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Relationship between susceptibility and genotype frequency of *Oncomelania* spp. to *Schistosoma japonicum* from mainland China analysed by logistic regression.

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The close association of schistosomes and snails is often shown by the specific and indeed local nature of the host-parasite relationship, which can be considered in terms of overall compatibility, comprising both infectivity of the parasite and susceptibility of the snail.[25].

It is well known that *Oncomelania* snails from different localities possess varying degrees of susceptibility to infection with geographic strains of *Schistosoma japonicum*. *O. formosana* from Taiwan was challenged with different strains of *S. japonicum* from Japan, but no successful infections resulted[12]. Since then, various comprehensive studies on susceptibility of *O. hupensis* from different geographic areas to strains of *S. japonicum* have been published by a number of investigators[1,7,8,9,10,11,16,26,27,28]. However, little is known about the genetic basis for these differences in host-parasite compatibility.

The present study was carried out in order to relate the intraspecific variation in susceptibility of natural populations of *Oncomelania* snails to genetic variability using a logistic regression model.

Snails were collected in 33 localities covering 9 provinces in southern China. Uninfected and mature snails were selected for susceptibility studies. Miracidia of *S. japonicum* (Wuxi strain) were harvested from eggs in the livers of rabbits infected with cercariae more than 35 days earlier in the laboratory. 100 snails from each population were exposed to miracidia of *S. japonicum* in groups (20 miracidia per snail) in petri dishes of 15 cm in diameter with 500 ml volume of water for 4 hours under conditions of continuous light at a temperature of 28 ± 2°C. All live snails were crushed and examined under a microscope 45–60 days after infection. The snails were marked as infected if the daughter sporocyst or cercaria of *S. japonicum* appeared in the digestive gland during the examination. The infection rate of each population of snails was calculated. If no infection was apparent a further four exposures were made.

Enzyme electrophoresis was carried out following standard methods[6,24]. A total of 13 enzymes were studied: Aldehyde dehydrogenase (ALDH, EC 1.2.1.5), Aldehyde oxidase (AO, EC 1.2.3.1), Alkaline phosphatase (AP, EC 3.1.3.1), Esterase (EST, EC 3.1.1.1), Glycerol-6-phosphate dehydrogenase (G6PDH), EC 1.1.1.49), Glutamate-oxaloacetate (GOT, EC 2.6.1.1), Glycerol-3-phosphate dehydrogenase (∝GPDH, EC 1.1.18), β-hydroxybutyrate dehydrogenase (HBDH, EC 1.1.1.30), Isocitrate dehydrogenase (IDH, EC 1.1.1.42), Lactate dehydrogenase (LDH, EC 1.1.1.27), Malate dehydrogenase (MDH, EC 1.1.1.37), Sorbitol dehydrogenase (SDH, EC 1.1.1.14) and Xanthine dehydrogenase (XDH, EC 1.2.1.37). After staining, enzyme activity patterns were recorded and photographed immediately. The loci were numbered in order of decreasing anodal mobility. Isoenzyme mobilities (Rf) were calculated relative to a bromophenol blue dye marker. Allele frequencies were estimated from the observed phenotype frequencies[4]. Only the frequencies of the commonest alleles in polymorphic loci were used in the regression analysis.

The infection rate of each snail population was included in logistic regression analyses as a dependent variable, and genotype frequencies of polymorphic loci as independent variables. Susceptibility of the population was scored as 0 when no snail became infected or as 1 when infection was positive. The model can be written as

$$\text{Prob (event)} = e^z / (1 + e^z),$$

or equivalently

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Prob (event) = $1 / (1 + e^{Z})$.
where Z is the linear combination,
$Z = B_0 + B_1X_1 + B_2X_2 + \ldots + B_pX_p$.
where B_0, B_1, B_2, ..., B_p were coefficients estimated from the data, X_1, X_2, ..., X_p were the independent variables, and e is the base of the natural logarithms.

In the logistic regression the parameters of the model were estimated using the maximum-likelihood method. All the computing procedures followed the model of Norusis[20]. The goodness of fit of the model was tested and the correct classification percent was calculated.

The infection rates in 27 susceptible populations of the 33 included in the susceptibility test, varied from 3.6 to 93.9% and in six populations the infection rate was zero (Dusheng, Dangling, Yuleng, Guanyi, Dali and Yiuanjing).

From the 13 enzymes examined, 17 presumptive loci were found following electrophoresis. 10 were monomorphic and invariant in all samples (ALDH, AO, EST-2, EST-3, EST-6, G6PDH, GPDH, HBDH, LDH and SDH), and seven were polymorphic(AP, EST-4, EST-5, GOT, MDH-2, IDH-2 and XDH). The commonest allele frequencies in the seven polymorphic loci are shown in Table 1.

