

## ヌマエビ地域集団間におけるMDHアイソザイムの遺伝的分化

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## Different expression in MDH isozymes among local populations in freshwater shrimp, *Paratya compressa* (Decapoda: Atyidae)

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### ABSTRACT

Malate dehydrogenase (MDH) activity in freshwater shrimps, *Paratya compressa*, *Caridina leucosticta* and *Neocaridina denticulata* (Decapoda: Atyidae), was examined by starch gel electrophoresis. In accordance with the previous results, most populations of *P. compressa* had only one activity zone (MDH-1). The Biwa Lake population, however, was found to have two activity zones (MDH-1 and MDH-2). Since both had a triple-banded phenotype and since densitometric measurements showed the expected activity pattern (1:2:1), it is most likely that they are dimeric enzymes and are encoded by two independent loci (*Mdh-1* and *Mdh-2*). *C. leucosticta* and *N. denticulata* examined had the two activity zones. But some individuals of both species showed a null phenotype in either of the two zones but not in both. Possibility that in most populations of *P. compressa* null allele at the *Mdh-2* locus was fixed was discussed.

### 1. INTRODUCTION

Malate dehydrogenase (MDH; EC1.1.1.37) activity has been detected in only one activity zone (MDH-1) on starch gel upon electrophoresis in the freshwater shrimp, *Paratya compressa* (Decapoda; Atyidae), common in Japan, including two subspecies *P. c. compressa* and *P. c. improvisa* (Ikeda et al., 1992, 1993). This result is based on the nearly 1,200 individuals in 22 local populations. Many species in decapod families other than Atyidae, on the other hand, have been shown to have two activity zones (MDH-1 and MDH-2) controlled by two independent loci (*Mdh-1* and *Mdh-2*) (Hedgecock et al., 1982; Chow and Fujio, 1987).

In the present study, we thus made further examinations on *P. compressa* and also on two other species of Atyidae, *Caridina leucosticta* and *Neocaridina denticulata*. Three populations in *P. c. compressa* and one population in *P. c. improvisa* showed only the MDH-1 activity. Biwa Lake population of *P. c. compressa*, however, was found to have two activity zones. Although many individuals of two other species, *C. leucosticta* and *N. denticulata*, examined

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showed the presence of two activity zones, some individuals of both species had a null phenotype in either of the two activity zones but not in both. A possibility that null allele of *Mdh-2* was fixed in most local populations of *P. compressa* was discussed.

## 2. MATERIALS AND METHODS

### *Animals*

Adults of three freshwater shrimp species were collected in various localities as shown in Table 1. *Paratya compressa compressa* lives mainly in the southern Japan, while *P. c. improvisa* is found mainly in the northern Japan (Kamita, 1970). Samples were kept frozen at  $-70^{\circ}\text{C}$  until use.

Table 1. Information on collection sites for four freshwater shrimp species

Species	Collection Site	Date
<i>Paratya compressa compressa</i>	Kubota River (Kagoshima Pref.)	Aug., 1990
	Hiki River (Wakayama Pref.)	Mar., 1992
	Takaoka (Toyama Pref.)	May, 1992
	Biwa Lake (Shiga Pref.)	May, 1992
<i>Paratya compressa improvisa</i>	Tama River (Ibaraki Pref.)	Mar., 1992
<i>Caridina leucosticta</i>	Shigenobu River (Ehime Pref.)	Sep., 1991
<i>Neocaridina denticulata</i>	Shigenobu River (Ehime Pref.)	Jan., 1992

### *Electrophoresis and measurement of relative activity*

Starch gel electrophoresis was carried out and the malate dehydrogenase (MDH) activity was detected by staining, as described previously (Ikeda et al., 1992). After the staining, gels were sheeted as described by Numachi (1981), and relative density was measured with a recording densitometer (Jooko Sangyo, Model-pan).

