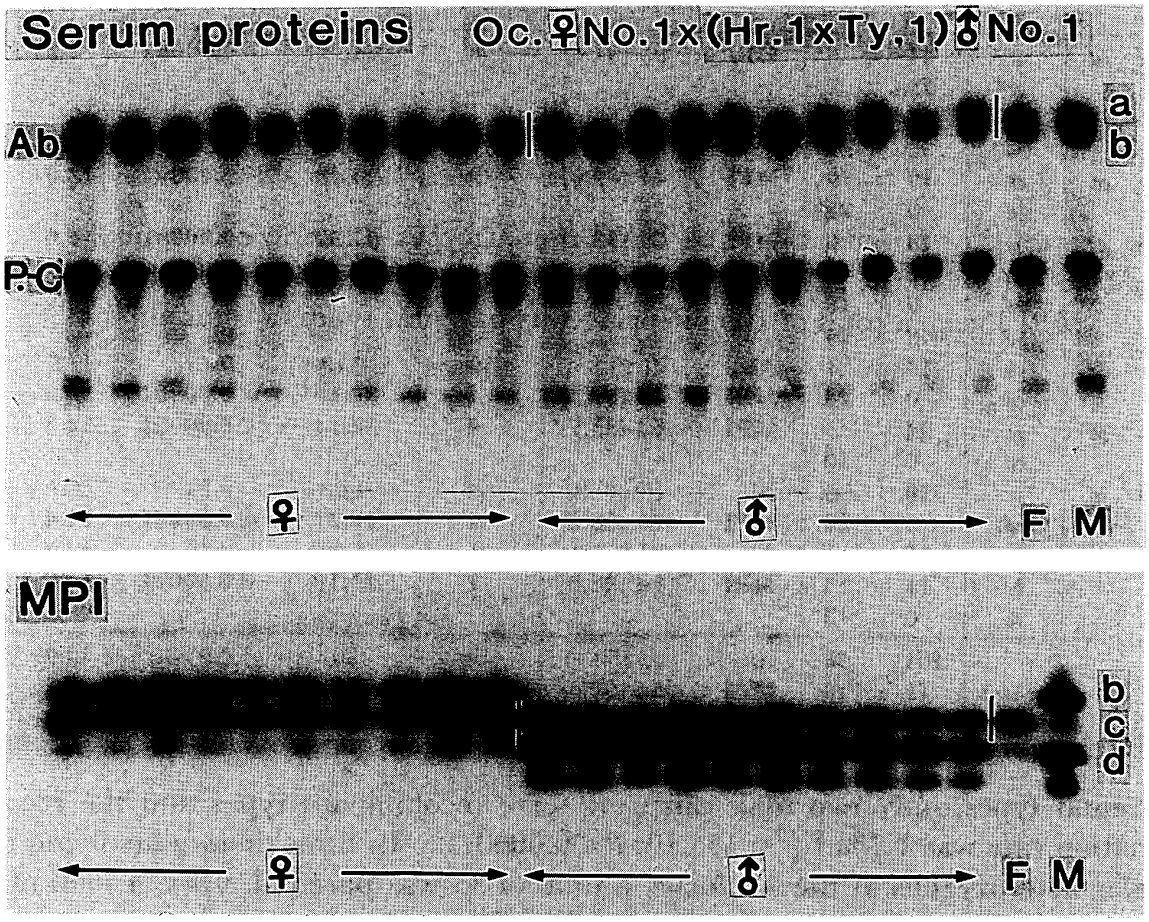

Fig. 3. Electrophoretic patterns of serum proteins and MPI from female and male offspring, Oc.♀No. 1 × 88 (Hr. 1 × Ty. 1)♂No. 1, and their parents. At the Ab locus, the genotypes of female (F) and male (M) parents were *bb* and *ab*, respectively. At the MPI locus, the genotypes of female (F) and male (M) parents were *cc* and *bd*, respectively. All the female offspring were *cb* heterozygous and all the male offspring were *cd* heterozygous at this locus.

