MALACOLOGIA, 1988, 29(2): 309–317

GENETIC DIFFERENTIATION AMONG WEST INDIAN POPULATIONS OF THE SCHISTOSOME-TRANSMITTING SNAIL *BIOMPHALARIA GLABRATA*

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ABSTRACT

Genetic variation and population differentiation are described for *Biomphalaria glabrata* from four islands in the West Indies. Estimates of enzyme polymorphism and individual heterozygosity were low relative to many other molluscs and consistent with the geographic isolation of these populations. Total genetic variance for the populations was partitioned as follows: 78% between islands, 2% between samples on islands and 20% between individuals in a sample.

Key words: *Biomphalaria glabrata, Schistosoma mansoni*, West Indies, enzyme polymorphism, genetic differentiation.

INTRODUCTION

A study of genetic variation in 28 loci in laboratory stocks of *Biomphalaria glabrata* revealed greater differentiation between some West Indian samples than expected (Mulvey & Vrijenhoek, 1984). In particular, a stock derived from the Dominican Republic (DR) had Nei (1978) genetic distance values of up to 0.43 when compared with stocks derived from Puerto Rico, St. Lucia and Brazil. Most conspecific mollusc populations have genetic distance values of less than 0.10 (Selander & Ochman, 1983). Furthermore, Mulvey & Vrijenhoek (1984) observed partial reproductive incompatibilities between the DR stock and other laboratory stocks of *B. glabrata*. Goldman *et al.* (1984) described chromosomal differences between this DR stock and other *B. glabrata*. To establish the significance of findings based on laboratory stocks, we have examined genetic variation in field-collected populations from four West Indian islands, including the Dominican Republic.

Biomphalaria glabrata is widely distributed throughout northern and eastern South America and the West Indies where it is the most important intermediate host for the human blood fluke, *Schistosoma mansoni* (PAHO, 1968). These snails exist as a series of locally or regionally differentiated races with respect to susceptibility to infection with strains of schistosomes (Kagan & Geiger, 1965; Michelson & DuBois, 1978). Compatibility of host and parasite is often limited to sympatric forms (Basch, 1975, 1976; Woodruff, 1985). Loci differentiation may be promoted by genetic drift associated with ephemeral habitats and the life history characteristics of these snails. The natural history of *B. glabrata* is well known as a result of field studies in Puerto Rico (Jobin, 1979; Pimental & White, 1957) and St. Lucia (Jordan *et al.*, 1978; McKellop & Harrison, 1980, 1982; McKillop *et al.*, 1981). These snails inhabit freshwater marshes, drainage or irrigation ditches, small ponds and slow-flowing streams (McKillop & Harrison, 1980). Suitable aquatic habitat has an inherent patchiness, and dispersal of snails between patches may be restricted. Populations are likely to experience occasional demographic "bottlenecks" due to drought and flooding. As they are potentially self-fertilizing hermaphrodites, a single individual can repopulate a site following a population crash. With a minimum generation time of 8 weeks under optimal conditions and a reproductive potential of 30 eggs/snail/day (Perlowagora-Szumlewicz, 1958), populations are capable of rapid recovery following disturbance (McKellop *et al.*, 1981).

Population structure and genetic variability in *B. glabrata* are important features, as they might affect transmission of the major human parasite, *Schistosoma mansoni*. Michelson & DuBois (1978) suggested that random genetic drift would be important in local differen-

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tiation of snail populations and might be associated with local differences in snail susceptibility to strains of schistosomes. Mulvey & Vrijenhoek (1982) tested this hypothesis by examining seven populations in Puerto Rico. These populations were highly structured; snails exhibited relative genetic homogeneity within populations and differentiation among local populations. Moreover, the snails from the island of Puerto Rico were genetically less variable than those described from Brazil (Narang *et al.*, 1981). An association between reduced genetic variability and genetic differentiation in island populations is not unexpected. The present study was undertaken to ascertain the degree of genetic differentiation occurring among island populations and to clarify the systematic relationships of snails from the various islands, especially the Dominican Republic.