## 3. RESULTS

### *MDH zymogram in Paratya compressa*

Fig. 1 shows examples of the MDH zymogram in *Paratya compressa compressa* from Hiki River and Biwa Lake, and in *P. c. improvisa* from Tama River. As expected from the previous results, populations from all the localities except for Biwa Lake showed only one activity zone (MDH-1). Many individuals had only one band, while others had three bands. The MDH-1 is thus most probably a dimeric protein, controlled by the single locus (*Mdh-1*) with two alleles, *Mdh-1<sup>A</sup>* and *Mdh-2<sup>B</sup>*. The triple-banded pattern then represent AA, BB homodimers and

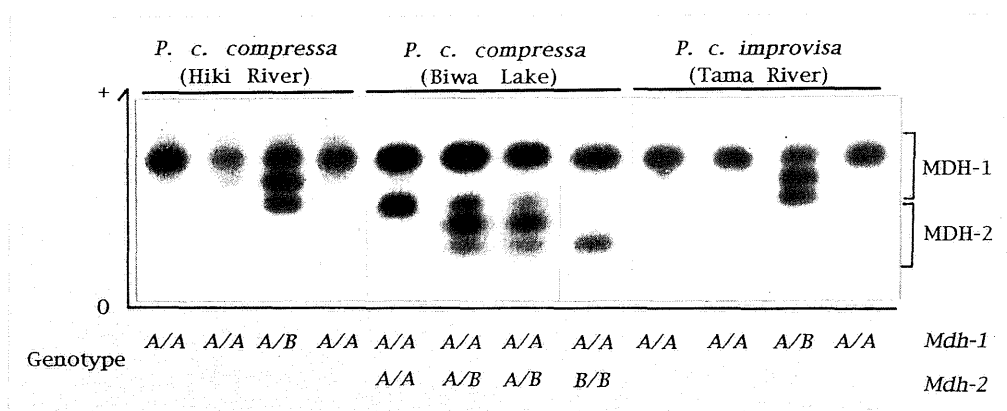


Fig. 1. Examples of MDH zymogram for three populations of *Paratya compressa*. Presumed genotypes at each locus are shown below the respective banding patterns.

an AB heterodimer. If so, the isozyme amount ratio in the individuals with triple-banded pattern would be AA:AB:BB=1:2:1. Densitometric measurements on individuals from Kubota River, Hiki River and Takaoka (*P. c. compressa*) and from Tama River (*P. c. improvisa*) in fact showed the ratio of 1:1.7:0.9 on the average, supporting the above notion. Allele frequencies were thus calculated and are presented in Table 2. In every population, allele *Mdh-1<sup>A</sup>* was found in high frequencies.

Table 2. Allele frequencies at *Mdh-1* and *Mdh-2* in *Paratya compressa*

	Population	N	Allele frequency at <i>Mdh-1</i>		Allele frequency at <i>Mdh-2</i>	
			A	B	A	B
<i>P. c. compressa</i>	Kubota R.	93	0.989	0.011	—	—
	Hiki R.	32	0.984	0.016	—	—
	Biwa L.	32	1.000	0	0.469	0.531
	Takaoka	50	0.970	0.030	—	—
<i>P. c. improvisa</i>	Tama R.	40	0.975	0.025	—	—

Unexpectedly, all individuals (32) of the Biwa Lake population (*P. c. compressa*) showed in addition the another activity zone with either the single or triple-banded pattern (MDH-2) (Fig. 1). Densitometric measurements on individuals with the triple-banded phenotype showed the isozyme ratio to be 1:1.9:0.8 on the average, again approximating the expected 1:2:1. The allele frequencies, A and B, at the *Mdh-2* locus was calculated and are presented in Table 2. It is noted that no variations existed in the *Mdh-1* locus in the Biwa Lake population.

*MDH zymograms in the other atyid shrimps*

MDH activities were also examined in two other atyid species: *Caridina leucosticta* and *Neocaridina denticulata*. In both species, two activity zones (MDH-1 and MDH-2) were observed each with single- and triple-banded phenotypes (Fig. 2), suggesting the control by two different loci (*Mdh-1* and *Mdh-2*).