whereas most of the male offspring (157) inherited the other paternal allele, *d*. The other male offspring (36) inherited paternal allele *c*. In these seven crosses, female offspring tended to inherit one paternal allele and male offspring the other. In total, 354 consisting of 161 females and 193 males were parental types, 38 males were recombinant types and the recombination rate was 9.7% ($\chi^2=254.74$, $P<0.00001$). Since all 38 recombinants were males, some of them are considered to be genetic females and sex-reversed males. In three crosses involving three males heterozygous at the Ab locus, a total of 67 was parental types, a total of 66 was recombinant types and the recombination rate was 49.6% ($\chi^2=0.01$, $P>0.9$). Thus, it is evident that the MPI locus was linked with the sex-determining genes, whereas the Ab locus was not linked with the latter.

10. Akita population

A total of 14 crosses involving six males heterozygous at eight loci, the Ab, AAT-B, ADA, α-GDH, LDH-B, ME-A, ME-B and MPI loci, was analyzed (Tables 8 and 9). In three crosses involving a male heterozygous at the ADA locus, a total of 38 offspring was parental types, a total of 24 offspring was recombinant types and the recombination rate was 38.7% ($\chi^2=3.16$, $P>0.07$). In three crosses involving a male heterozygous at the LDH-B locus, a total of 39 offspring was parental types, a total of 35 offspring was recombinant types and the recombination rate was 47.3% ($\chi^2=0.22$, $P>0.6$). In seven crosses involving two males heterozygous at the ME-A locus, a total of 59 offspring was parental types, a total of 58 offspring was recombinant types and the recombination rate was 49.6% ($\chi^2=0.01$, $P>0.9$). In nine crosses involving four males heterozygous at the MPI locus, a total of 105 offspring was parental types, a total of 80 offspring was recombinant types and the recombination rate was 43.2% ($\chi^2=3.38$, $P>0.06$). In four crosses involving two males heterozygous at all of the four loci, the Ab, AAT-B, α-GDH and ME-B loci, 24~28 were parental types, 18~21 were recombinant types and the recombination rate was 40.0~46.7% ($\chi^2=0.20\sim1.80$, $P>0.1\sim P>0.6$) (Table 9). These results show that none of the eight loci examined, the Ab, AAT-B, ADA, α-GDH, LDH-B, ME-A, ME-B and MPI loci, was linked with the sex-determining genes.

II. Summary of sex-linkage

Table 10 shows the linkage relationships between the sex-determining genes and 11 enzyme and blood protein loci in 10 populations of *Rana japonica*. The Ab locus was linked with the sex-determining genes in the following seven populations, the Munakata, Yamaguchi, Ochi, Saiki, Saijo, Sahara and Mobara populations, whereas the MPI locus was linked with the latter in two populations, the Ichinoseki and Toyama populations. In the Akita population, the sex-determining genes were linked with none of the eight loci examined including the Ab, AAT-B, ADA, α-GDH, LDH-B, ME-A, ME-B and MPI loci.

