GENETIC AND MORPHOLOGICAL VARIATIONS IN POPULATIONS OF *ONCOMELANIA* SPP IN CHINA

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Abstract. *Oncomelania* snails are the intermediate hoste of *Schistosoma japonicum* in Asian countries. In order to understand the genetic and morphological variation of *Oncomelania* snails in mainland China, field snails from 31 localities were collected and investigated by means of allele enzyme electrophoresis and numerical taxonomical technics. Results demonstrated that out of 17 loci examined, seven polymorphic loci were presented. Genetic distance (Nei, 1978) among the populations varied from 0.03 to 0.27. The phenogenetic tree based on UPGMA cluster analysis showed that genetic diversity corresponded to geographic distribution along the Yangtze River, which provided supplementary genetic data about the evolution of *Oncomelania* spp. A morphological study showed that Mahalanobis' morphological distance ranged from 1.53 to 346.7. Both genetic and morphological data indicated that the diversity among populations of smooth shelled snails was higher than that among populations of ribbed shelled snails. A positive correlation (r = 0.80) between Mahalanobis' morphological distance and genetic distance supports the hypothesis that the different shell phenotypes represent different species or subspecies.

INTRODUCTION

The taxonomy of *Oncomelania* spp, intermediate host of *Schistosoma japonicum,* has been unclear for a long time (Kang, 1985) and is still considered to be in a confusing state (WHO, 1993). Attempts have been made to solve taxonomical problems related to the *Oncomelania* spp using anatomical features, and some progress has been reached featuring biochemical methods such as protein isoenzyme electrophoresis and numerical taxonomy (Woodruff *et al,* 1986; Davis, 1994). At the population level of *Oncomelania* snails, for instance, some investigations have been performed to try to find shell characters such as the frequencies of shell indices and top angle of apex, in order to identify different geographic populations of *Oncomelania* snails in mainland China (Liu *et at,* 1981; Wang and Lu, 1985). Since only a few characters were employed and a few populations were used for analysis, it was difficult to apply these characters to identify all populations of *Oncomelania* snails in China.

A strong argument was recently voiced in a paper by Davis (1994), rising the question of whether or not *Oncomelania hupensis* is designated as a polytypic species with several subspecies or different species. He considered *Oncomelania hupensis* to be a polytypic species because there are small differences in susceptibility to different allopatric populations of *Schistosoma japonicum;* in size; in degree of shell varix formation and in the degree of gland formation about the medial aspects of the eyes. Davis (1968, 1979, 1980) hypothesized that *Oncomelania* or its immediate ascestor reached Asia on the Indian continental plate during the Miocene. During the concomitant Himalayan orogeny, the snails entered the newly developing Yangtze River system by way of northern Myanmar and Yunnan, and spread to the Pacific coast of China. From there they were dispersed to the continental islands of Japan and Taiwan and to the Philippine archipelago and Sulawesi, Indonesia. *O. hupensis* is therefore viewed as a taxon that arose, spread, and differentiated during the past six million years (Woodruff *et al,* 1988).

The relative recentness of the evolution of *O. hupensis,* coupled with its sexual mode of reproduction, suggests that if this species is genetically variable, measures of interpopulation allozyme differentiation could be used to test the above hypo thesis (Davis, 1980). However, the first step in testing Davis' hypotheses should be the interpopulation variation along the Yangtze River where the distribution of the snails is discontinued between the upper reaches and middle/lower reaches of the river. Therefore, it is a unique way to study the genetic population variation of *Oncomelania* snails in mainland China because: (1) mainland China is the most important distribution center of *Oncomelania* where natural conditions are very complex and spe-

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cies may be numerous (Kang, 1985); (2) variety of shell morphology only simpatrically appeared in mainland China (Zhou and Kristensen, 1992) and (3) the differential susceptibility to *Schistosoma japonicum* and variation in sensitivity to molluscicides between snail population has been reported in mainland China (He *et al,* 1991; Hong *et al,* 1995a,b; Zhou *et al,* 1995). It is difficult for scientists outside of China to collect snail specimens covering all of China and only a few populations were included in the studies which could not reflect all aspects of *Oncomelania* snail. To overcome this shortage, a project has been undertaken to reflect some aspects of *Oncomelania* snails in mainland China, and some papers related to different aspects have been published (Zhou 1992; Zhou and Kristensen 1992; Zhou *et al,* 1994a,b; Zhou *et al,* 1995a,b,c; Hong *et al,* 1995a,b). This report represents an attempt to search for interpopulation genetic variation and morphological variation with a view to testing Davis'historical hypothesis along the Yangtze River.

METERIALS AND MOTHODS

Snail sampling

Snails utilized in the study were all collected from the field in mainland China during March to

Table1

The localities of *Oncomelania* snails used in the study.

Locality code	Province	County	Locality	En	Latitude north	Longitude south
A1	Anhui	Guichi	Chuangjia	2	30.35	117.26
A2	Anhui	Guangde	Dusheng	1	30.53	119.25
A3	Anhui	Qingyang	Dinggiao	2	30.47	117.54
A4	Anhui	Qingyang	Dinggiao	1	30.47	117.54
A5	Anhui	Tongling	Chuzhang	1	30.52	117.51
A6	Anhui	Tongling	Datong	2	30.55	117.46
A7	Anhui	Tongling	Laozhou	2	30.58	117.46
F1	Fujian	Fuqing	Huanlu	1	25.50	119.24
H1	Hubei	Guoan	Donghuti	3	30.03	112.13
H2	Hubei	Hanchang	Muhe	2	30.39	113.48
H3	Hubei	Honghu	Yige	2	29.49	113.25
H4	Hubei	Puxi	Xingding	3	29.43	113.50
H5	Hubei	Qianjiang	Saoyiang	3	30.26	112.43
H6	Hubei	Shishou	Shishou	3	29.45	112.24
H7	Hubei	Wuchang	Qinjiang	2	30.21	114.08
HN1	Hunan	Xihu Farm	No. 5	2	29.05	112.07
J1	Jiangsu	Dongtai	Xuhe	3	32.53	120.36
J2	Jiangsu	Gaoyou	Xinming	2	33.02	119.21
J3	Jiangsu	Jintai	Baitar	3	31.46	119.33
J4	Jiangsu	Jiangpu	Laihua	2	31.02	118.36
J5	Jiangsu	Qixia	Baoguazhou	2	32.10	118.47
J6	Jiangsu	Qixia	Huayuan	3	32.12	119.02
J7	Jiangsu	Wuxian	Lumar	3	31.23	120.36
JX1	Jiangxi	Pengze	Hurong	2	29.53	116.33
SH1	Shanghai	Jinshan	Xinta	3	30.53	121.04
S1	Sichuan	Danling	Farm	1	30.03	103.34
S2	Sichuan	Dayi	Yuleng	1	30.39	103.31
S3	Sichuan	Pengshan	Guanyi	1	30.12	103.51
S4	Sichuan	Puge	Pugi	1	27.23	102.30
Y1	Yunnan	Weishang	Weishang	1	25.32	100.15
Y2	Yunnan	Weishang	Yiuanjing	1	25.15	100.20