MATERIALS AND METHODS

Specimens

Biomphalaria glabrata were collected with dip net or forceps from 6 locations in the West Indies between 20 August and 4 September 1983 as follows (arranged in geographic sequence from northwest to southeast):

A. Quisquisya, Dominican Republic	roadside ditch
B. Piedra Blanca, Dominican Republic	roadside ditch by cane field
C. Malpica, Puerto Rico	rural stream
D. Gosier, Guadeloupe	small farm pond
E. Bexon, St. Lucia	plantation irrigation ditch
F. Americ, St. Lucia	small hillside stream

Snails were abundant ($> 200/m^2$) at all locations except B and F. In addition, *Biomphalaria havenesis* were collected from a roadside ditch near Las Piedra in the Dominican Republic to serve as an outgroup for analytical purposes. Samples were transported alive to San Diego or stored on dry ice following collection. Voucher specimens are deposited at the University of Georgia's Savannah River Ecology Laboratory.

Electrophoresis

Snails were crushed individually; tissues were removed from the shell and homogenized in 0.2 ml of grinding solution (0.01 M Tris, 0.001 M EDTA, 0.05 mM NADP; pH 7.0). Homogenate fluid was absorbed onto filter paper wicks and inserted into 12.5% horizontal starch gels. Electrophoretic methods, including combinations of buffers and stains, are given elsewhere (Mulvey & Vrijenhoek, 1981a) and, in the case of five previously unstudied proteins, in Table 1. The M stock of *B. glabrata* (NIH albino) was used as a laboratory reference material and mobilities of electromorphs are reported relative to the common allozyme of the M stock which is arbitrarily assigned a value of 100. Typically, individuals from several populations as well as M stock standards were run on each gel to facilitate comparison of alleles across populations.

Statistical Analyses

Data consisting of multilocus genotypes for individual snails were analyzed using the BIOSYS-1 computer program (Swofford & Selander, 1981). A locus was considered polymorphic (P) if more than one allele was detected. Mean heterozygosity per individual (H) was estimated by direct count. A χ^2 statistic was used to test the fit of the observed data to expectations under a model of panmixia. For loci with three alleles, χ^2 statistics were calculated by pooling the least common alleles. Expected frequencies were calculated using Levene's (1949) correction for small sample sizes. Population structure was examined using F-statistics (Wright, 1978). The total genetic variance was partitioned into the following components: between islands, between subpopulations on islands, and heterozygosity within island subpopulations. Estimates of genetic distances were obtained by the method of Nei (1978) which is unbiased by sample size. Genetic distance values were clustered using the unweighted pair group averaging method.

RESULTS

Eighteen proteins were examined and provided information on 21 loci. Genetic interpretation of electromorph patterns is based on breeding experiments in *B. glabrata* (Mulvey

TABLE 1. Methods used to resolve additional enzyme systems in *Biomphalaria glabrata*.

Enzyme	Abbreviation	Buffer[a]		Stain[b]
Alkaline phosphatase	ALP	TC6.8	50	ml 0.2 M Tris/HCl, pH 8.0
			50	mg β-naphthyl acid phosphate
			50	mg Fast Blue BB
Haemoglobin	HB	TC6.8		Stain: 1.25 g Coomassie blue in fixative (250 ml methanol, 250 ml water, 46 ml acetic acid)
				Destain: several changes of fixative
Isocitrate dehydrogenase	IDH	TC6.8	50	ml 0.2 M Tris/HCl, pH 8.0
			120	mg isocitric acid
			140	mg $MgCl_2$
			10	mg NADP
			10	mg MTT
			5	mg PMS
Nucleoside phosphorylase	NP	TBE	50	ml 0.1 M K-phosphate, pH 6.5
			30	mg inosine
			10	units xanthine oxidase
			10	mg MTT
			5	mg PMS
Xanthine dehydrogenase	XDH	TBE	50	ml 0.2 M Tris/HCl, pH 8.0
			25	mg hypoxanthine
			10	mg NAD
			10	mg MTT
			5	mg PMS