TABLE 8

Inheritance of the ADA, LDH-B, ME-A and MPI loci in crosses

Year	Parents		No. of metamorphosed frogs	No. of offspring examined			ADA and sex			
							Parents		Offspring	
	Female	Male		Total	♀	♂ (%)	Genotype ♀	♂	Genotype	♀	♂
1991	Ak.1	Ak.1	27	11	6	5 (45.5)	—	—	—	—	—
	Ak.2	Ak.2	78	53	33	20 (37.7)	—	—	—	—	—
	Ak.3		87	27	6	21 (77.8)	—	—	—	—	—
1993	91(Ak.2 ×Ak.2)1	91(Ak.2 ×Ak.2)1	103	33	8	25 (75.8)	—	—	—	—	—
	Je.1		50	10	4	6 (60.0)	—	—	—	—	—
	Ic.1		81	19	8	11 (57.9)	—	—	—	—	—
	Ic.2		105	20	9	11 (55.0)	—	—	—	—	—
	91(Ak.2 ×Ak.2)2	91(Ak.2 ×Ak.2)2	34	24	13	11 (45.8)	*bd*	*bd*	*bb*	2	6
									bd	8	4
									dd	3	1
	Ic.1		60	20	10	10 (50.0)	*ad*	*bd*	*ab*	3	2
									db	3	4
									ad	3	1
									dd	1	3
	Ic.2		100	30	22	8 (26.7)	*ac*	*bd*	*ab*	5	2
									cb	2	2
									ad	3	4
									cd	12	0
	91(Ak.2 ×Ak.2)1	92(Je.1 ×Ak.3)2	11	11	6	5 (45.5)	—	—	—	—	—
	91(Ak.2 ×Ak.2)2		6	6	1	5 (83.3)	—	—	—	—	—
Total			742	264	126	138 (52.3)				15	16
										8	4
										22	9
		Parental (%)						38 (61.3)			
		Recombinant						24			
		χ^2						3.16			
		P						0.08			
		Recombination rate (%)						38.7			

DISCUSSION

Sex-linked genes have been reported in several species of amphibians over the past decade. A sex-linked gene was first reported by FERRIER, JAYLET, CAYROL, GASSER and BUISAN (1980) and FERRIER, GASSER, JAYLET and CAYROL (1983) in

with heterozygous males of the Akita population

LDH-B and sex				ME-A and sex				MPI and sex			
Parents	Offspring			Parents	Offspring			Parents	Offspring		
Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂
— —	—	—	—	— —	—	—	—	*dd de*	*dd*	3	0
									de	3	5
— —	—	—	—	— —	—	—	—	*dd cd*	*dc*	25	9
									dd	8	11
— —	—	—	—	— —	—	—	—	*dd cd*	*dc*	4	12
									dd	2	9
— —	—	—	—	*ab ab*	*aa*	1	6	*dd cd*	*dc*	3	10
					ab	4	11		*dd*	5	15
					bb	3	8				
— —	—	—	—	*aa ab*	*aa*	4	2	*cc cd*	*cc*	3	4
					ab	0	4		*cd*	1	2
— —	—	—	—	*aa ab*	*aa*	2	3	*cc cd*	*cc*	2	4
					ab	3	2		*cd*	6	7
— —	—	—	—	*aa ab*	*aa*	3	7	*cc cd*	*cc*	4	6
					ab	6	4		*cd*	5	5
bb ab	*ba*	6	7	*ab ab*	*aa*	1	0	— —	—	—	—
	bb	7	4		*ab*	7	7				
					bb	5	3				
bb ab	*ba*	6	3	*aa ab*	*aa*	6	4	— —	—	—	—
	bb	4	7		*ab*	4	6				
bb ab	*ba*	14	6	*aa ab*	*aa*	12	5	— —	—	—	—
	bb	8	2		*ab*	10	3				
— —	—	—	—	— —	—	—	—	*dd cd*	*dc*	4	3
									dd	2	2
— —	—	—	—	— —	—	—	—	*cd cd*	*cc*	0	0
									cd	1	4
									dd	0	1
	ba	26	16			29	27			48	48
	bb	19	13			11	18			1	4
						31	30			32	57
	39 (52.7)				59 (50.4)				105 (56.8)		
	35				58				80		
	0.22				0.01				3.38		
	0.64				0.93				0.07		
	47.3				49.6				43.2		

the salamander *Pleurodeles waltl.* In this species, the Pep–A locus was linked with the sex-determining genes. This locus was also demonstrated to be linked with the latter in the closely-related *Pleurodeles poireti* (DOURNON, GUILLET, BOUCHER and LACROIX, 1984). In the hybrids between female *Rana clamitans* and male *R.*