* En: refers the type of environment where snails infested as

1. mountainous and hilly regions,
2. lake and river regions and
3. plain (water-network) regions.

June 1992. A total of 31 populations of *Oncomelania* snails were collected and Table 1 lists the names of the 31 localities covering nine provinces along the Yangtze River in southern China. The active adult snails were maintained in the laboratory for more than one month and were checked for natural infections by cercariae shedding prior to experiments.

Allele enzyme electrophoresis

Horizontal starch gels were prepared for the analysis of allozyme patterns. Starch gel was made in a concentration of 12% (w/v) starch in gel buffer. Three electrophoresis buffer trays containing a buffered electrolyte solution were prepared (Steiner and Joslyn, 1979). All chemicals for enzyme staining were purchased from Sigma Chemical Company.

Preferably fresh samples from whole adult snails were used for allozyme studies. The individual living snail was quickly homogenized in 0.1-0.2 ml of distilled water and then centrifuged at 1,500 rpm for 5 minutes. The supernatant fluid was absorbed onto filter paper wicks (9 x 5 mm) and the wicks were inserted into a vertical slot cut 2 cm from the cathodal end of the gel. The gel loaded by sample wicks was placed at 4°C. The power supply was turned on at a certain level of voltage depending on the different buffer systems (Woodruff *et al,* 1988). The moving zone was made visible by including a wick (inserted) containing a solution of 1% bromophenol blue marker dye as the last sample member. The following 13 enzymes were studied using the appropriated buffer: aldehyde oxidase (AO 1.2.3.1), aldehyde dehydrogenase (ALDH 1.2.1.5), alkaline phosphatase (AP 3.1.3.1), esterase (EST 3.1.1.1), glutamate-oxaloacetate transaminase (GOT 2.6.1.1), glycerol-3-phosphate dehydrogenase (α-GPD 1.1.1.8), glycerol-6-phosphate dehydrogenase (G6PD 1.1.1.49), ß-hydrozybutyrate dehydrogenase (HBDH 1.1.1.30), isocitrate dehydrogenase (IDH 1.1.1.42), lactate dehydrogenase (LDH 1.1.1.27), malate dehydrogenase (MDH 1.1.1.37), sorbitol dehydrogenase (SDH 1.1.1.14), xanthine dehydrogenase (XDH 1.2.1.37). Once electophoresis was completed, the gels were sliced and then placed in individual plastic boxes for histochemical staining using standard methods (Ferguson, 1980; Richardson *et al,* 1986).

The isozyme patterns were documented either by photography or by immediately drawing observed patterns on paper, because all NADP-and NAD-dependent enzymes fade at room temperature over a period of serveral months. "Electromorph" is used in referring to a band of enzyme activity (King and Ohta, 1975), while genetic designations for the electromorphs were assigned using abbreviations for the particular enzymes. When several forms of the same enzyme existed, the loci were numbered in order of decreasing anodal mobility. Isozyme mobilities (Rf) were calculated relative to the bromophenol blue marker dye. Data consisting of multilocus genotypes for individual snails were analyzed. A locus was considered polymorphic (P) if more than one allele was detected. Mean heterozygosity per individual (H) was estimated by direct count.

The allele frequencies were computed by the formula of Ferguson (1980). To determine if the animal organisms came from a randomly mating population on the basis of the Hardy-Weinberg model, the observed frequencies of heterozygotes for each group were compared to the expected frequencies. The difference between the observed and expected values was tested for statistical significance, using both chi-square test and G-test (the log likelihood ratio test) for goodness-of-fit (Ferguson, 1980). The G-test is especially pertinent if the sample sizes are small (Sokal and Rohlf, 1969), The allele frequency data were loaded in the computer programe G-test (Siegismund, 1992) and "Phylip" Version 3.5 (Felsenstein, 1993) to compute genetic variability, to genetic distance (Nei, 1978), and to construct phylogenetic trees using UPGMA (unweighted pair-group method using arithmetic averages) cluster analysis.

Morphological study

Methods of analyzing the variation in shell features followed those of Brown *et al,* (1971), with the following modifications. Individual shells from each population were photographed under stereomicroscope after the shells were cleaned in 2% oxalic acid. The photographs of the shells were scored by Summagraphics (Bit Pad Two) controlled by BITPAD5.BAS computer program. A total of 16 shell characters were measured and recorded (Fig 1). Thirteen shell measurements were carried out on each specimen and recorded to the nearest 0.1 mm. Three other features counted on each were: ribs' number of body whorl, ribs' number of penultimate whorl and number of whorls.

