ALLOZYME VARIATION AMONG SIX POPULATIONS OF THE FRESHWATER-SNAIL *ONCOMELANIA HUPENSIS* IN ZHEJIANG, CHINA

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Abstract. Thirteen enzymes encoded by 16 loci of six population of *Oncomelania hupensis* in Zhejiang, China, were investigated by means of starch gel electrophoresis. Ten loci (AO, 6PGD, ME, AKP, OCT-1, HBDH-1, HBDH-2, XDH, MDH and MPI) were monomorphic and 6 loci (OCT-2, PGI, AAT, PGM-1, PGM-2 and ACP) were polymorphic. Three enzymes (OCT, HBDH and PGM) were encoded by 2 loci. The results indicated that there were allozyme variations in two subspecies, *O.h. hupensis* and *O.h. fausti* in Zhejiang, China. Nei's multilocus genetic distances (D) between subspecies ranged from 0.167 to 0.265. Minor genetic distances were detected between populations of the same subspecies. The results indicated that the enzyme acid phosphatase (ACP) is a possible marker to measure the degree of susceptibility of *O. hupensis* to *S. japonicum*.

INTRODUCTION

Oncomelania hupensis is the intermediate host snail of the zoonotic Asian blood fluke *Schistosoma japonicum*. Six subspecies of *O. hupensis* have been recognized in Asia by Davis (1980), and according to morphology, physiology, biochemistry and distribution, Liu (1981) concluded that there are seven subspecies of *Oncomelania* in China. The subspecies *O.h. hupensis* is markedly different from the other subspecies because of its ribbed shell. *O. hupensis* is a polytypic species. The classification of the subspecies of the *Oncomelania* snail in Chinese mainland still remains unsolved. Viyanat *et al* (1987) and Woodruff *et al* (1988) pointed the need to establish the degree of genetic differentiation among the seven subspecies found in China. Also, they noted the close parallel between the genetic differentiation of the parasite and the snail hosts.

Using the allozyme electrophoresis technique, Viyanat *et al* (1987) and Woodruff *et al* (1988) provided evidence that *O. hupensis* from China is genetically widely different from *O.h. quadrasi* from the Philippines, and that there were minor variations between samples of *O.h. quadrasi* representing four Philippine islands. Thus, they recommended that the Chinese and Philippine taxa deserved recognition as full species, *ie O. hupensis* and *O. quadrasi*. In a similar study, Tsukamoto *et al* (1988) compared allozyme variation in *Oncomelania* snails from Japan, Taiwan and Philippines and suggested that *O. nosophora* and *O. formosa* might also warrant elevation to species rank. More recently, *O. hupensis* snails were collected from a total of 33 localities covering nine provinces along the Yangtze River in southern China (Zhou, 1994). Morphological and anatomical studies together with allozyme electrophoresis provided more evident to support the opinion that snails with ribbed shells distributed along the Yangtze River were different from the snails with smooth shells found in other areas of the country, possibly at species level. However, Davis (1994) disagreed, arguing that large genetic distances indicated by enzyme analysis does not serve to define species.

Differences in susceptibility of *O. hupensis* to the geographic strains of *S. japonicum* have been reported (Lee *et al*, 1986; He *et al*, 1991). Cross infection studies indicated that there might be wider genetic differences among the various subspecies of *O. hupensis* (McManus and Hope, 1993). Within China, researchers noticed that the difference in shell morphology correlated with allozymes mobility and susceptibility (Chen *et al*, 1992; Zhou, 1992; Zhang *et al*, 1994). Using logistic regression, Zhou and Kristensen (1995) suggested a correlation between susceptibility of O.h. hupensis to *S. japonicum* and genotype frequency of MDH-2 in *O. hupensis*.

The aim of this study was to elucidate possible genetic variations in two *Oncomelania* subspecies, *O.h. hupensis* and *O.h. fausti*, in Zhejiang, China. Furthermore, the allozyme loci in relation to susceptibility of snail host to parasite infection is assessed.

MATERIALS AND METHODS

Six populations of *O. hupensis* belonging to two subspecies, *O.h. hupensis* and *O.h. fausti*, were collected in the field from Zhejiang, China. Population 1: *O.h. hupensis* from Jiashan County, with ribbed shell and distributed in plain area of north Zhejiang; Population 2: *O.h. hupensis* from Changshan County, with slightly ribbed shell and distributed in the hilly area of west Zhejiang; Population 3: *O.h. hupensis* from Kaihua County, with slightly ribbed shell and distributed in the hilly area of west Zhejiang; Population 4; *O.h. fausti* from Jiaxing County, with smooth shell and distributed in the plain area of north Zhejiang; Population 5: *O.h. fausti* from Jinghua County, with smooth shell and distributed in the mountainous area of middle Zhejiang; Population 6: *O.h. fausti* from Shenxen County, with smooth shell and distributed in the mountainous area of middle Zhejiang.