TABLE 9

Inheritance of the Ab, AAT-B, α-GDH and ME-B loci in crosses

Year	Parents		No. of metamorphosed frogs	No. of offspring examined			Ab and sex			
							Parents	Offspring		
	Female	Male		Total	♀	♂ (%)	Genotype ♀ ♂	Genotype	♀	♂
1993	91(Ak.2 ×Ak.2)1	92(Je.1 ×Ak.3)1	41	18	10	8 (44.4)	*cc* *bc*	*cb*	5	4
								cc	5	4
	91(Ak.2 ×Ak.2)2		12	12	7	5 (41.7)	*cc* *bc*	*cb*	2	3
								cc	5	2
	91(Ak.2 ×Ak.2)1	92(Je.1 ×Ak.3)2	11	11	6	5 (45.5)	*cc* *bc*	*cb*	4	5
								cc	2	0
	91(Ak.2 ×Ak.2)2		6	6	1	5 (83.3)	*cc* *bc*	*cb*	0	3
								cc	1	2
Total			70	47	24	23 (48.9)		*cb*	11	15
								cc	13	8
Parental (%)							28 (59.6)			
Recombinant							19			
χ^2							1.72			
P							0.19			
Recombination rate (%)							40.4			

catesbeiana, the LDH-B locus was reported to be linked with the sex-determining genes, apparently on the *R. catesbeiana* Y chromosome (ELINSON, 1981). ELINSON (1983) has shown that in *R. clamitans*, the Acon-1 locus was linked with the sex-determining genes, whereas the LDH-B, MPI, Est and probably SOD-1 loci appeared not to be linked with the latter. In *Rana pipiens*, the Pep-C and SOD-1 loci were demonstrated to be linked with the sex-determining genes, whereas 10 other loci including Acon-2, API, ADA, Est-1, Est-2, GLO, MPI, Pep-A, Pep-D and PGM-1 were not linked with the latter (WRIGHT and RICHARDS, 1983). The Pep-C and SOD-1 loci were also found to be linked with the sex-determining genes in the hybrids between female *Rana pipiens* and male *R. palustris*, and the SOD-1 locus was linked with the sex-determining genes in the hybrids between female *Rana sphenocephala* and male *R. blairi* (WRIGHT, RICHARDS, FROST, CAMOZZI and KUNZ, 1983). In the hybrids between female *Rana sphenocephala* and male *R. berlandieri*, the Pep-C and SOD-1 loci were not linked with the sex-determining genes, although the ADH-2 locus was found to be linked with the latter. According to these authors, all the loci closely linked with the ADH-2 locus, including the Ab, PGM-1, F16DP and β-GLU loci were also linked with the sex-determining genes in the hybrids between female *Rana sphenocephala* and male *R. berlandieri*, and the Acon-1 locus was linked with the sex-determining genes in *R. sphenocephala*.

In three populations, the Kure, Kumano and Kaita populations of *R. nigromaculata*, and the Okayama population (Typical race) of *R. brevipoda*, the sex-determining genes were linked with the LDH-B, Pep-B, SORDH, HK, ENO and

with heterozygous males of the Akita population

AAT-B and sex				α-GDH and sex				ME-B and sex			
Parents	Offspring			Parents	Offspring			Parents	Offspring		
Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂	Genotype ♀ ♂	Genotype	♀	♂
aa *ab*	*aa*	2	4	*bb* *ab*	*ba*	8	3	*aa* *ab*	*aa*	4	1
	ab	8	2		*bb*	2	3		*ab*	6	5
aa *ab*	*aa*	5	3	*bb* *ab*	*ba*	4	2	*aa* *ab*	*aa*	2	2
	ab	2	2		*bb*	3	3		*ab*	5	3
aa *ab*	*aa*	1	2	*bb* *ab*	*ba*	3	2	*aa* *ab*	*aa*	2	1
	ab	5	3		*bb*	3	3		*ab*	4	4
aa *ab*	*aa*	1	3	*bb* *ab*	*ba*	0	4	*aa* *ab*	*aa*	0	4
	ab	0	2		*bb*	1	1		*ab*	1	1
	aa	9	12		*ba*	15	11		*aa*	8	8
	ab	15	9		*bb*	9	10		*ab*	16	13
27 (60.0)				25 (55.6)				24 (53.3)			
18				20				21			
1.80				0.56				0.20			
0.18				0.46				0.65			
40.0				44.4				46.7			