All the morphological data from the same populations which were analyzed genetically were performed by discriminant analysis employing all of 16 variables. In the discriminant analysis program, the variables that lend the greatest separation of groups are automatically chosen for inclusion in the discriminant functions calculated. They were used to sort snails into groups representing the popula-

tions under consideration. The analysis produced a matrix of population relatedness or Mahalanobis' morphlogical distance (D^2) based on shell dimensions. The values of the D^2 will be transformed to the G-stat program for Mantel test to observe the correlation between morphological D^2 and genetic distance through comparing matrices pairwise. The tree of morphological variation among 31 populations was constructed by including D^2 in the program UPGMA from the package Phylip, version 3.5.

RESULTS

Allele enzyme electrophoresis

A total of 13 proteins were examined electrophoretically, which provided evidence for variation at 20 genes. Seventeen of these gave consistent and genetically interpretable results. Of these 17 presumptive loci, 10 were monomorphic and invariant in samples: ALDH, AO, Est-2, Est-3, Est-6, G6PD, GPDH, HBDH, LDH and SDH. Seven loci were variable, with four loci showing the appearance of a dimer: AP, GOT, Mdh-2 and Idh-2, and three loci showing the appearance of a monomer: Est-4, Est-5 and XDH. The remaining three loci are not reported since they showed inconsistent or uninterpretable banding patterns (Est-1, Idh-1, Mdh-1). A total of seven monomorphic enzymes were observed in ALDH, AO, G6PD, GPDH, HBDH, LDH and SDH being controlled by a single gene locus, which was evident in alleles $Aldh^{0.274}$, $Ao^{0.305}$, $G\text{-}6\text{-}pd^{0.290}$, $Gpd^{0.175}$, $Hbdh^{0.278}$, $Ldh^{0.290}$ and $Sdh^{0.258}$, and showed no detectable variation.

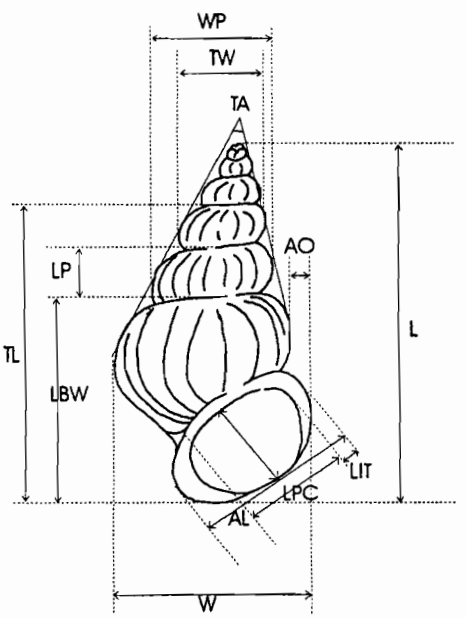

Fig 1–Measurement on the shell of *Oncomelania* snails. Aperture length (AL), Aperture overhang body whorl (AO), Aperture width (AW), Shell length (L), Width of shell (W), Length of penultimate whorl (LP), Length of body whorl (LBW), Lip thickness (LIT), Length of parietal callus (LPC), Top angle of apex (TA), Length of last three whorls (TL), Width of last three whorls (TW), Width of penultimate whorl (WP).

A total of six polymorphic enzymes were observed in AP, EST, GOT, IDH, MDH and XDH. One locus was scored in the enzyme AP (alkaline phosphatase) composed of two alleles ($Ap^{0.320}$ and $Ap^{0.231}$) encoding for three allozymes, of which $Ap^{0.320}$ was predominant. Heterozygotes appeared in three bands indicating a dimer of this locus.

A total of six loci encoded for the enzyme EST (esterase). Est-1 was excluded from this study since it was either not shown in some specimens or too faint to be scored. Est-2, Est-3 and Est-6 were presented as monomorphic enzymes in every population at alleles of $Est\text{-}2^{0.493}$, $Est\text{-}3^{0.406}$ and $Est\text{-}6^{0.072}$, respectively. Est-4 was polymorphic with two alleles ($Est\text{-}4^{0.348}$ and $Est\text{-}4^{0.314}$) encoding for three allozymes. Est-4 appeared as a monomer due to the occurrence of two bands indicative of heterozygotes. Est-5 was also polymorphic with two alleles ($Est\text{-}5^{0.290}$ and $Est\text{-}5^{0.232}$) giving a total of three allozymes. This enzyme exhibited variability in all populations and heterozygotes with two bands indicated a monomeric enzyme of the locus.

GOT (glutamate-oxaloacetate transaminase) exhibited two alleles of $Got^{0.470}$ and $Got^{0.400}$ giving a total of three allozymes. Some homozygotes showed two bands due to a sub-band or conformational type of the locus. The occurrence of three-banded heterozygotes revealed a dimer of the Got locus.

Two loci were encoded in the enzyme IDH (isocitrate dehydrogenase), but only one locus, Idh-2, could be scored, and Idh-1 appeared to be blurred and was not shown in many samples. Hence, Idh-1 was excluded from this study. Idh-2 was represented by alleles $Idh\text{-}2^{0.250}$ and $Idh\text{-}2^{0.073}$, of which the former was predominant in all populations. In some populations such as A5, two bands appeared in Idh-2 alleles due to a sub-band or conformational type of the locus. A few heterozygotes were found, which appeared in three bands, indicating a dimer of the Idh-2 locus.

MDH (malate dehydrogenase) was encoded by two loci, but Mdh-1 was either not shown in many

specimens or was too faint to score. Hence, Mdh-1 was excluded from this study. Mdh-2 consisted of two alleles, Mdh-$2^{0.150}$ and Mdh-$2^{0.040}$, of which Mdh-$2^{0.040}$ was predominant, and most populations exhibited Mdh-2 as a monomorphic enzyme at allele Mdh-$2^{0.040}$. In some samples, two bands appeared in either allele Mdh-$2^{0.150}$ or allele Mdh-$2^{0.040}$ due to a sub-band or conformational type of the Mdh-2 locus. Heterozygotes were observed in some populations (such as A5, A2 and Y1) with three bands indicating a dimer of the Mdh-2 locus.

XDH (xanthine dehydrogenase) was composed of two alleles, $Xdh^{0.280}$ and $Xdh^{0.243}$, of which $Xdh^{0.280}$ was predominant in all populations. A few heterozygotes were observed with two bands indicating a monomeric of the XDH locus.