[a]TC6.8 = 0.188 M tris, 0.065 M citrate, pH 6.8; dilute 1:20 for gels and 1:10 for electrode chambers. TBE = 0.5 M tris, 0.65 M borate, 0.2 M EDTA; pH 8.0; dilute 1:10 for gels and use undiluted for electrode chambers.
[b]NAD = nicotinamide adenine dinucleotide, NADP = nicotinamide adenine dinucleotide phosphate, MTT = methyl thiazolyl blue, PMS = phenazine methosulphate

& Vrijenhoek, 1984; Mulvey & Woodruff, 1985; Mulvey *et al.*, in press) and descriptions given by Harris & Hopkinson (1976) and Richardson *et al.* (1986). Each of the proteins not previously described for *B. glabrata* (ALP, IDH, HB, NP and XDH) appeared as a single region of activity. Patterns observed for heterozygous individuals were consistent with known subunit structures. Electromorph patterns were identical in tissues frozen in the field or never frozen.

Allozyme frequencies for the seven samples are presented in Appendix A, and summary statistics describing genetic variation are presented in Table 2. Eight loci were monomorphic for all *B. glabrata* examined. Thirteen loci were variable; *Gap, Got-1, Ldh, Mdh-1, Me, Np, Pgd* and *Xdh* had two alleles, and *Est-2, Idh, Pgm-1* and *Pgm-2* had three alleles among populations of *B. glabrata*. The Dominican Republic population of *B. havenensis* had 16 monomorphic loci and was diallelic at five loci (*Gpd, Got-1, Pgd, Pgm-1* and *Pgm-2*). Average individual heterozygosity (*H*) ranged from 0.000 to 0.037 for populations of *B. glabrata* and was 0.006 for the *B. havenensis* population.

Genotype frequencies in each sample were generally in agreement with expectations for random mating. Twenty-eight X^2 tests for fit to Hardy-Weinberg expectations were performed and no statistically significant deviations were observed. Values for F-statistics for the six populations of *B. glabrata* are presented in Table 3. The mean F_{it} value of 0.838 reflects a larger contribution by F_{st} (between population differentiation) and little contribution by F_{is} (within population differentiation) to the overall fixation index. A hierarchical G-statistic analysis indicates that 78% of the total genetic variance can be accounted for between islands, 2% between samples on islands, and 20% between individuals in samples.

Genetic distance values are presented in Table 4. Values for populations of *B. glabrata* ranged from 0.00 to 0.18. The interspecific genetic distances for *B. glabrata* and *B. havenensis* were all greater than 0.47.

The two St. Lucia samples exhibited no detectable enzyme activity for the *Idh* locus. When tissue from St. Lucia snails was electrophoresed and stained for IDH activity, the gel remained unstained; M stock controls or snails from other islands run alongside showed good activity. An attempt to demon-

TABLE 2. Summary statistics of genetic variation among West Indian populations of *Biomphalaria glabrata*. See text for locations. *P* = % polymorphic loci; > one allele detected. *H* = mean heterozygosity by direct count

Population	Sample Size	Mean No. alleles/locus (± 0.1)	*P*	*H*
Biomphalaria glabrata				
A. Dominican Republic	33.4 ± 1.0	1.2	19.0	0.027
B. Dominican Republic	38.8 ± 1.2	1.3	23.8	0.022
C. Puerto Rico	29.4 ± 1.5	1.3	23.8	0.011
D. Guadeloupe	32.5 ± 2.8	1.1	9.5	0.003
E. St. Lucia	36.9 ± 3.0	1.1	9.5	0.003
F. St. Lucia	37.5 ± 3.2	1.2	23.8	0.037
Biomphalaria havenesis				
G. Dominican Republic	33.0 ± 2.2	1.3	23.8	0.006

strate IDH activity in St. Lucia snails was made by varying conditions of electrophoresis and modifying the staining solution but no activity could be detected. As the St. Lucia samples showed strong activity when stained for all other enzyme systems, an allelic designation of "null" has tentatively been given to these snails for the *Idh* locus.

