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Distribution of Transferrin Phenotypes in *Rana nigromaculata* from Japan and Korea

By

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ABSTRACT

Transferrins present in sera of *Rana nigromaculata* collected from 27 locations in Japan and one location in Korea were analyzed by acrylamide-gel electrophoresis. The results showed that there were 10 different phenotypes controlled by four codominant alleles, *a*, *b*, *c* and *d*. Of these alleles, allele *c* was the highest in frequency as a whole and particularly predominant in the Honshu, Chubu, Kinki and Sanin regions. All the four alleles were distributed in the Sanyo and Shikoku regions, and the three other than allele *d* were found in the Kyushu region and Korea.

INTRODUCTION

Electrophoretic studies have been made on transferrin polymorphism of amphibian sera in *Pleurodeles* by CHALUMEAU-LE FOULGOC, FINE and L. GALLIEN (1966, 1972), FINE, CHALUMEAU-LE FOULGOC and AMOUCH (1967), CHALUMEAU-LE FOULGOC (1968, 1969) and others, in *Bufo* by GUTTMAN (1967, 1969, 1972, 1973), GUTTMAN and WILSON (1973) and others, in *Acris* by DESSAUER and NEVO (1969), in *Rana* by KASHIWAGI (1981) and in *Xenopus* by SCHONNE, PETIT, LIZEN and PICARD (1988). From these studies, it was obvious that a number of transferrin phenotypes controlled by a single or multiple codominant alleles were present in one and the same species.

NISHIOKA, SUMIDA and OHTANI (1992) have recently examined enzymes of skeletal muscles and blood proteins by starch-gel electrophoresis to clarify intra- and interspecific differentiation in the *Rana nigromaculata* group in Japan.

The present study attempts to confirm the distribution of transferrin phenotypes in *Rana nigromaculata* from 27 locations of Japan and one location of Korea.

MATERIALS AND METHODS

Rana nigromaculata HALLOWELL were collected from 1974 through 1978 from 27 localities in Japan, Akita, Sakata, Shibata, Kashiwazaki, Joetsu, Toyama, Kanazawa, Mikuni, Okaya, Sudama, Iida, Maibara, Shingu, Tottori, Matsue, Gotsu, Himeji, Konko, Kure I, Kure II, Kure III, Hiroshima, Yamaguchi,

Takamatsu, Matsuyama, Munakata and Sasebo, and one locality in Korea, Suwon.

Before bleeding, each frog received an intra-abdominal injection of 1 ml heparin (100,000 units of heparin sodium in 50 ml RINGER's solution for amphibian adults) and was kept *in situ* for five minutes. After etherization blood samples were collected from the heart and then centrifuged at 2300 r.p.m. for three minutes at room temperature. The supernatant was put in 2 ml analyzer cups and stored at −20°C until use. Separation and identification of transferrins from preserved sera followed the method of MORIWAKI, SADAIE and HIRASAWA (1974).

OBSERVATION

Serum transferrins of *Rana nigromaculata* specimens collected from 28 localities were examined by electrophoresis. As shown in Fig. 1, a total of 10 transferrin phenotypes, AA, BB, CC, DD, AB, AC, AD, BC, BD and CD, was discovered. They were controlled by four alleles, *a*, *b*, *c* and *d*. Table 1 and Fig. 2 show the number of individuals analyzed in each *R. nigromaculata* population, the transferrin