MPI loci situated on chromosome No. 4, whereas the sex-determining genes were linked with the ME-B locus situated on chromosome No. 3 in the Maibara population (Nagoya race) of *R. brevipoda* (NISHIOKA and SUMIDA, 1989, 1994). The sex-determining genes were demonstrated to be linked with the mME locus in *Xenopus laevis* which was of the ZZ-ZW type in sex-determining mechanism (GRAF, 1989). In view of these reports, it is likely that there is no conserved sex-linkage group in amphibians (SCHMID, NANDA, STEINLEIN, KAUSCH, EPPLEN and HAAF, 1991).

The present study revealed two kinds of sex-linked genes in 10 local populations of *Rana japonica*. One of them was the Ab locus which was linked with the sex-determining genes in seven southern populations, the Munakata, Yamaguchi, Ochi, Saiki, Saijo, Sahara and Mobara populations. The other was the MPI locus which was linked with the sex-determining genes in two northern populations, the Ichinoseki and Toyama populations. In the Akita population, one of the northern populations, none of the eight loci examined including the Ab, AAT-B, ADA, α-GDH, LDH-B, ME-A, ME-B and MPI loci was linked with the sex-determining genes.

NISHIOKA, OHTANI and SUMIDA (1987) reported the following chromosomal localization in the *Rana nigromaculata* group. The loci for Ab and ADH-A were assumed to be located on chromosome No. 1, those for α-GDH, Pep-C, SOD-B and ME-A on chromosome No. 2, those for ME-B and MDH-B on chromosome No. 3, those for LDH-B, HK, MPI and Pep-B on chromosome No. 4, that for Pep-A on chromosome No. 5, those for Hb and IDH-B on chromosome No. 6,

TABLE 10

Summary of the linkage relationships between the sex-determining genes and 11 enzyme or blood protein loci in 10 populations of *Rana japonica*

Population		Mn.	Ym.	Oc.	Sk.	Sj.	Sh.	Mb.	Ic.	Ty.	Ak.
No. of analyzed males		1	3	2	3	4	2	1	3	5	6
Ab—Sex	No. of frogs	**23**	**159**	**69**	**130**	**164**	**38**	**106**	66	133	47
	Parental	**21**	**157**	**62**	**117**	**136**	**36**	**106**	35	67	28
	Recombinant	**2**	**2**	**7**	**13**	**28**	**2**	**0**	31	66	19
	Rec. rate (%)	**8.7**	**1.3**	**10.1**	**10.0**	**17.1**	**5.3**	**0**	47.0	49.6	40.4
AAT-B—Sex	No. of frogs	—	—	—	—	—	—	—	—	—	45
	Parental										27
	Recombinant										18
	Rec. rate (%)										40.0
ADA—Sex	No. of frogs	—	—	—	—	—	—	—	—	—	62
	Parental										38
	Recombinant										24
	Rec. rate (%)										38.7
α-GDH—Sex	No. of frogs	—	—	—	—	—	—	—	—	—	45
	Parental										25
	Recombinant										20
	Rec. rate (%)										44.4
LDH-B—Sex	No. of frogs	—	—	—	—	—	—	—	—	—	74
	Parental										39
	Recombinant										35
	Rec. rate (%)										47.3
MDH-B—Sex	No. of frogs	—	—	—	108	—	—	—	—	—	—
	Parental				55						
	Recombinant				53						
	Rec. rate (%)				49.1						
ME-A—Sex	No. of frogs	—	—	—	—	—	—	—	—	—	117
	Parental										59
	Recombinant										58
	Rec. rate (%)										49.6
ME-B—Sex	No. of frogs	—	—	—	—	—	—	—	—	—	45
	Parental										24
	Recombinant										21
	Rec. rate (%)										46.7
MPI—Sex	No. of frogs	—	—	—	273	—	53	150	**163**	**392**	185
	Parental				145		27	80	**161**	**354**	105
	Recombinant				128		26	70	**2**	**38**	80
	Rec. rate (%)				46.9		49.1	46.7	**1.2**	**9.7**	43.2
Pep-A—Sex	No. of frogs	—	—	—	51	—	—	—	—	—	—
	Parental				26						
	Recombinant				25						
	Rec. rate (%)				49.0						
Pep-C—Sex	No. of frogs	—	—	—	114	—	—	—	—	—	—
	Parental				58						
	Recombinant				56						
	Rec. rate (%)				49.1						