DISCUSSION

The levels of genetic polymorphism among populations of *B. glabrata* from four islands in the West Indies are in the lower range of values reported for 28 terrestrial and freshwater pulmonate snails (Selander & Ochman, 1983). Mean individual heterozygosity (*H*) in populations of *B. alexandrina* in Egypt (Graven, 1984) and *B. straminea* in Hong Kong (Woodruff *et al.*, 1985) were 0.04–0.09 and 0.06–0.10, respectively. Narang *et al.* (1981) reported that populations of *B. glabrata* in Brazil had levels of allozyme polymorphisms (*P*) that ranged from 0.18 to 0.48 and levels of individual heterozygosity of 0.076 to 0.211. Although there is only partial overlap for the enzymes used in these studies, the lower levels of genetic variation in the West Indian populations are consistent with their island distribution as geographic isolation reduces the probability of dispersal among populations. Estimates of average individual heterozygosity ranged from 0.00 to 0.04. These values are also at the lower end of the range reported for molluscs (Selander & Ochman, 1983). As discussed by Simon & Archie (1985), estimates of *P* and *H* are strongly dependent on the loci chosen as well as sample size. Thus differences in proteins studied in the studies of *Biomphalaria* may account for some of the differences in *P* and *H* estimates.

Selfing is probably not a significant contributor to population structure among these island populations. Although a functional hermaphrodite, *Biomphalaria* is a preferential outcrosser, capable of multiple matings and sperm storage (Mulvey & Vrijenhoek, 1981b). These attributes tend to balance the pressures of small population size which would otherwise reduce genetic diversity. Population structure, as determined by F-statistics, followed a predictable pattern: differentiation among islands > among local populations > within populations.

As expected, Nei's genetic distance values were highest for the between species comparison, D = 0.54. Values for conspecific populations of *B. glabrata* ranged from 0.00 to 0.18. Estimates of genetic distances involving the Dominican Republic and St. Lucia populations have bearing on questions raised by previous work about the relationships of these populations to other *B. glabrata*. Mulvey & Vrijenhoek (1984) studied ten laboratory stocks of *B. glabrata* and found average genetic distance values for stocks originating from the Dominican Republic (DR) and St. Lucia (L-311) to be 0.29 and 0.21, respectively from other stocks of *B. glabrata*. These values are quite high for conspecific comparisons and are more often reported for comparisons of congeneric species. The laboratory stocks previously examined had, however, been isolated many generations and may have undergone genetic divergence reflecting one or more founder events and/or genetic drift or selection associated with maintenance under artificial laboratory conditions. Snails collected in the

TABLE 3. F-statistics for six West Indian populations of *Biomphalaria glabrata* based on 13 polymorphic loci.

Locus	F_{is}	F_{it}	F_{st}
Est-2	0.056	0.888	0.881
Gap	0.200	0.234	0.043
Got-1	−0.014	−0.002	0.011
Idh	–	1.00	1.00
Ldh	−0.231	−0.032	0.161
Lap	−0.036	−0.006	0.029
Mdh-1	−0.014	−0.002	0.011
Me	0.114	0.154	0.045
Np	−0.032	−0.005	0.026
Pgd	0.361	0.976	0.962
Pgm-1	0.344	0.724	0.579
Pgm-2	0.646	0.651	0.013
Xdh	−0.014	−0.004	0.009
Mean	0.171	0.838	0.805

TABLE 4. Genetic distance values (Nei, 1978) for six populations of *B. glabrata* and one population of *B. havenensis*.

	B	C	D	E	F	G
B. glabrata						
A. Dominican Republic	0.002	0.003	0.164	0.143	0.142	0.532
B. Dominican Republic	–	0.000	0.174	0.134	0.132	0.547
C. Puerto Rico		–	0.180	0.139	0.138	0.554
D. Guadeloupe			–	0.091	0.093	0.469
E. St. Lucia				–	0.002	0.560
F. St. Lucia					–	0.555
B. havenesis						
G. Dominican Republic						–

field from the Dominican Republic and St. Lucia and compared with M stock snails had genetic distance values of 0.17 and 0.16, respectively. Field samples thus showed lower levels of genetic differentiation. The DR stock apparently derived from collections made from ponds at the Botanical Gardens in Santo Domingo. The Quisquisya and Piedra Blanca sites are approximately 135 and 150 km respectively, from Santo Domingo, therefore, these samples may not be directly comparable to the DR stock. Among the collections of *B. glabrata* from four islands the genetic distance values were all less than or equal to 0.20; such values are similar to those obtained for conspecific populations of mammals, rodents and molluscs (Nevo, 1978).