The percentage of polymorphic loci (P) among the 17 presumptive loci in each sample varied from 5.9 to 29.4 between samples from different areas (Table 2). The most variable population was S2 with five variable loci, followed by S1, S3, F1, A2 and A4, with four variable loci. Among these variable populations, S2, S1, S3 and F1 were characterized by multiple alleles at Est-4 and Got. A2, A4, and A5 from hilly regions adjacent to the plains regions also showed a higher proportion of polymorphic loci (p = 17.6-23.5). The proportion of polymorphic loci in populations of snails from the plains and lake regions was slightly lower (5.9-17.6%). Mean number of alleles per locus lies in the range 1.059-1.294 (±0.243 - 0.470 SE). Mean heterozygosity (H) varied from 0.021 to 0.121 among samples and displayed a pattern similar to that for P. The population from mountainous regions presented higher H values (H = 0.064 - 0.101), followed by the ad-

Table 2
Genetic variability at 17 loci in all populations (standard errors in parentheses).

Population	Mean sample size per locus	Mean No. of alleles per locus	Percentage of loci polymorphic	Mean heterozygosity		
				Direct count	Hdy-Wbg expected	P
A1	18.5 (7.86)	1.176 (0.39)	17.6	0.047 (0.12)	0.046 (0.12)	> 0.5
A2	17.4 (8.12)	1.235 (0.44)	23.5	0.093 (0.19)	0.097 (0.19)	> 0.5
A3	12.6 (5.85)	1.059 (0.24)	5.9	0.024 (0.10)	0.019 (0.08)	> 0.5
A4	17.2 (9.22)	1.235 (0.44)	23.5	0.121 (0.23)	0.121 (0.23)	> 0.5
A5	16.8 (3.51)	1.176 (0.39)	17.6	0.052 (0.13)	0.043 (0.11)	> 0.5
A6	18.5 (7.86)	1.118 (0.33)	11.8	0.040 (0.13)	0.037 (0.12)	> 0.5
A7	16.7 (3.55)	1.118 (0.33)	11.8	0.032 (0.09)	0.042 (0.13)	> 0.5
F1	8.5 (2.94)	1.235 (0.44)	23.5	0.082 (0.17)	0.068 (0.14)	> 0.5
H1	18.5 (7.86)	1.118 (0.33)	11.8	0.040 (0.13)	0.039 (0.13)	> 0.5
H2	12.6 (5.85)	1.059 (0.24)	5.9	0.024 (0.10)	0.030 (0.12)	> 0.5
H3	12.6 (5.89)	1.059 (0.24)	5.9	0.026 (0.11)	0.021 (0.09)	> 0.5
H4	15.8 (4.10)	1.118 (0.33)	11.8	0.046 (0.14)	0.037 (0.11)	> 0.5
H5	8.4 (2.92)	1.235 (0.44)	17.6	0.051 (0.13)	0.071 (0.17)	> 0.5
H6	15.4 (4.06)	1.059 (0.24)	5.9	0.021 (0.09)	0.018 (0.08)	> 0.5
H7	15.7 (4.15)	1.118 (0.33)	11.8	0.029 (0.08)	0.039 (0.12)	> 0.5
HN1	18.5 (7.86)	1.176 (0.39)	11.8	0.032 (0.09)	0.042 (0.13)	> 0.5
J1	18.1 (9.81)	1.118 (0.33)	11.8	0.036 (0.13)	0.055 (0.16)	> 0.5
J2	12.6 (5.90)	1.059 (0.24)	5.9	0.026 (0.11)	0.024 (0.10)	> 0.5
J3	17.3 (8.07)	1.118 (0.33)	11.8	0.036 (0.11)	0.030 (0.09)	> 0.5
J4	15.4 (4.58)	1.059 (0.24)	5.9	0.025 (0.10)	0.029 (0.12)	> 0.5
J5	12.6 (5.89)	1.059 (0.24)	5.9	0.029 (0.12)	0.030 (0.12)	> 0.5
J6	20.2 (10.6)	1.118 (0.33)	11.8	0.069 (0.20)	0.057 (0.16)	> 0.5
J7	17.1 (7.92)	1.176 (0.39)	17.6	0.077 (0.18)	0.086 (0.19)	> 0.5
JX1	17.0 (7.90)	1.118 (0.33)	11.8	0.062 (0.17)	0.057 (0.16)	> 0.5
SH1	8.5 (2.94)	1.176 (0.39)	11.8	0.063 (0.17)	0.055 (0.14)	> 0.5
S1	19.3 (1.01)	1.235 (0.44)	23.5	0.101 (0.22)	0.096 (0.19)	> 0.5
S2	17.8 (7.42)	1.294 (0.47)	29.4	0.082 (0.15)	0.090 (0.16)	> 0.5
S3	8.5 (2.94)	1.235 (0.44)	23.5	0.098 (0.25)	0.084 (0.18)	> 0.5
S4	12.6 (5.89)	1.176 (0.39)	17.6	0.097 (0.26)	0.076 (0.17)	> 0.5
Y1	18.0 (10.5)	1.125 (0.34)	12.5	0.064 (0.18)	0.047 (0.13)	> 0.5
Y2	8.5 (2.94)	1.118 (0.33)	11.8	0.071 (0.21)	0.055 (0.16)	> 0.5

jacent populations (A2, A4 and A5) (H = 0.052 - 1.121), while populations from the plains and lake regions presented slightly lower values (0.021 - 0.082). Multilocus genetic differences between these samples estimated by using Nei's (1978) unbiased coefficient genetic destance (D) are shown in Table 3. The genetic distance (D) ranged from 0.01 to 0.22, showing that obvious differentiation occurred among populations.