Further variations were observed in *C. leucosticta* and *N. denticulata* as

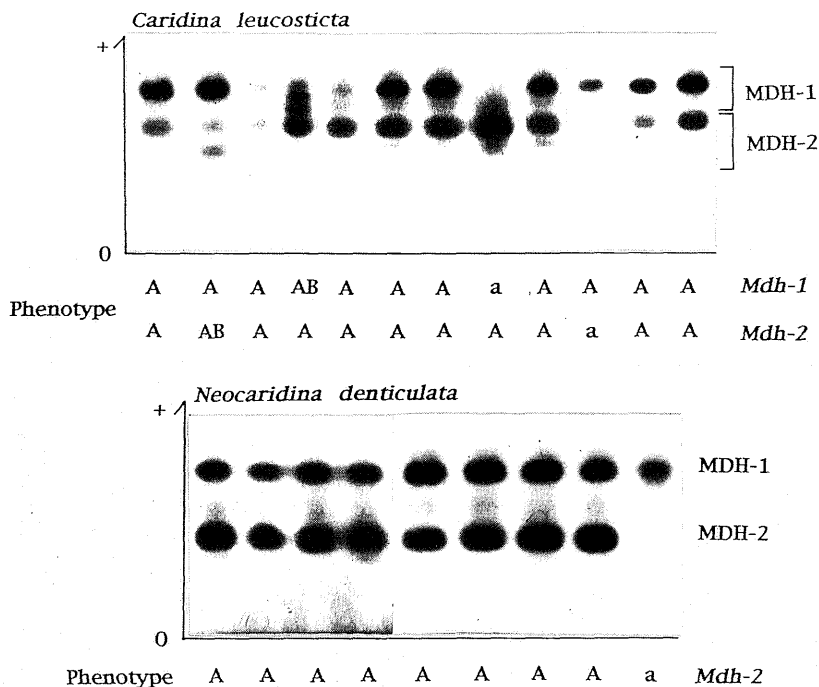


Fig. 2. MDH zymograms in two atyid shrimp species. Phenotypes at each locus are shown below the respective banding patterns. A and AB designate the banded phenotypes. a designates the null band phenotype.

Table 3. Allele frequencies at *Mdh-1* and *Mdh-2* in *Caridina leucosticta* and *Neocaridina denticulata*

Species	N	Allele frequency at <i>Mdh-1</i>			Allele frequency at <i>Mdh-2</i>		
		A	B	$a^*$	A	B	$a^*$
<i>C. leucosticta</i>	32	0.767	0.047	0.186	0.653	0.031	0.316
<i>N. denticulata</i>	32	1.000	0	0	0.823	0	0.177

$a^* = \sqrt{a/N}$ , where a presents the number of shrimps which lack the activity at the locus, and N presents the total number of shrimps.

evidenced by the absence of activity in some individuals either in MDH-1 or MDH-2, but never in both simultaneously. Assuming the occurrence of null allele, *a*, the allele frequencies were calculated (Table 3). The null allele frequencies were 0.186 at *Mdh-1* and 0.316 at *Mdh-2* in *C. leucosticta*, and 0.177 at *Mdh-2* in *N. denticulata*.

#### 4. DISCUSSION

The present results have shown rather unexpectedly the existence of two zones of MDH activity (MDH-1 and MDH-2) on starch gel in the Biwa Lake population of *Paratya compressa compressa*. All shrimps collected from three other populations (Kubota River, Hiki River and Takaoka) of *P. c. compressa* and one population (Tama River) of *P. c. improvisa* showed only one activity zone (MDH-1) as expected from the previous study (Ikeda et al., 1992, 1993). The present results have also shown that there are null allele polymorphisms in the *Mdh-1* and *Mdh-2* loci in two other atyid species, *Caridina leucosticta* and *Neocaridina denticulata*, examined.

Taken together, it may be reasonable to assume that the null allele for the *Mdh-2* locus in *P. c. compressa* has been fixed in most local populations other than the Biwa Lake populations. One possible explanation may be that in habitats of *P. compressa* such as rivers and small ponds populations experience large fluctuations and the *Mdh-2* null allele is fixed by simple genetic drift, while in the Biwa Lake the population may never undergo such drastic changes in number and hence no fixation. This, however, does not explain why in the Biwa Lake population the *Mdh-1<sup>A</sup>* allele is fixed, while in other populations the *Mdh-1* locus is constantly polymorphic. Alternatively, the Biwa Lake population may have a different origin from all other populations which may in turn have a single origin. The Biwa Lake population with genetic differentiation not immediately apparent morphologically may nevertheless be better fit in competition, and may have eradicated the older populations. The present results thus warrant further studies on the genetic differentiation in *P. compressa*.

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