ACKNOWLEDGEMENTS

The authors are grateful to Liscencio Mercedes Vargas de Gomez, Instituto de Bilharzia, Universidad Autónomo de Santo Domingo, Dominican Republic; Jean-Pierre Pontier, Laboratoire de Biologie Marine et Malacologie, Ecole Pratique des Hautes Etudes, Paris, France; and the Honorable Mr. Allan Bousquet and Anthony Callender, Ministry of Health, Castries, St. Lucia, for assistance in locating sampling sites. This research was supported by USPHS fellowship No. GM 0719909 to MM at the University of California, San Diego, and NSF grant PCM 83-11210. Data analysis and preparation of the manuscript were supported by contract DE-AC09-76SR00819 between the U.S. Department of Energy and the University of Georgia's Savannah River Ecology Laboratory.

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Revised Ms. accepted 7 March 1988

Appendix A. Allele frequencies in West Indian populations of *B. glabrata* and *B. havenensis*. – designates cathodal migration.

Locus/Allele	Population[a]						
	A	B	C	D	E	F	G
Aconitase-2							
100	1.00	1.00	1.00	1.00	1.00	1.00	
130							1.00
Alkaline Phosphatase							
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Aspartate amino transferase-1							
100	1.00	1.00	1.00	1.00	1.00	1.00	0.95
167							0.05
Aspartate amino transferase-2							
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Esterase-2							
50	1.00	1.00	1.00	0.03			
80							1.00
100				0.95	0.83	1.00	
160				0.02	0.17		
Glyceraldehyde-3-phosphate dehydrogenase							
118		0.07	0.04				
110							1.00
100	1.00	0.93	0.96	1.00	1.00	1.00	
α-Glycerophosphate dehydrogenase							
140							0.05
100	1.00	1.00	1.00	1.00	1.00	1.00	0.95
Hemoglobin							
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Isocitrate dehydrogenase							
100						1.00	1.00
114	1.00	1.00	1.00				
Null				1.00	1.00		
Lactate dehydrogenase							
100	1.00	1.00	1.00	1.00	0.81	1.00	1.00
86					0.19		
Leucine amino peptidase							
100	0.97	1.00	1.00	1.00	1.00	1.00	1.00
96	0.03						
Malate dehydrogenase-1							
235							1.00
100	0.99	1.00	1.00	1.00	1.00	1.00	
22	0.01						
Malate dehydrogenase-2							
–100	1.00	1.00	1.00	1.00	1.00	1.00	
–115							1.00
Malic enzyme							
58	0.02	0.09	0.06				
100	0.98	0.91	0.94	1.00	1.00	1.00	
122							1.00
Mannose-6-phosphate isomerase							
100	1.00	1.00	1.00	1.00	1.00	1.00	
132							1.00
Nucleoside phosphorylase							
100	1.00	1.00	1.00	1.00	0.97	1.00	1.00
117					0.03		
6-Phosphogluconate dehydrogenase							
92	1.00	1.00	0.94				
100			0.06	1.00	1.00	1.00	
283							0.97
317							0.03

Appendix A continued

Locus/Allele	Population[a]						
	A	B	C	D	E	F	G
Phosphoglucomutase-1							
92	0.07	0.32	0.08	0.02	0.02	0.98	0.99
100	0.89	0.68	0.88	0.98	0.98	0.02	0.01
106	0.04		0.04				
Phosphoglucomutase-2							
100	1.00	1.00	1.00	1.00	0.98	0.99	
229						0.01	0.04
200							0.94
187							0.02
133					0.02		
Phosphoglucose isomerase							
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Xanthine dehydrogenase							
100		0.02	0.01				
82	1.00	0.98	0.99	1.00	1.00	1.00	1.00

[a]*Biomphalaria glabrata* (A–F): A = Quisquisya, DR; B = Piedra Blanca, DR; C = Malpica, PR; D = Gosier, Guadeloupe; E = Bexon, St. Lucia; F = Americ, St. Lucia; *Biomphalaria havenensis*, G = La Cambia, DR.