—, not examined

those for Prot-C and ALD on chromosome No. 9, those for Est-1, Est-2, Est-3, Est-5 and Pep-D on chromosome No. 10 and that for ADA on chromosome No. 11. In *Rana clamitans*, the LDH-B locus was linked with the MPI locus (ELINSON, 1983). WRIGHT, RICHARDS and NACE (1980) and WRIGHT, RICHARDS, FROST, CAMOZZI and KUNZ (1983) found the following linkage groups in *Rana pipiens* complex. The first group included the Ab, PGM-1, F16DP, ADH-2 and β-GLU loci, the second group included the MDH-2, Pep-C and SOD-1 loci, the third group included the MPI, Pep-B, LDH-B and HK-2 loci, the fourth group included the IDH-1 and Hb-1 loci, and the fifth group included the GPI and Pep-D loci. From the foregoing linkage groups, it is evident that many linkage relationships have been retained in these *Rana* species having 26 chromosomes.

If the similarities of linkage relationships within the foregoing *Rana* species are also conserved in *R. japonica*, the sex-determining genes of the southern and northern populations examined in the present study are assumed to be located on separate chromosomes: the sex-determining genes of the seven southern populations which were linked with the Ab locus are considered to be situated on chromosome No. 1, and those of the two northern populations which were linked with the MPI locus are assumed to be located on chromosome No. 4. MIURA (1984) reported that in the Wakuya population of *Rana japonica*, the sex chromosomes were chromosomes No. 4 which had a sex-specific C-band. Thus, the three northern populations including the Ichinoseki, Toyama and Wakuya populations seem to have the same sex chromosomes. On the other hand, none of the eight loci, the Ab, AAT-B, ADA, α-GDH, LDH-B, ME-A, ME-B and MPI loci, was linked with the sex-determining genes in the Akita population. In the foregoing *Rana nigromaculata* group, these loci are assumed to be located on chromosomes Nos. 1, 2, 3, 4 and 11, although the linkage relationships of AAT-B are still unknown. It may be probable that the sex-determining genes in the Akita population are considered to be situated on chromosomes other than those of Nos. 1, 2, 3, 4 and 11, although further examination will be necessary to clarify this point.

SUMIDA (1981) and SUMIDA and NISHIOKA (1991, 1993, 1994) reported that whereas the eastern and western groups of *Rana japonica* were not distinctly differentiated morphologically, they could be easily distinguished karyologically and biochemically from each other. When the crossings were carried out between them, it was found that males were remarkably numerous in the reciprocal hybrids and these male hybrids were more or less abnormal in the inner structure of the testes and also in their spermatogenesis (SUMIDA and NISHIOKA, 1991; SUMIDA, 1994). The genetic differentiation between them was distinctly recognizable at the Hb-II and Pep-A loci. Biochemical data elucidated by electrophoretic analyses of 25 loci controlling 15 enzymes and three blood proteins showed that *Rana japonica* was roughly divided into the eastern and western groups, and thereafter they probably came into contact with each other in the northwestern region including the Akita population (SUMIDA and NISHIOKA, 1991, 1994). The differences in the sex-linked genes between the northern and southern populations

are not always considered to correspond with the differentiation stated above, but it is evident that the locus linked with the sex-determining genes differs with the local populations.

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