Twenty to fifty living snails of each population were washed with snail water and the shell crashed, Extracts were prepared by individually homogenizing the snail body without shell in 10 μl distilled water. The homogenized samples were centrifuged at 3,000*g* for 2 minutes at room temperature. The supernatant obtained was used for electrophoresis.

The starch gel electrophoresis buffer system, conditions and enzyme staining methods used were essentially the same as those described by Jelnes *et al* (1979). Bromophenol blue was used as marker and the mobility (RF) of each band was calculated using standard procedures. The following 13 enzymes were examined: malate dehydrogenase (MDH E.C. 1.1.1.37), aldehyde oxidase (AO E.C. 1.2.3.1.), 6-phosphogluconate dehydrogenase (6PGD E.C. 1.1.1.14), malic enzyme (ME E.C. 1.1.1.40), alkaline phosphatase (AKP E.C. 3.1.3.1.), octopine dehydrogenase (OCT E.C. 2.1.3.3.), hydroxybutyrate dehydrogenase (HBDH E.C. 1.1.1.30), phosphoglucomutase (PGM E.C. 2.7.5.1.), phosphateglucose isomerase (PGI E.C. 5.3.1.9.), aspartate aminotransferase (AAT E.C. 2.6.1.1.), mannose-6-phosphate (MPI E.C. 5.3.1.8.), xanthine dehydrogenase (XDH E.C. 1.2.3.2.), acid phosphatase (ACP E.C. 3.1.3.2.).

The mean number of alleles per locus, the proportion of polymorphic loci (P) and the mean heterozygosity (H) were calculated for each population according to the method described. Nei's genetic distance coefficients (D) and the genetic similarity coefficients were analyzed using the BIOSYS-1 computer program (Swofford and Selander, 1989).

RESULTS

Thirteen enzymes encoded by 16 loci were resolved by means of horizontal starch gel electrophoresis. A locus was considered polymorphic at the 95% criterion. The electrophoretic patterns of 13 enzymes (16 loci) are shown in Fig 1. Three enzymes (OCT, HBDH and PGM) were encoded by 2 loci. Ten loci (AO, 6PGD, ME, AKP, OCT-1, HBDH-1, HBDH-2, XDH, MDH and MPI) were monomorphic and 6 loci (OCT-2, PGI, AAT, PGM-1, PGM-2 and ACP) were polymorphic. The allele frequencies of the 16 loci in the six snail populations are summarized in Table 1. The allele frequencies in OCT-2, PGI, AAT, PGM-1, PGM-2, and ACP were variable both within and among populations. In three of six single-loci *ie* OCT-2,

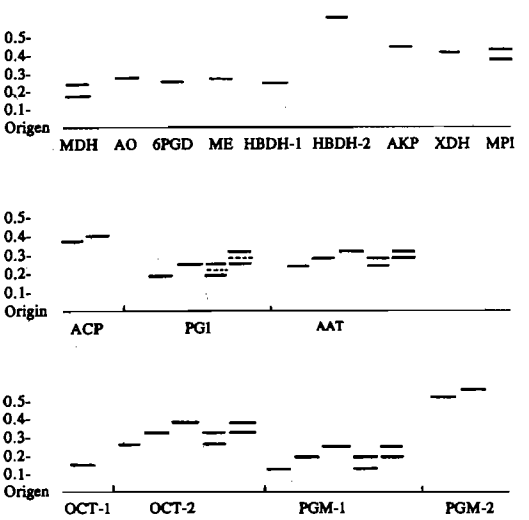

Fig 1–Electrophoretic patterns for 13 enzymes system (16 loci) in six populations of *O. hupensis*.

Table 1

Comparison of allele frequencies at 16 loci of six populations of *Oncomelania hupensis*.