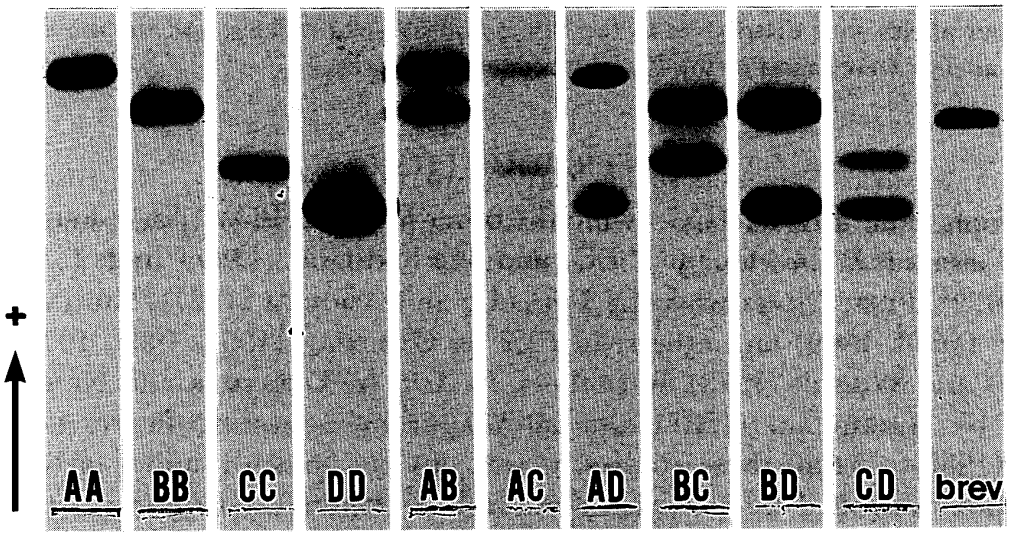

Fig. 1. Ten kinds of transferrin phenotypes in *Rana nigromaculata* and one transferrin phenotype in *R. brevipoda*.
brev, *R. brevipoda* from Konko

phenotypes recognized, and the frequencies of genes determining them.

Three frogs from Akita and five from Sakata were examined electrophoretically and all possessed CC. Of nine frogs from Shibata, four had CC, four had BC, and the remaining one had DD. Allele *c* was found most often in this locality. In the Kashiwazaki population, 16 of 17 individuals had CC and the remaining one had BC. Twenty-one frogs from Joetsu and 23 frogs from Toyama all showed CC. Twelve of 21 individuals from Kanazawa had CC, eight had BC, and the remaining one had BB, while six frogs from Mikuni, 30 from Okaya, nine from