Forward and backward stepwise methods[20] were utilized in the logistic regression analysis between susceptibility and genotype frequency of dominant alleles in the seven polymorphic loci. Results showed that only the MDH-2 locus was of significance to susceptibility (P = 0.0001). The regression coefficient was 13.71 (SE = 5.40, P = 0.01). The constant was −8.74 (SE = 3.74, P = 0.019). Therefore, given X as the genotype frequency of MDH-2, the logistic regression equation for the probability of infection can be written as: Prob (infection) = $1/ (1 + e^{Z})$, where Z = 13.71X − 8.74.

The test of the model showed no significant dif-

Table 1. Infection rate and the commonest allele frequencies of 7 polymorphic loci among 33 populations of *O. hupensis*. Number of snails infected was 100–500 per sample; > 100 in samples with low or negative infecton after first exposure.

Province	Populations	Inf.rate %	$AP^{0.32}$	$EST\text{-}4^{0.36}$	$EST\text{-}5^{0.23}$	$GOT^{0.47}$	$MDH\text{-}2^{0.04}$	$IDH\text{-}2^{0.25}$	$XDH^{0.28}$
Anhui	Laozhou	36.90	0.87	1.00	0.07	1.00	0.75	1.00	1.00
Anhui	Chuzhang	25.00	0.87	1.00	0.36	1.00	1.00	1.00	1.00
Anhui	Datong	31.10	0.92	1.00	0.62	1.00	1.00	1.00	1.00
Anhui	Dusheng	0.00	0.90	1.00	0.54	1.00	0.67	1.00	0.58
Anhui	Chuangjia	40.90	0.92	1.00	0.60	1.00	1.00	1.00	0.93
Anhui	Dingqiao (hilly)	14.30	0.50	1.00	0.46	0.71	0.84	1.00	1.00
Anhui	Dingqiao	17.00	1.00	1.00	0.20	1.00	1.00	1.00	1.00
Fujian	Huanlu	3.60	0.80	0.25	0.20	1.00	1.00	0.95	0.00
Hubei	Yige	82.70	1.00	1.00	0.23	1.00	1.00	1.00	1.00
Hubei	Muhe	85.90	1.00	1.00	0.55	1.00	1.00	1.00	1.00
Hubei	Saoyiang	36.40	0.38	1.00	0.50	1.00	1.00	0.95	0.97
Hubei	Donghuti	5.60	0.92	1.00	0.54	1.00	1.00	1.00	1.00
Hubei	Sisou	44.10	1.00	1.00	0.18	1.00	1.00	1.00	1.00
Hubei	Qinjiang	35.10	0.88	1.00	0.29	1.00	1.00	1.00	1.00
Hubei	Xingding	20.40	0.88	1.00	0.27	1.00	1.00	1.00	1.00
Hunan	Jizen	26.60	0.76	1.00	0.66	1.00	1.00	1.00	0.94
Hunan	Xihu Farm	38.50	0.92	1.00	0.48	1.00	1.00	1.00	0.98
Jiangsu	Borguazhou	93.80	1.00	1.00	0.50	1.00	1.00	1.00	1.00
Jiangsu	Xinming	41.70	1.00	1.00	0.28	1.00	1.00	1.00	1.00
Jiangsu	Lumar	15.20	0.52	0.56	0.68	1.00	1.00	1.00	1.00
Jiangsu	Huayan	21.40	0.65	1.00	0.46	1.00	1.00	1.00	1.00
Jiangsu	Baitar	54.70	0.80	1.00	0.90	1.00	1.00	1.00	1.00
Jiangsu	Laihua	28.00	1.00	1.00	0.36	1.00	1.00	1.00	1.00
Jiangsu	Nuidan	91.70	0.92	1.00	0.60	1.00	1.00	1.00	1.00
Jiangsu	Zhaopi	26.70	0.80	0.65	0.65	1.00	1.00	1.00	0.00
Jiangxi	Hurong	78.60	0.66	1.00	0.53	1.00	1.00	1.00	1.00
Shanghai	Xinta	64.10	0.35	1.00	0.75	1.00	1.00	1.00	0.97
Sichuan	Dangling	0.00	1.00	0.00	0.34	0.63	0.39	1.00	0.11
Sichuan	Yuleng	0.00	1.00	0.14	0.18	1.00	0.63	0.75	0.05
Sichuan	Pugi	6.00	1.00	0.18	0.40	1.00	0.50	1.00	0.00
Sichuan	Guanyi	0.00	1.00	0.05	0.65	1.00	0.50	1.00	0.20
Yunnan	Dali	0.00	1.00	0.00	0.18	1.00	0.33	1.00	1.00
Yunnan	Yiuanjing	0.00	0.70	0.00	0.00	1.00	0.40	1.00	1.00

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ference from the logistic regression model (P = .9999). Overall, 93.9% of the population (31) were correctly classified based on the model in 0.5 probability level, and among them, 83.3% resistant and 96.3% susceptible populations were correctly classified. These results suggested that the logistic regression model established fitted the data well.