Loci	Allele	RF	Population					
			Po. 1	Po. 2	Po. 3	Po. 4	Po. 5	Po. 6
MDH	(N)		30	30	30	30	30	30
	a	0.196	0.500	0.500	0.500	0.500	0.500	0.500
	b	0.255	0.500	0.500	0.500	0.500	0.500	0.500
AO	(N)		30	30	30	30	30	30
	a	0.191	1.000	1.000	1.000	1.000	1.000	1.000
6PGD	(N)		30	30	30	30	30	30
	a	0.169	1.000	1.000	1.000	1.000	1.000	1.000
ME	(N)		30	30	30	30	30	30
	a	0.189	1.000	1.000	1.000	1.000	1.000	1.000
AKP	(N)		30	30	30	30	30	30
	a	0.366	1.000	1.000	1.000	1.000	1.000	1.000
OCT-1	(N)		30	20	20	30	30	30
	a	0.169	1.000	1.000	1.000	1.000	1.000	1.000
OCT-2	(N)		30	20	20	30	30	20
	a	0.277	0.517	0.450	0.550	0.050	0.017	
	b	0.339	0.483	0.525	0.450	0.400	0.517	0.600
	c	0.400		0.025		0.550	0.467	0.400
HBDH-1	(N)		30	30	30	30	30	30
	a	0.140	1.000	1.000	1.000	1.000	1.000	1.000
HBDH-2	(N)		30	30	30	30	30	30
	a	0.574	1.000	1.000	1.000	1.000	1.000	1.000
PGI	(N)		50	30	30	40	50	30
	a	0.175	0.050	0.183	0.167	0.250	0.310	0.033
	b	0.250	0.750	0.667	0.700	0.625	0.570	0.700
	c	0.313	0.200	0.150	0.133	0.125	0.120	0.267
XDH	(N)		30	30	30	30	30	30
	a	0.321	1.000	1.000	1.000	1.000	1.000	1.000
AAT	(N)		40	20	20	40	40	20
	a	0.275				0.325	0.438	0.075
	b	0.303	0.063	0.100	0.175	0.675	0.563	0.925
	c	0.349	0.938	0.900	0.825			
PGM-1	(N)		50	30	30	40	50	30
	a	0.126	0.130	0.200	0.033	0.725	0.010	0.017
	b	0.207	0.780	0.667	0.683	0.275	0.690	0.467
	c	0.261	0.090	0.133	0.283		0.300	0.517
PGM-2	(N)		40	20	20	40	40	20
	a	0.559			0.050	1.000	0.900	1.000
	b	0.586	1.000	1.000	0.950		0.100	
MPI	(N)		30	30	30	30	30	30
	a	0.279	0.500	0.500	0.500	0.500	0.500	0.500
	b	0.341	0.500	0.500	0.500	0.500	0.500	0.500
ACP	(N)		30	30	30	30	30	30
	a	0.385	1.000	1.000	1.000	1.000		
	b	0.426					1.000	1.000

AAT and PGM-2, the genetic distance coefficients between the two subspecies were much higher than the coefficients within subspecies (Table 2). The enzyme acid phosphatase (ACP) was encoded by one locus with two alleles. Two single bands with different mobility, ACP-a (RF = 0.385) and ACP-b (RF = 0.426), were specific to population 1, 2, 3 and 4 and population 5 and 6, respectively.

The mean number of alleles per locus ranged from 1.5-1.6. The percentage of polymorphic loci (P) was 37.5-43.8. The mean heterozygosity per locus (H) was 0.161-0.199. Nei's unbiased genetic distances (D) were 0.000-0.001 within *O.h. hupensis* and 0.016-0.119 within *O.h. fausti*. The genetic distances between the two subspecies were 0.167-0.265 (Table 3).

DISCUSSION

The present study focused on six populations of *O. hupensis* collected in Zhejiang Province, China. The results demonstrated allozyme variations in the two subspecies of *O.h. hupensis* and *O.h. fausti*. Previously, Selander and Ochman (1983) suggested that there may be a relationship between allozyme variation and the degree of genetic differentiation and taxonomy within a group of related populations. The Nei's multilocus genetic distances (D) were calculated to estimate intra- and inter- subspecies divergence. Recent studies by Zhou (1994) and Qian (1994) indicated that the genetic distance between two populations of snails with smooth shells and with ribbed shells was 0.15 and that the

Table 2

Single locus genetic distance coefficient range between two subspecies of *O. hupensis* in Zhejiang.

Locus	*O.h. hupensis* Population (1, 2, 3)	*O.h. fausti* Population (4, 5, 6)	Between two subspecice
OCT-2	0.021-0.115	0.090-0.182	0.472-0.624
PGI	0.023-0.130	0.043-0.267	0.053-0.233
AAT	0.044-0.114	0.074-0.281	0.696-0.812
PGM-1	0.081-0.199	0.145-0.657	0.054-0.577
PGM-2	0.000-0.143	0.000-0.204	0.624-0.900
ACP	0.000-0.000	0.000-0.900	0.000-0.900

Table 3

Genetic similarity and distance coefficients between populations.