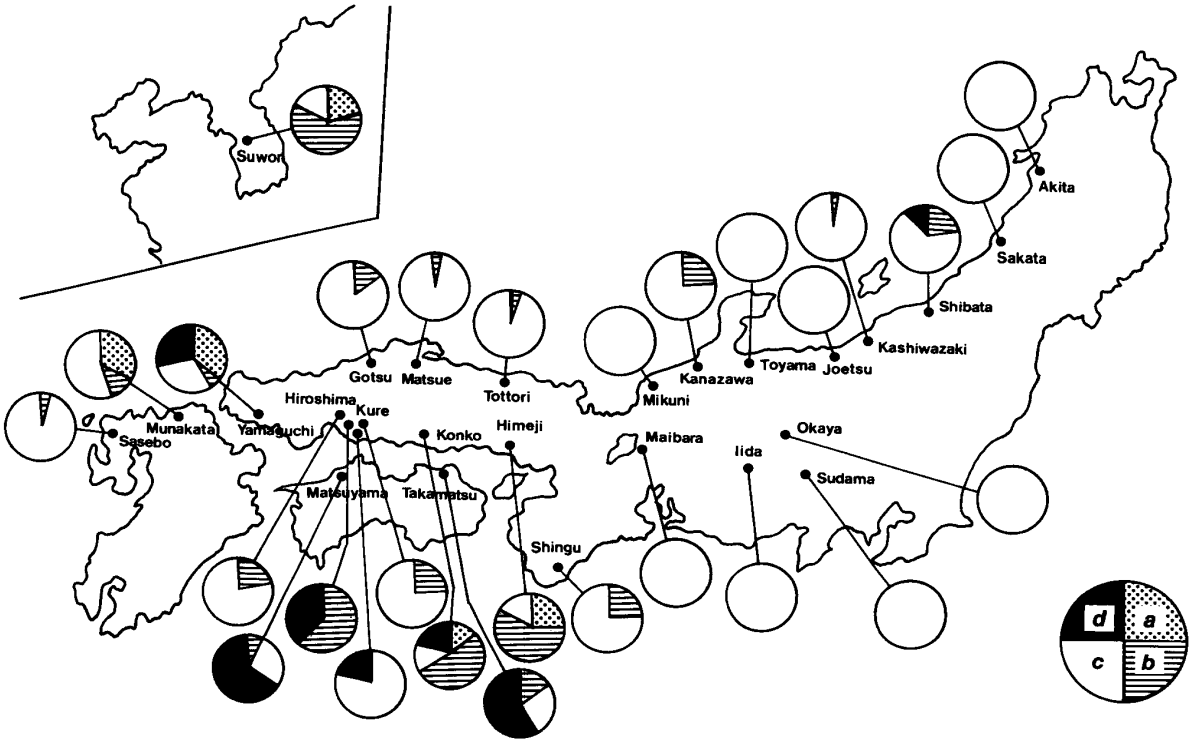

Fig. 2. Geographic distribution of transferrin alleles among populations of *Rana nigromaculata* in Japan and Korea.

Sudama, 22 from Iida and seven from Maibara showed CC. In Shingu, four of eight frogs examined had CC, while the other four had BC. Forty-four of 49 frogs from Tottori had CC, four had BC, and one had CD. Of 71 frogs from Matsue, 65 showed CC and the remaining six had BC. Of 18 frogs from Gotsu, 13 showed CC and five showed BC.

Of six individuals from Himeji, two had BB, two had BC, one had AA and one had AB. Of 12 individuals from Konko, five had BD, three had BB, two had AC and the remaining two had AB and BC. Four kinds of alleles occurred in this locality. Frogs were collected from three different sites of Kure. Twelve of 23 frogs from Kure I had CC, while the remaining 11 had BC. Of the 38 individuals collected from Kure II, 22 had CC and 16 had CD, while 12 of the 16 frogs collected from Kure III had BD and four had BB. Twenty-three of the 41 frogs collected from Hiroshima had CC and 18 had BC. Of the 47 frogs collected at Yamaguchi, 13 had AD, 12 had CD, nine had AC, five had AA, four had BC and two had CC, while the remaining two had AB and DD. A total of eight different transferrin phenotypes was found in this locality.

Of the 17 frogs collected at Takamatsu, seven had CD, five had DD, three had BD, one had BB and the remaining one had CC. A total of 13 frogs from Matsuyama was examined. Five of them had DD, five had CD, two had BD and the remaining one had CC. In Munakata, 14 of 35 frogs had AC, 10 had CC, four had BC, three had AA, three had AB and the remaining one had BB. Of the 39 frogs collected from Sasebo, 35 showed CC and four showed BC.

TABLE 1

Frequencies of transferrin phenotypes and alleles in *Rana nigromaculata* populations