In order to clearly reveal the relationship among populations, the best tree of genetic diversion was constructed by employing seven polymorphic allele frequencies among 31 populations with the program CONTML (maximum likelihood) from the package Phylip, version 3.5 (Felsenstein, 1993). It is clearly seen in the phylogenetic tree (Fig 2) that the genetic diversion among these populations corresponds to their geographic distribution along the Yangtze River, and two distinguishable groups were presented in the tree: Group I: Y2, Y1, S1, S3, S4, S2, F1, and A2, which are the populations from mountainous regions and all snails without a ribbed shell; group II: the other 22 populations mainly from lake and plains regions, except for the populations of A4 and A5 from adjacent hilly regions, and all snails with ribbed shells. The phylogenetic tree shows that variations in group I are larger than in group II. Four sub-groups in group I can be further classified as Yunnan (Y1, Y2), Sichuan (S1, S2, S3, S4), east coast (F1, J1) and adjacent (A2) sub-groups. In contrast, the populations in group II are close to each other, and difficult to classify as sub-groups corresponding to the geographic distribution of the populations.

Morphological study

The measurement data of 1,113 individual shells from 31 populations were performed in discriminant analysis employing 16 variables. The results showed that 68.19% of the total shells were classified correctly. When those shells were divided into two groups based on shell with or without ribs, a very high classification rate (80.44%) appeared in the smooth shells. In contrast, only 59.17% of the ribbed shells were classified correctly. Less overlaps occurred in the smooth shells than in the ribbed shells, indicating that much difference/variation in shell morphology existed among populations with smooth shells.

Mahalanobis' morphological distance (D^2) between populations by discriminant analysis is shown in Table 3. The variation of D^2 between populations was at a very high level (ranging from 1.53 to 346.7). The tree (Fig 3) of morphological variation among 31 populations was constructed by putting D^2 in the program UPGMA and showed that two distinct groups were separated from each other,

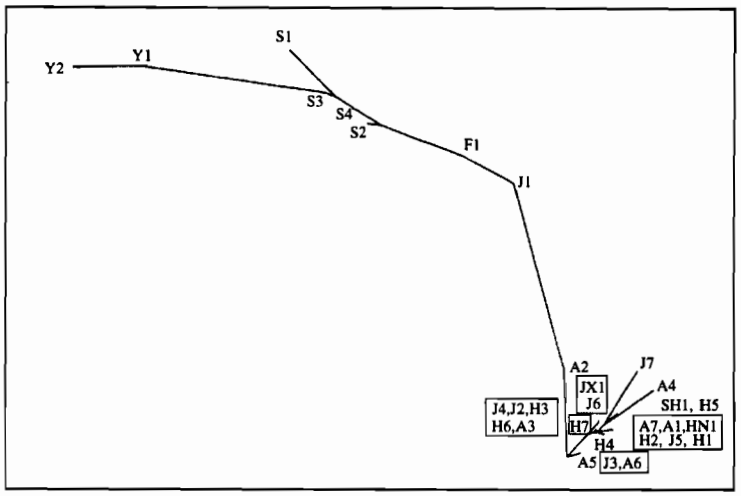

Fig 2–Phylogenetic tree based on allele frequencies generated by UPGMA.

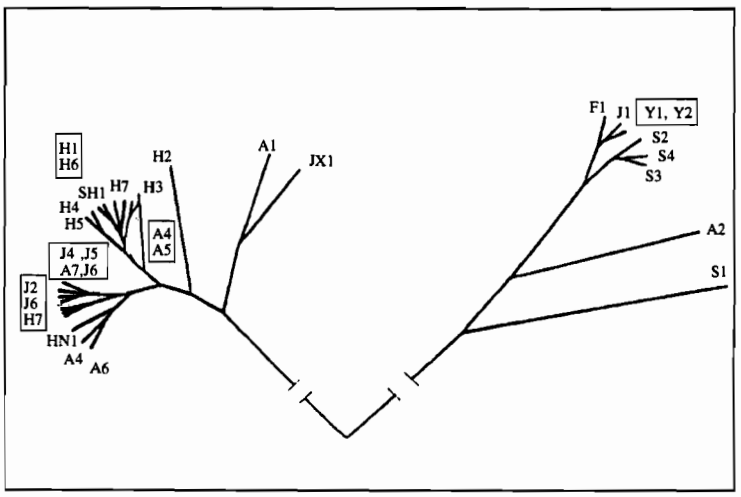

Fig 3–Phylogenetic tree based on Mahalanobis (D^2) distances generated by UPGMA.

Table 3

The genetic distance (Nei's 1978) (above diagonal) and Mahalanobis' morphological distance (D^2) (below diagonal) between 31 populations of *Oncomelania* spp.