The observations suggest a correlation between susceptibility and genotype frequency of MDH-2 in *O. hupensis*. Genetically determined susceptibility or resistance to parasitism has been found in other snail-trematode relationships: *Biomphalaria glabrata/ Schistosoma mansoni*[22,23], and *Helisoma anceps/ Halipegus occidualis*[17].

One variable (gene frequency of MDH-2), out of seven polymorphic loci, entered the logistic regression model at a significant level in the present study. Hence, there may be a possible linkage between susceptibility and the MDH-2 locus. Some form of association was also shown in *Biomphalaria glabrata* and *Schistosoma mansoni* by Fletcher *et al.*[5] who analysed the phenotype frequencies rather than allele frequencies, and reported correlation between the LDH locus and the degree of infection caused by five isolates of *S. mansoni*.

The test of the model showed no significant difference (P>0.05) and a highly correct classification (93.94%), indicating that the model fits the data well.

The logistic regression model established clearly indicates a quantitative relationship between susceptibility of *Oncomelania* snails and frequency of the MDH-2. MDH-2 is a dimeric locus with two alleles: MDH-$2^{0.15}$ and MDH-$2^{0.04}$, the latter being the commonest allele and the one analysed by the model. It was found that most susceptible populations were monomorphic for allele MDH-$2^{0.04}$. In contrast, most resistant populations were polymorphic for MDH-2. Only a few individuals were homozygous for MDH-$2^{0.15}$. The results indicated that the MDH-$2^{0.04}$ allele might be associated with susceptibility, and MDH-$2^{0.15}$ with resistance.

The influence of parasites on population structure may result from parasite resistance having a genetic basis, as shown in some laboratory stocks of snails[21]. However, experimental results have shown that resistant snails were selectively disadvantaged in the presence of both susceptible snails and schistosome parasites[15]. The present study indicates that the MDH-$2^{0.15}$ allele is associated with resistance of *O. hupensis* to *S. japonicum*. In six resistant snail populations, the frequencies of MDH-$2^{0.15}$ ranged from 0.33 to 0.67. The reason that some resistant snails still remain in populations with lower frequencies of MDH-$2^{0.15}$, may be that more direct selection pressure acts on the susceptible rather than resistant populations, resulting in more greater heterozygosity in resistant populations.

The most popular model of frequency-dependent selection assumes that host individuals carrying a recently arisen mutant allele have a selective advantage[19]. Damian[3] proposed a model of molecular mimicry of a peptide that mimics the host MHC (major histocompatibility complex) molecule, and he suggested that this mechanism generates polymorphism in immune system loci in the host. The present results provided evidence for the theory that there is much more heterozygosity at the MDH-2 locus in resistant rather than in susceptible populations. A greater number of polymorphic genomes may be more resistant to infection, as reported by Mulvey *et al.*[17] based on a study of infected *Helisoma anceps* from natural populations in a highly infected pond, showing that the infected snails had a lower protein electrophoretic variability than uninfected snails. The present studies suggesting a genetic association in *Oncomelania* snails showed that susceptible populations had a higher frequency of the allele MDH-$2^{0.04}$ than resistant populations. This corresponds to the hypothesis that random genetic drift and low rates of migration might be responsible for the patchy distribution of snail susceptibility to schistosomes.[18]. However, genetic differences between infected and uninfected snails could also reflect spatial heterogeneity of the snail distribution, for example local subdivision of uninfected and infected snail groups.[13].

The correlation does not indicate that the MDH-2 marker itself plays a direct role in snail susceptibility or resistance to *S. japonicum*, because there are probably additional genetic factors for susceptibility in intermediate host snails and for infectivity in parasite strains. Since both snail populations and parasite populations may vary qualitatively in the alleles present and quantitatively in gene frequencies, variations in host-parasite relations are to be expected when populations from different geographic areas are compared. This subject is to be dealt with more specifically in a later publication.

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