Locality of collection	No. of frogs	Phenotype										Allele (%)			
		AA	BB	CC	DD	AB	AC	AD	BC	BD	CD	*a*	*b*	*c*	*d*
Akita	3	0	0	3	0	0	0	0	0	0	0			100	
Sakata	5	0	0	5	0	0	0	0	0	0	0			100	
Shibata	9	0	0	4	1	0	0	0	4	0	0		22.2	66.7	11.1
			(0.4)	(4.0)	(0.1)				(2.7)	(0.4)	(1.3)				
Kashiwazaki	17	0	0	16	0	0	0	0	1	0	0		2.9	97.1	
				(16.0)					(1.0)						
Joetsu	21	0	0	21	0	0	0	0	0	0	0			100	
Toyama	23	0	0	23	0	0	0	0	0	0	0			100	
Kanazawa	21	0	1	12	0	0	0	0	8	0	0		23.8	76.2	
			(1.2)	(12.2)					(7.6)						
Mikuni (Fukui)	6	0	0	6	0	0	0	0	0	0	0			100	
Okaya	30	0	0	30	0	0	0	0	0	0	0			100	
Sudama (Yamanashi)	9	0	0	9	0	0	0	0	0	0	0			100	
Iida	22	0	0	22	0	0	0	0	0	0	0			100	
Maibara (Shiga)	7	0	0	7	0	0	0	0	0	0	0			100	
Shingu	8	0	0	4	0	0	0	0	4	0	0		25.0	75.0	
			(0.5)	(4.5)					(3.0)						
Tottori	49	0	0	44	0	0	0	0	4	0	1		4.1	94.9	1.0
			(0.1)	(44.1)					(3.8)	(0.1)	(0.9)				
Matsue	71	0	0	65	0	0	0	0	6	0	0		4.2	95.8	
			(0.1)	(65.2)					(5.7)						
Gotsu	18	0	0	13	0	0	0	0	5	0	0		14.0	86.0	
			(0.4)	(13.3)					(4.3)						
Himeji	6	1	2	0	0	1	0	0	2	0	0	25.0	58.3	16.7	
		(0.4)	(2.0)	(0.2)		(1.7)	(0.5)		(1.2)						
Konko (Okayama)	12	0	3	0	0	1	2	0	1	5	0	12.5	54.2	12.5	20.8
		(0.2)	(3.5)	(0.2)	(0.5)	(1.6)	(0.4)	(0.6)	(1.7)	(2.7)	(0.6)				
Kure I (Hiroshima)	23	0	0	12	0	0	0	0	11	0	0		23.9	76.1	
			(1.3)	(13.3)					(8.4)						
Kure II	38	0	0	22	0	0	0	0	0	0	16			79.0	21.0
				(23.7)	(1.7)						(12.6)				
Kure III	16	0	4	0	0	0	0	0	0	12	0		62.5		37.5
			(6.3)		(2.2)					(7.5)					
Hiroshima	41	0	0	23	0	0	0	0	18	0	0		22.0	78.0	
			(2.0)	(24.9)					(14.1)						
Yamaguchi	47	5	0	2	1	1	9	13	4	0	12	35.1	5.3	30.9	28.7
		(5.8)	(0.1)	(4.5)	(3.9)	(1.8)	(10.2)	(9.5)	(1.5)	(1.4)	(8.3)				
Takamatsu	17	0	1	1	5	0	0	0	0	3	7		14.7	26.5	58.8
			(0.4)	(1.2)	(5.9)				(1.3)	(2.9)	(5.3)				
Matsuyama	13	0	0	1	5	0	0	0	0	2	5		7.7	26.9	65.4
			(0.1)	(0.9)	(5.6)				(0.5)	(1.3)	(4.6)				
Munakata (Fukuoka)	35	3	1	10	0	3	14	0	4	0	0	32.9	12.8	54.3	
		(3.8)	(0.6)	(10.3)		(2.9)	(12.5)		(4.9)						
Sasebo (Nagasaki)	39	0	0	35	0	0	0	0	4	0	0		5.1	94.9	
			(0.1)	(35.1)					(3.8)						
Suwon (Korea)	26	1	10	0	0	6	3	0	6	0	0	21.2	61.5	17.3	
		(1.2)	(9.8)	(0.8)		(6.8)	(1.9)		(5.5)						

Parentheses indicate the expected number.

Twenty-six frogs collected from Suwon, Korea, were examined. Ten of them had BB, six had AB, six had BC, three had AC and the remaining one had AA.

Allele *c* was widely distributed in all populations except Kure III. This allele was very high in frequency, being 0.667~1.000, in 20 populations, while it was relatively low, being 0.125~0.543, in seven other populations. Allele *b* was

0.029~0.625 in frequency in 18 populations. Allele *d* was 0.010~0.654 in frequency in eight populations, and allele *a*, 0.125~0.351, in five populations (Table 1; Fig. 2).