	A1	A2	A3	A4	A5	A6	A7	F1	H1	H2	H3	H4	H5	H6	H7	HN1
A1	-	0.01	0.01	0.02	0.02	0.00	0.00	0.10	0.00	0.00	0.01	0.01	0.02	0.01	0.01	0.00
A2	198	-	0.03	0.03	0.03	0.02	0.02	0.08	0.02	0.02	0.02	0.02	0.04	0.03	0.02	0.02
A3	16.4	210	-	0.03	0.01	0.01	0.00	0.10	0.01	0.01	0.00	0.00	0.03	0.00	0.00	0.01
A4	31.2	195	9.39	-	0.02	0.02	0.02	0.12	0.02	0.02	0.03	0.02	0.01	0.03	0.02	0.02
A5	38.9	206	15.6	4.43	-	0.02	0.01	0.11	0.02	0.02	0.01	0.01	0.03	0.01	0.01	0.01
A6	12.6	192	7.78	17.1	28.8	-	0.01	0.12	0.00	0.00	0.01	0.01	0.02	0.01	0.01	0.00
A7	20.1	201	10.4	14.7	16.0	14.1	-	0.10	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
F1	177	24.7	189	185	190	175	179	-	0.11	0.11	0.10	0.10	0.12	0.10	0.10	0.11
H1	39.2	240	13.7	12.0	8.33	30.4	18.2	209	-	0.00	0.01	0.00	0.01	0.01	0.00	0.00
H2	36.9	237	17.5	20.7	22.5	30.8	35.6	217	26.5	-	0.01	0.01	0.02	0.01	0.01	0.00
H3	57.6	270	21.7	20.0	15.8	42.3	32.5	237	10.3	27.4	-	0.00	0.03	0.00	0.00	0.00
H4	47.2	235	12.6	7.20	7.47	30.7	26.5	219	10.3	15.2	8.31	-	0.02	0.00	0.00	0.00
H5	63.0	284	20.1	13.8	11.4	42.1	32.1	262	12.6	23.7	15.9	6.92	-	0.03	0.02	0.02
H6	39.3	235	10.7	10.6	12.3	24.4	21.0	210	5.21	28.0	22.0	12.3	12.2	-	0.00	0.00
H7	27.3	198	6.89	9.25	16.4	13.9	17.2	177	20.0	17.4	34.0	15.1	18.2	11.1	-	0.00
HN1	21.5	226	8.41	20.9	24.8	11.9	13.4	186	14.0	28.9	26.1	26.2	30.3	13.9	15.3	-
J1	182	41.0	185	174	175	180	172	8.68	190	212	217	206	240	195	173	178
J2	25.2	196	9.38	9.31	17.8	10.5	8.96	183	23.9	37.0	41.7	24.2	28.3	15.0	7.29	18.2
J3	22.2	200	10.7	8.38	6.72	19.9	5.78	180	11.8	22.3	28.1	17.5	22.6	14.9	11.8	16.2
J4	24.8	193	17.6	17.9	17.1	19.9	6.61	166	23.4	33.0	42.1	31.8	36.5	23.7	14.8	17.0
J5	28.5	205	13.1	19.2	23.7	17.0	4.70	188	28.9	41.0	46.8	32.2	34.2	24.3	13.1	19.3
J6	35.9	188	10.8	6.23	10.9	18.5	14.2	171	16.1	23.1	29.8	13.8	12.7	8.69	3.90	18.5
J7	44.3	265	12.5	16.8	15.9	27.5	11.9	246	14.5	42.9	19.8	18.0	18.8	18.2	25.0	19.8
JX1	20.0	183	17.7	30.7	46.4	10.4	33.1	180	56.4	35.3	66.3	43.4	59.1	44.4	19.6	31.3
SH1	43.2	236	11.3	9.97	7.40	27.5	10.5	218	9.49	33.3	19.8	12.0	13.6	11.5	16.8	20.7
S1	234	93.2	252	260	270	240	215	71.0	291	305	330	305	347	292	239	258
S2	189	57.6	200	201	210	187	176	24.6	225	240	263	242	279	223	185	196
S3	186	52.5	195	190	192	188	169	16.6	205	229	242	227	262	210	180	187
S4	184	49.8	205	198	202	188	172	19.7	222	245	258	244	281	228	193	197
Y1	178	28.1	187	177	181	177	167	6.10	199	218	230	214	253	203	175	183
Y2	169	35.7	181	173	176	171	163	6.24	192	207	223	209	246	196	169	173

	J1	J2	J3	J4	J5	J6	J7	JX1	SH1	S1	S2	S3	S4	Y1	Y2
A1	0.07	0.01	0.01	0.00	0.00	0.01	0.02	0.00	0.02	0.15	0.13	0.11	0.12	0.11	0.12
A2	0.05	0.02	0.03	0.02	0.02	0.02	0.04	0.02	0.04	0.10	0.08	0.07	0.07	0.09	0.11
A3	0.10	0.00	0.03	0.00	0.01	0.01	0.04	0.01	0.05	0.16	0.12	0.13	0.13	0.09	0.10
A4	0.09	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.16	0.15	0.14	0.15	0.11	0.10
A5	0.11	0.01	0.05	0.01	0.02	0.02	0.05	0.02	0.05	0.15	0.12	0.13	0.12	0.08	0.07
A6	0.08	0.01	0.01	0.00	0.00	0.01	0.02	0.00	0.02	0.16	0.14	0.12	0.13	0.11	0.12
A7	0.09	0.00	0.02	0.00	0.00	0.00	0.03	0.00	0.03	0.16	0.13	0.13	0.13	0.10	0.10
F1	0.07	0.10	0.14	0.11	0.11	0.11	0.09	0.11	0.13	0.04	0.02	0.04	0.02	0.10	0.10
H1	0.08	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.02	0.16	0.13	0.12	0.13	0.10	0.11
H2	0.08	0.00	0.01	0.00	0.00	0.01	0.03	0.01	0.03	0.16	0.13	0.11	0.13	0.10	0.11
H3	0.09	0.00	0.03	0.00	0.00	0.01	0.04	0.01	0.04	0.16	0.12	0.13	0.13	0.09	0.10
H4	0.09	0.00	0.02	0.00	0.00	0.01	0.03	0.01	0.03	0.16	0.12	0.13	0.13	0.09	0.09
H5	0.10	0.03	0.02	0.03	0.02	0.01	0.02	0.01	0.00	0.19	0.15	0.15	0.15	0.13	0.11
H6	0.10	0.00	0.03	0.00	0.01	0.01	0.04	0.01	0.05	0.16	0.12	0.13	0.13	0.09	0.09
H7	0.09	0.00	0.02	0.00	0.00	0.00	0.03	0.01	0.03	0.16	0.12	0.13	0.13	0.09	0.10
HN1	0.08	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.02	0.16	0.13	0.12	0.13	0.10	0.11
J1	-	0.09	0.08	0.09	0.08	0.09	0.11	0.09	0.10	0.10	0.10	0.09	0.08	0.20	0.22
J2	179	-	0.03	0.00	0.00	0.01	0.04	0.01	0.04	0.16	0.12	0.13	0.13	0.09	0.10
J3	169	12.1	-	0.02	0.01	0.01	0.02	0.01	0.01	0.18	0.16	0.13	0.15	0.13	0.14
J4	158	11.5	6.41	-	0.00	0.01	0.03	0.01	0.04	0.16	0.12	0.12	0.13	0.09	0.10
J5	184	5.77	11.5	9.31	-	0.01	0.03	0.01	0.03	0.16	0.13	0.12	0.13	0.10	0.11
J6	161	5.69	10.1	13.6	11.0	-	0.02	0.00	0.01	0.17	0.14	0.13	0.14	0.11	0.10
J7	231	19.3	17.0	28.4	16.8	19.1	-	0.01	0.01	0.14	0.12	0.09	0.12	0.08	0.08
JX1	193	22.3	36.3	37.1	29.6	29.7	48.6	-	0.01	0.17	0.14	0.13	0.14	0.11	0.11
SH1	203	15.0	8.66	19.8	14.2	11.0	4.24	48.1	-	0.20	0.17	0.15	0.16	0.15	0.14
S1	83.3	229	238	208	213	222	307	244	282	-	0.02	0.02	0.01	0.06	0.07
S2	29.9	182	187	160	179	174	256	199	227	23.7	-	0.02	0.01	0.07	0.08
S3	14.6	180	182	153	176	167	242	204	212	36.9	6.46	-	0.01	0.06	0.08
S4	20.7	185	169	157	182	179	252	203	226	33.8	9.16	6.44	-	0.07	0.08
Y1	5.63	175	173	154	177	163	234	187	207	56.3	17.4	6.96	9.85	-	0.01
Y2	5.36	171	163	147	173	158	231	182	203	57.6	15.3	5.31	8.91	1.53	-