Population	1	2	3	4	5	6
1. Jiashan	–	0.981	0.971	0.808	0.774	0.762
2. Changshan	0.000	–	0.975	0.825	0.787	0.766
3. Kaihua	0.001	0.000	–	0.820	0.798	0.776
4. Jiaxing	0.184	0.167	0.167	–	0.875	0.859
5. Jinghua	0.234	0.223	0.210	0.113	–	0.938
6. Shenxen	0.265	0.255	0.236	0.119	0.016	–

Above diagonal: Rogers (1972) genetic similarity
Below diagonal: Nei (1978) unbiased genetic distance

genetic distances between three populations of *O.h. hupensis* were 0.005-0.015. In this study, genetic distances between subspecies ranged from 0.167-0.265, which were much higher than D = 0.000-0.001 and D = 0.016-0.119 of within population of *O.h. hupensis* and *O.h. fausti*, respectively. Minor genetic distances were detected between three populations of the same subspecies. Furthermore, in three of six single-loci *ie* OCT-2, AAT and PGM-2, the genetic distance coefficients between the two subspecies were much higher than the coefficients within subspecies. The allozyme electrophoresis results supported the observation by Liu *et al* (1980), suggesting that although *O.h. fausti* and *O.h. hupensis* are sympatric, they are different in the morphological characters and ecological conditions. Furthermore, *O.h. fausti* represents a microgeographical subspecies. In Zhejiang Province, the terrain slopes from southwest to northeast: mountainous region in the middle and in the southwest, plains in northeast. *O.h. fausti* with smooth shell (Population 5 and 6) inhibits upland regions and *O.h. hupensis* with ribbed shell (Populations 1, 2 and 3) inhibits lowland regions. The observed allozyme variations between the two subspecies possibly related to shell characters, as well as ecological condition. There is no simple relationship between genetic distance and taxonomic level, and careful interpretation of allozyme electrophoresis patterns is required to avoid incorrect estimates of genetic variability. It is therefore doubtful if the different populations or subspecies of *O. hupensis* deserve recognition as full species according to allozyme results.

Acid phosphatase (ACP) has been associated with susceptibility and refractoriness of *Biomphalaria glabrata* populations to *S. mansoni* infection (Michelson and Dubois, 1981). They associated the slow allele ACP-S with susceptibility, and the fast allele ACP-F with refractoriness. The quantitative relationship between susceptibility of *Oncomela-nia* snails and frequency of the MDH-2 has been assessed by Zhou and Kristensen (1995). Most susceptible populations were monomorphic for allele MDH-2 (RF = 0.04) and most resistant populations were polymorphic for MDH-2 (RF = 0.15). In the present study, one locus was observed studying ACP of the six populations of *O. hupensis*. Two single bands with different mobility were specific to each population. ACP-a (RF = 0.385) was identified in population 1, 2, 3 and 4, and ACP-b (RF = 0.426) in populations 5 and 6. Preliminary data of susceptibility had shown that the populations 1, 2, 3 and 4 were susceptible to isolates of *S. japonicum* from Zhejiang, Anhui, Jiangxi, Hunan and Hubei, and that the population 5 and 6 were resistant to the isolates of *S. japonicum* mentioned above (Lin *et al*, 1994; Qian, 1995, unpublished data). This suggests that the degree of susceptibility of the six populations examined may be associated with ACP.

In the past, both Jinghua and Shenxen County, where population 5 and 6 were collected, were highly endemic areas of schistosomiasis (Anonymous, 1992). The susceptibility between the parasite and the snail host depends not only on the *Oncomelania* snail but also on the *Schistosoma* parasite and further studies examing more isolates of *S. japonicum* from Zhejiang is necessary before conclusive evidence can be reached on the use of ACP as a possible marker to measure the degree of susceptibility of *O. hupensis* to *S. japonicum*.

In conclusion, six populations of *O. hupensis* collected from Zhejiang Province, China, belonging to the two subspecies of *O.h. hepensis* or *O.h. fausti*, are different in the shell character, geographic distribution, the degree of susceptibility to different isolates of *S. japonicum* and allozyme loci. Acid phosphatase may be associated with the degree of susceptibility of *O. hupensis* to *S. japonicum*.

ACKNOWLEDGEMENTS

This study was supported by Danish Bilharziasis Laboratory and Zhejiang Natural Sciences Foundation. The authors are grateful to Prof Song Changcun and Dr Samson Mukuratirwa for advice. We also thank Mz Xu Dong-mei for her participating in parts of the work.

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