DISCUSSION

Serum transferrin polymorphism was reported for *Bufo* by DESSAUER, FOX and HARTWIG (1962), GUTTMAN (1967, 1969, 1972), BROWN and GUTTMAN (1970), GUTTMAN and WILSON (1973) and others. According to GUTTMAN (1969), six species of *Bufo americanus* each possessed several different transferrin phenotypes, and there were 13 transferrin phenotypes in a total of 77 individuals examined. *B. woodhousei* from different localities showed remarkable differences in transferrin phenotype. GUTTMAN and WILSON (1973) collected 182 male and three female *B. americanus* from agricultural reservoirs in southwestern Ohio and analyzed their transferrin. It was found that there were 36 transferrin phenotypes controlled by 13 different codominant alleles.

Transferrin polymorphism was also reported by DESSAUER and NEVO (1969) in *Acris crepitans*, collected from 27 locations in 20 states, and *A. gryllus* from five locations in four states. The results showed that five kinds of alleles were present in *A. crepitans*, and two in *A. gryllus*. The authors classified *A. crepitans* into three large population groups on the basis of regional differences found in their transferrin genes.

KASHIWAGI (1981) examined transferrin components for *Rana japonica* collected from 12 localities of western Japan, and discovered three phenotypes determined by two codominant alleles.

Transferrin polymorphism in urodeles was examined in *Pleurodeles waltl* by FINE, CHALUMEAU-LE FOULGOC and AMOUCH (1967), CHALUMEAU-LE FOULGOC (1969), CHALUMEAU-LE FOULGOC, FINE and L. GALLIEN (1972) and others. Of the natural populations, approximately 70% had BB and the others had AB. No AA was detected.

In the present investigation, electrophoretic analyses were performed on sera of *R. nigromaculata* collected from 27 locations of Japan and one location of Korea, and 10 phenotypes controlled by four codominant alleles, *a*, *b*, *c* and *d*, were found. It was possible to divide *R. nigromaculata* populations into three groups by the distribution of transferrin genes (Fig. 2). The first group included populations ranging from the northern end of Honshu throughout the Chubu and Kinki regions, and extending into the Sanin region. The second group included populations in the Sanyo and Shikoku regions. The third group included populations found in the Kyushu region. Allele *c* predominated in the first general group. Most populations possessed this gene exclusively, but some populations had alleles *b* and *d* to some extent. Allele *a* was not found to be present. Alleles *b* and *c* occurred in almost all populations in the second group, with allele *d* being observed to a lesser extent, and allele *a* occurring only rarely. Alleles *b* and *c* were most frequent in the third group. Frogs from Munakata, in the northern end of

the Kyushu region, had allele *a*, but not allele *d*. In Korea, allele *b* occurred in a high frequency, while alleles *a* and *c* were found in a lesser extent. Allele *d* was not observed.

Populations of *R. nigromaculata* possessing only one allele, *c*, were found to be distributed in the colder areas of Japan where the climate was not so compatible to species. On the other hand, populations displaying a variety of transferrin genes inhabit comparatively warmer areas.

This finding agrees well with the observations of DESSAUER and NEVO (1969) on *Acris crepitans* in the United States. Moreover, NISHIOKA, SUMIDA and OHTANI (1992) made electrophoretic analyses of enzymes and blood proteins for the purpose of clarifying the differentiation of 47 populations of *R. nigromaculata* and 23 populations of *R. brevipoda* distributed in Japan.

In the geologic age, *R. nigromaculata* presumably migrated from the Asian continent, across the Korean Peninsula, or lands formed in the vicinity, then into northern Kyushu to increase in number and finally spread to every corner of Japan. The reason why allele *d* was not present in the populations from Kyushu and Korea is still unknown, since the present study investigated frogs gathered from only two locations in eastern Kyushu and only one location in Korea. Populations in central and southern Kyushu are not known at all, and those in southern Shikoku remain uninvestigated. Future study of *R. nigromaculata* distributed in Korea and China will undoubtedly resolve the detailed distribution and differentiation of this species throughout eastern Asia.

According to NISHIOKA, SUMIDA and OHTANI (1992), *R. brevipoda* invaded Japan earlier than *R. nigromaculata* and produced *R. b. porosa* by frequent hybridization with *R. nigromaculata* mainly in the Shibata district.

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