corresponding highly to one of the shell characters, with or without ribs.

In addition, the comparison between matrices of morphological Mahalanobis distance and genetic distance by utilization of Mantel test showed a correlation between morphology and genetics at a significant level ($p < 0.001$, $r = 0.80$).

DISCUSSION

The genetic variability of *Oncomelania* snails from China has been studied and a high variability of the snails from different types of regions was found by Zhou (1992); Zhou *et al* (1994b; 1995b); Hope and McManus (1994); Zhang *et al* (1994); Qian *et al* (1996). On the other hand, morphological studies showed that the degree of morphological variation was parallel to the geographic variation (Zhou and Kristensen, 1992), and particularly that ribbed shells, which were only distributed along the Yangtze River in mainland China, differ from the snails with smooth shells in other areas of the country, possibly at the species level (Qian *et al*, 1996). However, the comparison between genetic and morphological variation has not been reported.

In the present study, a total of 17 loci were examined and seven polymorphic loci were presented in the 31 natural snail populations covering nine Provinces, in almost all of the current endemic areas of schistosomiasis in China. Results from the present study demonstrate that the genetic variation of *O. hupensis* from China is slightly variable within populations, suggesting that these snails are outbreeding at random in the field. Mean number of alleles per locus (1.059 - 1.294), and the estimates of P and H showed above, are minimum estimates based on single-gel electrophoretic determinations. They probably reflect 80% of the true variability at the loci examined (Selander and Whittam, 1983). Seven out of 17 presumptive polymorphic loci which were presented, agreed with Tsukamoto *et al* (1988) who found that the emzymes of LAP, PGM, ACP, GOT, HK, G6PD, LDH, ALDOX, XDH, MDH, ME and EST were variable among three populations of *Oncomelania* spp (*O. nosophora O. formosana, O. quadrasi*). All data and data treatment therefore provided reliable results on genetic diversity of *Oncomelania* spp in China and were suitable for testing Davis' historical hypothesis (Davis, 1968, 1979, 1980) along the Yangtze River.

The accuracy of phylogenetic trees established by different methods was evaluated by Fiala and Sokal (1985). The most commonly used method is UPGMA, strongly recommended by Prager and Wilson (1978) and Nei *et al* (1983). In the present study, the best phylogenetic tree clustered by UPGMA showed that the groups clustered based on allele frequency, corresponding to their geographic distribution along the Yangtze River. From the phylogenetic tree, it was found that the diversity among populations of smooth shelled snails is higher than that among populations of ribbed shelled snails, the reason perhaps being that the populations of ribbed shelled snails are distributed mainly in the middle and lower reaches of the Yangtze River. In the flooding season the snails are dispered along the river, resulting in frequent gene exchange, while the smooth shelled popultions, normally distributed in mountainous regions or along the coastline where geographic barriers exist between them. This indicates that populations of ribbed shelled snails represent a group with higher genetic identity, while populations of smooth shelled snails represent a group which might be divided into subgroups because of higher diversity. In this sense, more subspecies can be found in the group of smooth shelled snails, depending on the genetic distance and geographic information. Thus it is necessary to include populations from the complete area of distribution when studying the evolution of *Oncomelania* spp in mainland China and in testing Davis' historical hypothesis.

Keeping in mind that the Tethys Sea covered Tibet and areas of Southeast Asia and western China until the Oligocene and early Miocene (Pascoe, 1950), and that the uplift of the Tibetan Plateau initiated the drainage patterns that were to become the main rivers of Asia (Yangtze, Mekong, Salween, Irrawaddy and Brahmaputra), Davis (1979) considered that subsequent to the introduction of the Pomatiopsids to the Asian mainland, there was a dispersal of pomatiopsine stock (*Oncomelania* spp) down the Yangtze River, leading to eventual colonization in Japan. The present data have provided supplementary genetic data about the evolution of *Oncomelania* spp along the whole Yangtze River. The phylogenetic tree based on UPGMA cluster analysis (Fig 2), shows the genetic evolution among 31 populations, from which it is presumed that *Oncomelania* spp first arrived in Yunnan Province coming down from the Himalayas, then moved on to the Sichuan basin which was connected to the Yangtze River. After that, the snails were dispersed to the east coast of China.

The fact is that some snail populations are scattered in hilly regions and adjacent to the plains regions

along the middle or lower reaches of the Yangtze River. Normally the shell of these populations is without ribs, (for example, population A2), but in some of them their shells are slightly ribbed. Apparently these belong to a taxon between ribbed shell and smooth shell. Therefore, in view of snail evolution, a few snails from the adjacent populations occasionally dispersed down to the lake regions where genetic drift happened dramatically, due to a different environment, and ribbing appeared to be controlled by a new mutation gene with natural selection force.

Genetic variation is considered to be related to environmental variability (Levintion, 1973; Selander and Kaufman, 1973), and the major forces in the evolution of the Pomatiopsidae have been environmental selective forces operating on morphological features. New morphological features marking the entrance to a new adaptive zone have been the basis for adaptive radiation (Davis, 1979). Therefore, based on the results of the present study that the differences in shell morphological features between *Oncomelania* spp were quite large in terms of Mahalanobis' morphological distance, and a positive correlation (r = 0.80) with the genetic distance, it seems likely that environmental factors are important in contributing to both morphological variation and genetic variation of *O. hupensis* populations. This idea has been supported by some studies (Roper, 1969; Cohen, 1976; Kristensen, 1982). It is likely to assume that the different shell phenotypes represent different species or subspecies.

The phenotype tree constructed by UPMGA based on the Mahalanobis distance in the study showed that two distinguished groups were separated and corresponded entirely to the shell feature with or without ribs. Ribbing is controlled by a single gene, dominant to smooth (Davis and Fuff, 1973), and the degree of ribbing is controlled by multiple alleles (Davis, 1980). Liu *et al* (1981) and Lou (1982) found that the degree of ribs on the shells of snails from mainland China showed a continuous changing with geographic distribution, and they considered ribbed shells to be an important basis for classification of subspecies. They concluded that it was the easiest way to divide *Oncomelania* spp found in mainland China. Although Davis (1994) argued that large genetic distances indicated by enzyme analysis do not serve to define species, and ribbing was hardly a criterion for regarding taxa with ribs as a distinct species different from taxa without ribs, recent genetic data have provided more evidence that genetic distance between snails with ribbed shells and snails with smooth shells in mainland China is large and has probably reached the species level (Zhou, 1992; Hope and McManus, 1994; Zhou *et al*, 1995a; Qian *et al*, 1996). Therefore, more evidence needs to be studied by modern technic to explain the separation at species level affected by factors of environment and genetics.

Woodruff *et al.* (1986) were the first to study allozyme differences in populations of *Oncomelania* from China and the Philippines and came to the conclusion that the two taxa, which had been considered as sub-species, actually had reached the species level. Woodruff *et al* (1998) subsequently reported a geographically widespread polytypic species, *O. hupensis*, with a fragmented range on numerous isolated islands. They found no inter-population variation on a single island in the Philippines (Leyte), minor variation between samples representing four Philippine islands (Leyte, Luzon, Mindoro and Mindanao), but great differences between samples of *O. h. quadresi* from the Philippines and samples of *O. h. hupensis* from Guizhi in Anhui Province, China (Nei's genetic distance, D = 0.62). Since the Chinese *Oncomelania* from Guizhi had alleles not found in the Philippines at 14 out of 21 loci and no alleles are shared between the two areas at five loci, they recommended recognition as full species for the Chinese and Philippine taxa, *O. hupensis* and *O. quadrasi*, despite morphological similarity and a lack of strong post-mating reproductive isolation. Viyanant *et al* (1987) examined six populations of *O. h. quadrasi* from the Philippine islands of Luzon, Mindor and Leyte, compared to *O. h. hupensis* from Guizhi in the Anhui Province of China. Using isoelectric focusing technic on polyacrylamide gel to examine the allozyme patterns of eight enzymes, they found that only minor genetic variation was detected among various populations of snails from the Philippines. On the other hand, large differences were detected between *O. h. hupensis* and *O. h. quadrasi*. Of the eight enzymes examined, six were found to be different between the Chinese and Philippine snails. Tsukamoto *et al* (1998) compared allozyme variation in *Oncomelania* from Japan, Formosa and the Philippines and suggested that *O. nosophora* and *O. formosana* might also warrant elevation to species rank. *O. lindoensis* is very well differentiated as well, and deserves full species status according to Sobhon and Upatham (1990). In China, three populations of *O. hupensis* examined by allozyme technic showed minor allozyme variation between snails from Hubei and Jiangsu, but larger variation between Sichuan and two other

populations (Zhou, 1992). More reports (Zhang *et al,* 1994; Zhou *et al,* 1995a; Qian *et al,* 1996) showed that genetic variation of *Oncomelania* snails in mainland China was certainly large and corresponded to the geographic distribution of the snails. Our previous allozyme experiments suggested that the *Oncomelania* snails from Hubei and Jiangsu Provinces of China were quite similar genetically, while genetic distances between snails from the Philippines, Sichuan Province and Hubei/Jiangsu Provinces of China were quite large (Zhou, 1992), The present study provides more evidence to support the contention that snails with ribbed shells distributed along the Yangtze River are extremely different from the snails with smooth shells which are found all over the country and may well be a different species. In the group of smooth shelled snails, more subgroup can be clustered at subspecies level, those subgroups being snails from the Yunnan plateau, from the Sichuan basin, from the Fujian and Jiangsu coasts and from the hilly regions along the Yangtze River.

In conclusion, the present data studied in 31 natural populations of *Oncomelania* snails from mainland China provided supplementary genetic data about the evolution of *Oncomelania* spp along the whole Yangtze River. It is presumed that *Oncomelania* spp first arrived in Yunnan Province coming down from the Himalayas, then moved on to the Sichuan basin which was connected to the Yangtze River. After that, the snails were dispersed to the east coast of China. Some adjacent populations scattered in hilly regions along the middle or lower reaches of the Yangtze River occasionally dispersed down to the lake regions where genetic drift happened dramatically due to a different environment, and ribbing appeared to be controlled by a new mutation gene with natural selection force. A positive correlation (r = 0.80) between Mahalanobis' morphological distance and the genetic distance supports the hypothesis that the different shell phenotypes represent different species or subspecies. Our results thus underpin the theory that difference in species level has been reached between the snails with ribbed shells and the snails with smooth shells, while in the group of smooth shelled snails, more subgroups can be clustered at subspecies level.

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